Plant Biology 2009 Final Program

Phycological
Society of America

Final program and abstracts of symposia, plenaries, minisymposia, talks, and poster presentations at Plant Biology 2009

Joint Annual Meetings of the
American Society of Plant Biologists
and the Phycological Society of America

Hawaii Convention Center, Honolulu, Hawaii

Saturday July 18 thru Wednesday July 22, 2009

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THE MEETING AND ABSTRACTS

The program for Plant Biology 2009 (**Hawaii Convention Center, Honolulu, Hawaii)** was organized by the ASPB & PSA Program Committees:

Chairs: Danny J. Schnell, Terence Evans
Committee Members: Tuan-hua David Ho, Nicholas C. Carpita, Judy Callis, Georg Jander, Janet Braam, Todd Mockler and Alison Sherwood as our local PSA representative

The call for abstracts for poster presentations and minisymposia was published in December 2008. The deadline for abstracts was April 24, 2009. All of the abstracts were received electronically via the web. All abstracts received on time were accepted, and the program was arranged from those abstracts. A searchable online version of the abstracts has been available since May 2009 via http://abstracts.aspb.org/pb2009/public/. This abstract supplement was produced from the electronic files submitted directly by the authors (missing numbers represent withdrawn abstracts). The author of each abstract is responsible for the abstract's accuracy, appearance, and content.

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Minisymposium 5 – Reproductive Biology

Plant Biology 2009 Daily Schedule SATURDAY, JULY 18

Start Time	End Time	Event/Item	Location
9:00 AM	9:00 PM	**Registration Open**	Exhibit Hall II & III
8:00 AM	10:00 AM	**ASPB Education Committee Meetings**	325A
8:00 AM	12:00 PM	***Plant Physiology*® Editorial Board Meeting**	325B
9:00 AM	5:00 PM	**Exhibitor and Poster Set-up**	Exhibit Hall II & III
11:00 AM	12:00 PM	**Undergraduate Networking Poster Session –** Students move your posters to Exhibit hall afterwards	Maui 316B
11:00 AM	1:00 PM	**ASPB Program Committee Meeting**	325A
12:30 PM	5:00 PM	**PSA Bold Talks** 12:30 - P04002: Craig Aumack - *Impacts of filamentous algal epiphytes/endophytes on macroalgal productivity in the Western Antarctic Peninsula* 12:45 - P02010: Stephanie Brunelle - *Post-transcriptional regulation of the DNA Replication Fork Proteins in the Florida Red Tide dinoflagellate, Karenia brevis* 1:00 - P05018: Amy Lynn Carlile - *Molecular systematics of North Pacific Ceramiaceae species, with a focus on the tribe Ceramieae* 1:15 - P02009: Jillian Lynch - *Metacaspase activity in aging Karenia brevis cultures: preliminary insight into cellular mechanisms regulating bloom termination.* 1:30 - P05021: Kimberly Peyton - *Exotic threats to biodiversity: A new species of Udotea forms meadows in the deep-water of Hawaii with an invasive alga bordering its edges* 1:45 - P04015: Rosemary Romero - *Recruitment strategies of the early colonizing macroalgae Ulva (Linnaeus) and Porphyra perforata in central California* 2:00 - P04012: Heather Spalding - *Discovering deepwater algal meadows in Hawaii: Home is where the Halimeda is* 2:15 - P03001: Cheryl Squair - *Feast or famine? Evaluating the significance of nutrient enrichment to crustose coralline algae on Ofu, American Samoa.* **2:30-3:00 Coffee Break In Ala Halawai Hallway** 3:00 - P04020: Daniel O'Doherty - *Genetic assessment of the pantropical alga Acanthophora spicifera (Rhodophyta) as revealed by DNA sequencing and microsatellite genotyping, with a focus on Hawaiian populations* 3:15 - P06001: Yen-Chun Liu - *Mechanism for Differential Desiccation Tolerance in Porphyra Species* 3:30 - P05019: Bridgette Clarkston - *A comparison of two DNA barcode markers for species discrimination in marine red algae* 3:45 - P05017: Katy Hind - *Identification of the genus Corallina (Corallinales, Rhodophyta) in Canada* 4:00 - P06005: Erin Cox - *Taking tropical stress in stride? An investigation of the impact of low tides on the brown alga Padina sanctae-crucis distribution and physiology.* 4:15 - P03002: Thomas Sauvage - *Assessing impacts of the non-indigenous red alga Gracilaria salicornia on Waikiki's reef health: Algal distribution changes over a 40 year period.* 4:30 - P04007: Selena McMillan - *Grazing effects of the turban snail, Chlorostoma brunnea, on the productivity of the giant kelp, Macrocystis pyrifera, in central California.* 4:45 - P04004: Megan Wehrenberg - *Alternative life history of Gracilariopsis sp. (Rhodophyta) within the dynamic substrate regime of a central Californian estuary*	Kaua'I 311
1:00 PM	2:00 PM	**Opening Address and ASPB Awards Ceremony**	Kalakaua Ballroom B & C
2:00 PM	2:40 PM	**Symposium I: Charles Albert Shull Award Winner –** Abstract S011 - Sheng Luan, University of California Berkeley **-** *The CBL-CIPK calcium signaling network in plants*	Kalakaua Ballroom B & C
3:00 PM	6:50 PM	**Symposium II: ILLUMINATING PLANT PHOTOMORPHOGENESIS** Organizer: Richard Vierstra, Univ. Wisconsin 3:00 - S021: Winslow Briggs – Carnegie Institute of Washington - *The LOV domains of the phototropins: In loyal service to photoreceptors in bacteria, fungi, algae, and higher plants* 3:40 - S022: Mannie Liscum, University of Missouri - *Musings and cogitations on phototropic signaling* **4:20 – 4:50 Coffee Break Outside Ballroom** 4:50 - S023: Richard D. Vierstra, University of Wisconsin - *Atomic perspectives on phytochrome photochemistry* 5:30 - S024: Peter H. Quail, USDA-Plant Gene Expression Center, UC Berkeley - *Phytochrome signaling networks* 6:10 - S025: Xing-Wang Deng, Yale University - *Light Control of Plant Development: a role of proteolysis*	Kalakaua Ballroom B & C

Plant Biology 2009 Daily Schedule SATURDAY, JULY 18 (continued)

6:30 PM	8:00 PM	**PSA Talks -** **Algal Phylogenetics & Taxonomy-I** 6:30 - P05016: Lesleigh Kraft - *A study of Australian Ulva challenges notions of cosmopolitanism and the utility of anatomical species designations* 6:45 - P05023: Charles O'Kelly - *Molecular assessment of the species of Ulva (Ulvophyceae, Chlorophyta) in the Hawaiian Islands* 7:00 - P05015: Gerald Kraft - *The marine macroalgae of Lord Howe Island: 32 years on* 7:15 - P05013: Judith Broom - *Progress in documenting the common coralline algae of New Zealand* 7:30 - P05006: Haj Allali - *A biodiversity survey of subaerial algae from an African tropical Rainforest* 7:45 - P05002: Daryl Lam - *Epiphytic Biodiversity of the Raleighvallen Rainforest (Suriname, South America) Inferred from Environmental Sequencing*	Kaua'I 311
6:30 PM	7:45 PM	**PSA Talks -** **Applied Phycology-I** 6:30 - P01007: Maria Ghirardi - *Hydrogen Fuel Production by Microalgae: Issues and Future Directions* 6:45 - P01003: Matthew Timmins - *High-efficiency hydrogen production from green microalgae* 7:00 - P01006: Takashi Yamamoto - *Elevation of the hydrogenase activity to produce hydrogen by Synechocystis sp. strain PCC6803* 7:15 - P02008: Yunyun Zhuang - *Regulatory network of cell cycle in marine phytoplankton* 7:30 - P01005: Makoto Wakayama - *Elevation of the production rate of the intracellular D-glucose in Synechococcus sp. strain PCC6301*	Lana'I 314
6:30 PM	8:00 PM	**PSA Talks -** **Algal Ecology & Population Biology-I** 6:30 - P04013: Peter Thompson - *The phytoplankton ecology of Western Australia.* 6:45 - P04018: Dennis Hanisak - *Water quality in the Indian River Lagoon, Florida: Relationship to the macroalgal community* 7:00 - P04009: Jennifer Ress - *Bryophytic algal communites from Nu'uanu Pali, O'ahu, (Hawai'i, U.S.A.)* 7:15 - P04017: Hugh Forehead - *Effects of reduced physical disturbance and nutrient enrichment on the ecology of subtidal benthic microalgae in Western Australia* 7:30 - P04003: Rex Lowe - *Distribution and morphological variability of Cosmioneis (Bacillariophyceae) in Hawaii* 7:45 - P04024: Alan Millar - *Threatened seaweeds and how we could protect them?*	Moloka'I 315
7:00 PM	8:20 PM	**Perspectives of Science Leaders** - Speaker: Dr. William H. Danforth – Recipient of the 2009 ASPB Leadership in Science Public Service Award	Kalakaua Ballroom B & C
8:30 PM	10:00 PM	**Opening Reception/Mixer**	Exhibit Hall II & III

Plant Biology 2009 Daily Schedule SUNDAY, July 19

Start Time	End Time	Event/Item	Location
7:00 AM	6:00 PM	**Registration Open**	Exhibit Hall II & III
7:00 AM	8:30 AM	**ASPB International Committee Meeting**	325A
7:00 AM	8:30 AM	**ASPB Education Foundation Board Meeting**	325B
7:00 AM	8:30 AM	**Small Colleges/PUI Networking Breakfast**	Maui 316B
7:00 AM	11:00 PM	**Posters Open**	Exhibit Hall II & III
8:30 AM	12:20 PM	**Symposium III: ASPB-PSA JOINT SYMPOSIUM: GENOMICS APPROACHES FOR SYSTEMATICS, ENERGY METABOLISM AND ACCLIMATION IN ALGAE** Organizers: Sabeeha Merchant, UCLA Alison Sherwood, Univ. Hawaii 8:30 - S031: Debashish Bhattacharya, University of Iowa - *The evolution of photosynthesis on the tree of life* 9:10 - S032: Sabeeha Merchant, UCLA - *Functional analysis of trace nutrient homeostasis in chlamydomonas using next generation sequencers* **9:50 Coffee Break Outside Ballroom** 10:20 - S033: Chris Bowler, CNRS, Molecular Plant Biology, Paris - *Genomics-enabled approaches for revealing the molecular secrets of marine diatoms* 11:00 - S034: Simon Prochnik - *Comparative genomic analysis of Chlamydomonas and Volvox sheds light on the evolution of developmental complexity* 11:40 - S035: Mary Rumpho, University of Maine - *Sea slug-algal chloroplast symbiosis: is horizontal gene transfer driving the evolution of photosynthesis in an animal?*	Kalakaua Ballroom B&C
9:30 AM	7:00 PM	**Exhibits Open**	Exhibit Hall II & III
12:30 PM	3:00 PM	**Exclusive Poster & Exhibit Session –** Boxed lunch provided 1:00 - 2:00 - even number abstracts 2:00 - 3:00 - odd number abstracts	Exhibit Hall II & III
3:00 PM	6:50 PM	**Symposium IV: PLANT NATURAL PRODUCTS - CHEMICAL EVOLUTION IN TIME AND SPACE** Organizer: Robert Last, Michigan State University 3:00 - S041: Rick Dixon, Noble Foundation - *Changing the spatial accumulation of proanthocyanidins- what else do we need to know?* 3:40 - S042: Jonathan Gershenzon, Max Planck Institute for Chemical Ecology - *Glucosinolate hydrolysis products: Why they dare to be different* **4:20 Coffee Break Outside Ballroom** 4:50 - S043: Rob Last, Michigan State University - *Integrated approaches to understanding tomato glandular trichome metabolism* 5:30 - S044: Joe Noel, Salk Institute - *Peeling back the layers of time: Reconstructing the evolutionary history of nature's biosynthetic toolbox* 6:10 - S045: Anne Osbourn, John Innes Centre - *Metabolic diversification in plants*	Kalakaua Ballroom B & C
7:00 PM	8:30 PM	**ASPB Minority Affairs Committee Meeting**	325A
7:00 PM	8:00 PM	**USDA Reception**	325B
7:00 PM	10:00 PM	**Career Workshop I - How To Survive A New Job** (ticket required) Panelists: Judy Callis, Simon Gilroy, Winslow Briggs, C. Robertson McClung, Marta Laskowski	318 A & B
7:00 PM	10:00 PM	**Career Workshop II - Alternate Careers** (ticket required) Panelists: Frederick Perlak, Flo Paoli, Rick Dixon, Susanne Brink, Sharlene Weatherwax, Huishan Guo, Patrick Morgan	319 A & B
7:00 PM	9:00 PM	**NSF Awardee Workshop - Collaborating with Chinese Scientists**	Maui 316A
7:00 PM	9:30 PM	**PSA Endowment Auction**	O'ahu 313A
7:30 PM	9:30 PM	**Education Workshop - Talking Science in Public: Evolution, GMO's and other challenging issues.** (ticket required)	Maui 316B
7:30 PM	9:30 PM	**Guidelines for Preparing Digital Art - Beginner -** Speaker: Michael Hepp of The Sheridan Group	Lana'I 314

Plant Biology 2009 Daily Schedule MONDAY, July 20

Start Time	End Time	Event/Item	Location
7:00 AM	8:30 AM	**ASPB Women in Plant Biology Committee Meeting**	325A
7:00 AM	11:00 PM	**Posters Open**	Exhibit Hall II & III
8:00 AM	5:00 PM	**Registration Open**	Exhibit Hall II & III
8:30 AM	12:20 PM	**Symposium V: ASPB-CSPP JOINT SYMPOSIUM: CROP FUNCTIONAL GENOMICS** Organizers: Jeffrey Bennetzen, Univ. Georgia Xioaya Chen, Shanghai Institute of Biological Sciences 8:30 - S051: Qifa Zhang, Huazhong Agricultural University, China - *Progress in rice functional genomics research in China* 9:10 - S052: Bin Han, National Center for Gene Research, and Beijing Institute of Genomics, Chinese Academy of Sciences - *High-throughput genotyping of rice recombinant inbred lines by whole genome re-sequencing* **9:50 - 10:20 - Coffee Break Outside Ballroom** 10:20 - S053: Xiao-ya Chen, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Science, Chinese Academy of Sciences - *Transcriptome and metabolic analysis of cotton* 11:00 - S054: Katherine VandenBosch, University of Minnesota - *Transcriptomics of legumes: Medicago truncatula as a case study* 11:40 - S055: Jeff Bennetzen, University of Georgia - *Comparative genomic approaches to identify novel gene functions, and their origins, in cereals*	Kalakaua Ballroom B & C
8:30 AM	12:30 PM	**PSA Talks** - **Algal Phylogenetics & Taxonomy-II** 8:30 - P05008: Juan Lopez-Bautista - *New insights in the systematics of Trentepohliales based on rbcL and morphological analyses* 8:45 - P05027: Gabrielle Rocap - *The chloroplast genome of the marine picoplankter Pinguiococcus pyrenoidosus* 9:00 - P05026: Jeffrey Johansen - *Using secondary structure of the 16S rRNA molecule and associated 16S-23S ITS to examine phylogenetic relationships of Aulosira (Nostocaceae, Cyanobacteria).* 9:15 - P04019: Michael Jacobs - *Differential rate of divergence in mitochondrial and chloroplast genome sequences from geographically separate strains of Heterosigma akashiwo.* 9:30 - P05004: Michael Wynne - *The recognition of Caulerpa integerrima (Zanardini) comb. et stat. nov. (Bryopsidales, Chlorophyta) from the Red Sea* 9:45 - P04021: Chi Chiu Cheang - *The phylogeography of Sargassum fusiforme (Fucales, Heterokontophyta) in the northwestern Pacific* 10:00 - P04008: Kyle Demes - *Phenotypic plasticity reconciles incongruous molecular and morphological taxonomies: Macrocystis is a monospecific genus* **10:15 - 11:00 Coffee Break In Ala Halawai Concourse** **Algal Phylogenetics & Taxonomy-III** 11:00 - P05022: Michael Lynch - *Investigating deep phylogenetic relationships within the Rhodophyta by RNA secondary structure and nuclear gene sequence analysis* 11:15 - P05014: Sandra Lindstrom - *Contrasting phylogeographic patterns of two high intertidal dioecious species of Porphyra (Bangiales, Rhodophyta) in the northeast Pacific* 11:30 - P05055: Max Hommersand - *Molecular distance is correlated with biogeographic separation in marine red algae, whereas morphological variation is conditioned by environmental change, especially adaptations to different temperature isotherms* 11:45 - P05010: Craig Schneider - *Molecular investigations of foliose Rhodymeniophycidae (Rhodophyta) from Bermuda, western Atlantic*. 12:00 - P05003: Paul Geraldino - *Phylogenetic relationships within the genus Hypnea (Gigartinales, Rhodophyta) based on multigene data* 12:15 - P05042: Daniela Gabriel - *The red algal genus Titanophora (Schizymeniaceae, Nemastomatales) in the Gulf of Mexico*	Ni'ihau 312

8:30 AM	10:15 PM	**PSA Talks** **Algal Ecology & Physiology** 8:30 - P06008: Raymond Lewis - *Reduced salinity increases oogenesis in various kelps (Order Laminariales)* 8:45 - P06004: Regina Radan - *Differential toxin response of Pseudo-nitzschia multiseries as a function of nitrogen source: batch and continuous cultures* 9:00 - P04005: Philip Bucolo - *Phototactic responses of swarmers of the Antarctic epiphyte Elachista antarctica* 9:15 - P04011: Kathryn Van Alstyne - *The release of dopamine by Ulvaria obscura and its allelopathic effects on algae and invertebrate larvae* 9:30 - P06006: Sarah Kiemle - *What do the cell walls of two primitive true taxa of the charophycean green algae, Chlorokybus atmophyticus and Klebsormidium flaccidum, tell us about the evolution of the land plant cell wall?* 9:45 - P06010: Graham Peers - *An ancient light harvesting protein is critical for the regulation of algal photosynthesis* 10:00 - P06002: Yingjun Wang - *LCIB: a novel gene family involved in the microalgal CO2-concentrating mechanism*	Lana'I 314
8:30 AM	12:45 PM	**PSA Talks** **Algal Cellular & Molecular Biology** 8:30 - P02005: John La Claire - *Gene expression profiling growth stages of Prymnesium parvum (Haptophyta) in culture and in nature* 8:45 - P07002: Alejandra Gonzalez - *Morphometric and molecular studies identify cryptic species in the Lessonia nigrescens complex along the chilean coast* 9:00 - P02006: Rose Ann Cattolico - *Chloroplast genomes, genes and gene expression in two strains of Heterosigma akashiwo- a bloom forming alga.* 9:15 - P07001: Megan Black - *Heterosigma akashiwo world-wide population diversity* 9:30 - P07003: Michelle Casanova - *Can we use vegetative morphology for species determination in Nitella (Characeae, Charophyceae)?* 9:45 - P02002: Nedeljka Rosic - *Gene expression analysis and housekeeping genes selection for real-time RT-PCR in symbiotic dinoflagellates during thermal and light stress* 10:00 - P02003: Yusuke Matsuda - *Modeling of structure of CO2 responsive promoter in the marine diatom Phaeodactylum tricornutum.* 10:15 - P04006: Su Yeon Kim - *Molecular approach for the current distribution pattern of Pterocladiella capillacea (Gelidiales, Rhodophyta)* **10:30 - 11:00 Coffee Break In Ala Halawai Concourse** **Applied Phycology-II** 11:00 - P01009: Zackary Johnson - *Isolation and characterization of marine phytoplankton as a next generation biofuel* 11:15 - P01008: Lisa Pickell - *Mid-scale screening of marine phytoplankton for large scale production of biofuel* 11:30 - P02001: Rachel Miller - *Oil biosynthesis in chlamydomonas reinhardtii* 11:45 - P01004: Eugene Zhang - *Characterisation of australian microalgae for biodiesel production* 12:00 - P02007: Todd Lane - *Digital transcriptomic analysis of silicate starvation induced triacylglycerol* 12:15 - P06011: Skye Thomas-Hall - *Analysis of lipid accumulation in microalgae* 12:30 - P04016: Charles Yarish - *Multi-Component Evaluation to Minimize the Spread of Aquatic Invasive*	Moloka'I 315
9:30 AM	2:00 PM	**Exhibits Open**	Exhibit Hall II & III
12:20 PM	2:30 PM	**ASPB Women in Plant Biology Sponsored Lunch & Speaker -** Speaker: Mary Lou Guerinot (ticket required)	Maui 316B
12:20 PM	2:00 PM	**TAIR Workshop I - How to effectively use the tools and resources at TAIR to enhance your research -** Speakers: Phillipe Lamesch, Donghui Li, A.S. Karthikeyan	318 A & B
2:30 PM	4:10 PM	**Guidelines for Preparing Digital Art - Advanced -** Speaker: Michael Hepp of The Sheridan Group	319 A&B
2:30 PM	4:30 PM	**PSA Plenary 1 - Algal Biotechnology** 2:30 - P01001: David Chapman - *Algal biotechnology: an overview of past present and prognosis* 3:30 - P02020: Arthur Grossman - *Opening the floodgates: Comparative genomic analyses from a Chlamydomonas-centric perspective* 4:00 - P01002: Don Cheney - *Seaweed biotechnology: from phycocolloids to environmental bioremediation and renewable sources of biofuels*	Ni'iahu 312

Plant Biology 2009 Daily Schedule MONDAY, July 20 (continued)

2:30 PM	4:10 PM	**Minisymposium 1: Education Outreach - Evolution & Innovation in Plant Biology Outreach for Elementary, Community College, Undergraduate, & Professional Science Educators** - Chair: Jane Ellis 2:30 - M0101: Jeremy Pritchard - *Simple but Dangerous ideas; strategies to help in teaching evolution* 2:55 - M0102: Christina Reynaga-Pena - *Development and assessment of didactic packages including DVDs on plant biology experiments for rural schools in Mexico* 3:20 - M0103: Kabi Neupane - *Advances in biosciences education for community Colleges: The journey from summer workshop to year-round independent research project* 3:45 - M0104: Erin Dolan - *Undergraduate-level inquiry: Benefits and challenges of engaging in classroom-based research*	Lana'I 314
2:30 PM	4:10 PM	**Minisymposium 2: Gibberellins & Abscisic Acid** - Chair: Tai-ing Sun 2:30 - M0201: Kohji Murase & Rodolfo Zentella - *Structure-function analysis of GA receptor and DELLA protein in Arabidopsis* 2:55 - M0202: Camille M. Steber - *Relieving DELLA repression of stem elongation and flowering, evidence for a proteolysis independent mechanism for GA signaling* 3:20 - M0203: Wan-Chi Lin - *ABA Receptors? Not sure. Signaling molecules? Yes.* 3:45 - M0204: David H. Huizinga - *Isoprenylcysteine methylation and demethylation regulate abscisic acid signaling in Arabidopsis*	Kaua'I 311
2:30 PM	4:10 PM	**Minisymposium 3: Genome Integrity** - Chair: Anne Britt 2:30 - M0301: Igor Kovalchuk - *Progeny of stressed plants exhibit dramatic changes in genome stability, methylation pattern, stress tolerance and metabolites profile* 2:55 - M0302: Anne B. Britt - *The NAC domain transcription factor Suppressor of Gamma Response 1 (Sog1) governs programmed response to DNA damage* 3:20 - M0303: Sascha Biedermann - *The DDB1a interacting proteins CSA and DDB2 are critical factors for UV-B tolerance in Arabidopsis thaliana* 3:45 - M0304: Vipula K. Shukla - *Precise genome modification in the crop species Zea mays using zinc-finger nucleases*	Moloka'I 315
2:30 PM	4:10 PM	**Minisymposium 4: Secondary Metabolism** - Chair: Kazuki Saito 2:30 - M0401: Kazuki Saito - *Transcriptome coexpression analysis and comprehensive metabolite profiling led to decoding gene-metabolite correlations in Arabidopsis flavonoid metabolism* 2:55 - M0402: Hyun Joo Koo - *Evolution and biosynthesis of medicinally important terpenoids curlone and the turmerones in turmeric and ginger* 3:20 - M0403: Charles E. Stewart - *Convergent biosynthetic evolution in type III polyketide synthases* 3:45 - M0404: Hong Han - *The biosynthesis of triterpenoid glutinol and friedelin in kalanchoe daigremontiana*	Maui 316A
2:30 PM	4:10 PM	**Minisymposium 5: Reproductive Biology** - Chair: Mark Johnson  2:30 - M0501: Yongxian Lu - *Cation/proton transporters are key players in pollen tube guidance* 2:55 - M0502: Tetsuya Higashiyama - *Identification of pollen tube attractants derived from the synergid cell* 3:20 - M0503: Mily Ron - *Mis-regulation of a nat-siRNA pair in sperm cells results in single fertilizations.* 3:45 - M0504: Mark A. Johnson - *HAP2(GCS1) is a sperm-expressed component of a deeply conserved fertilization mechanism* **Sponsored by Elsevier - *Plant Science***	Maui 316C
2:30 PM	4:10 PM	**Minisymposium 6: Chloroplast Signaling & Gene Expression** - Chair: Maureen Hanson 2:30 - M0601: Masahiro Sugiura - *Novel termination-dependent translation in chloroplasts* 2:55 - M0602: Wade Heller - *A comparative genomics approach identifies RARE1: a pentatricopeptide repeat protein mediating chloroplast accD transcript editing* 3:20 - M0603: Takehito Inaba - *Coordination of plastid protein import and nuclear gene expression by plastid-to-nucleus retrograde signaling pathway* 3:45 - M0604: Barry Pogson - *Chloroplast-nuclear signaling: A tale of phosphatases, gene silencing and histone modifications*	O'ahu 313A
4:10 PM	4:45 PM	**Coffee Break**	Ala Halawai Concourse
4:45 PM	6:45 PM	**PSA Plenary 2 - Algal Species Concept in Molecular Ecology** 4:45 - P07006: Wayne Litaker - *Defining algal species in the 21st century: Morphology vs. genetics?* 5:45 - P07005: Dale Casamatta - *Towards a method of untangling cyanobacterial systematics in the age of genomics.* 6:15 - P07004: Robert Sheath - *What is a red algal species?*	Ni'ihau 312

4:45 PM	6:25 PM	**Minisymposium 7: Minority Affairs - Ka Hunaola Lā`au (Plant Cell Biology)** - Chair: John Harada 4:45 - M0701: Kawika Winters - *Seeing the waonāhele from amongst the trees: A culturally-based, whole-system view of plant biology* 5:10 - M0702: Beronda Montgomery-Kaguri - *Right place, right time: Spatiotemporal phytochrome regulation of plant growth and development* 5:35 - M0703: Elison Blancaflor - *Filling the gap between cytoskeletal remodeling and membrane trafficking in the regulation of tip growth in plants* 6:00 - M0704: Magdalena Bezanilla - *Controlling actin dynamics is required for tip growth*	Lana'I 314
4:45 PM	6:25 PM	**Minisymposium 8: Hormone Biology** - Chair: Yuji Kamiya 4:45 - M0801: Yuji Kamiya - *Indole-3-acetaldoxime dependent auxin biosynthesis in Arabidopsis* 5:10 - M0802: John G. Tallman -*Like heat, L-NG-monomethyl arginine (L-NMMA), an inhibitor of arginine-dependent nitric oxide (NO) production, blocks auxin signaling for gene expression and interferes with hormone-dependent cell expansion and division in cultured Nicotiana glauca guard cell protoplasts (GCP).* 5:35 - M0803: Abidur Rahman - *Transcytosis of PIN2 in arabidopsis is regulated by protein phosphatase 2A and PID kinase* 6:00 - M0804: Noriyuki Nishimura - *Identification of new ABI1-mediated ABA signaling components in arabidopsis.*	Kaua'I 311
4:45 PM	6:25 PM	**Minisymposium 9: Cell Cycle Regulation** - Chair: Dirk Inze 4:45 - M0901: Christine Foyer - *Redox homeostasis and regulation in the cell cycle* 5:10 - M0902: Dirk Inzé - *The molecular basis of organ growth* 5:35 - M0903: Hyun-Sook Pai - *Dual Functions of Nicotiana benthamiana Rae1 in interphase and mitosis* 6:00 - M0904: Yuh-Ru Julie Lee - *The WD40 repeat protein NEDD1 plays a role in microtubule organization during mitotic cell division in Arabidopsis thaliana*	Moloka'I 315
4:45 PM	6:25 PM	**Minisymposium 10: Emerging Model Systems** - Chair: Janet Slovin 4:45 - M1001: Todd Michael - *Duckweeds as model aquatic plants* 5:10 - M1002: Janet Slovin - *Diploid strawberry (Fragaria vesca) a reference species for the Rosaceae family* 5:35 - M1003: John Vogel - *Brachypodium distachyon: a new model for the grasses* 6:00 - M1004: Todd Mockler - *Brachypodium distachyon transcriptomics*	Maui 316A
4:45 PM	6:25 PM	**Minisymposium 11: Abiotic Stress** - Chair: Joerg Kudla 4:45 - M1101: Joerg Kudla - *Regulation and function of calcium sensor proteins and their interacting kinases in abiotic stress responses* 5:10 - M1102: June M. Kwak - *Two MAP kinases preferentially expressed in guard cells positively regulate ROS-mediated ABA signaling* 5:35 - M1103: Hargurdeep S. Saini - *Enhancement of salinity tolerance by engineering a chloride-volatilizing enzyme into plants* 6:00 - M1104: Shutian Li - *Nuclear activity of ROXY1, a glutaredoxin interacting with TGA factors, promotes petal development in Arabidopsis*	Maui 316C
4:45 PM	6:25 PM	**Minisymposium 12: Light Signalling** - Chair: Robert Larkin 4:45 - M1201: Robert M. Larkin - *Integration of light and plastid signals* 5:10 - M1202: Md. Sayeedul Islam - *Photoreceptor systems for light-dependent intracellular positioning of mitochondria in Arabidopsis thaliana* 5:35 - M1203: Meng Chen - *HEMERA, an essential regulator linking phytochrome nuclear bodies and light signaling in Arabidopsis* 6:00 - M1204: Hongtao Liu - *Blue light-specific regulation of CIB1 protein expression in Arabidopsis*	O'ahu 313A
6:30 PM	8:30 PM	**ASPB Minority Affairs Dinner and Sponsored Speaker:** Speaker: Cliff Poodry, Director, Division of Minority Opportunities and Research, National Institute of General Medical Sciences, NIH (ticket required)	Maui 316B
7:00 PM	8:30 PM	**TAIR Workshop II - TAIR, PMN, and SGn workshop: Focus on comparative genomics and new tools.** Speakers: Philip Lamesch, A.S. Karthikeyan, Lukas Muellar, Pankal Jaiswal	318 A & B
7:00 PM	8:30 PM	**Grantsmanship Workshop:** Representatives from Federal agencies will provide overviews of funding opportunities	319 A&B
7:00 PM	8:30 PM	**ASPB Fellows Reception**	325A
8:30 PM	11:00 PM	**Exclusive Poster & Exhibit Session** 9:00 - 10:00 PM - odd numbered abstracts 10:00 - 11:00 PM - even numbered abstracts	Exhibit Hall II & III

Plant Biology 2009 Daily Schedule TUESDAY, July 21

Start Time	End Time	Event/Item	Location
7:00 AM	8:30 AM	**ASPB Public Affairs Committee Meeting**	325A
7:00 AM	8:30 AM	**ASPB Membership Committee Meeting**	325B
7:00 AM	11:00 PM	**Posters Open**	Exhibit Hall II & III
8:00 AM	5:00 PM	**Registration Open**	Exhibit Hall II & III
8:00 AM	12:00 PM	**Symposium VI: DARWIN'S LEGACY: EVOLUTION AND PLANT BIOLOGY** Organizer: Barbara Schaal, Washington University, St. Louis 8:00 - S061: Michael Purugannan, NYU - *Adaptive radiation and regulatory gene evolution in the Hawaiian Silversword alliance (Asteraceae)* 8:40 - S062: Kenneth Olsen, Washington University - *Clover cyanogenesis: evolution and ecology of an adaptive polymorphism* **9:20 - 10:00 - Coffee Break Outside Ballroom** 10:00 - S063: Brandon Gaut, UC, Irvine - *A mechanism of selection against transposable elements in Arabidopsis thaliana* 10:40 - S064: Tzen-Yuh Chiang, Taiwan - *Ecological genomics of Miscanthus (Poaceae), a biofuel plant* 11:20 - S065: Leonie Moyle, Indiana University - *Insights into the origin of species from Solanum and other plant groups*	Kalakaua Ballroom B & C
9:30 AM	1:30 PM	**Exhibits Open**	Exhibit Hall II & III
12:00 PM	1:30 PM	**ASPB Executive Committee Meeting/Luncheon**	325A
12:00 PM	1:30 PM	**PSA *Journal of Phycology* Editorial Board Luncheon**	325B
12:00 PM	1:30 PM	**Exclusive Poster Session & Exhibit Session -** Open All Posters	Exhibit Hall II & III
1:30 PM	7:30 PM	**Exhibitor Breakdown**	Exhibit Hall II & III
1:30 PM	4:40 PM	**Symposium VII: *THE PLANT CELL* 20TH ANNIVERSARY** Organizer: Cathie Martin, John Innes Centre 1:30 - Cathie Martin - Introduction 1:40 - S071: Kazuo Shinozaki - *Transcriptional regulatory network in drought stress response and tolerance (Plant Cell 10: 1391–1406)* 2:10 - S072: Karin Schumacher - *pH in the endomembrane system: Moving on (Plant Cell 18: 715–730)* 2:40 - S073: John Ryals - *Systemic acquired resistance (Plant Cell 3: 1085–1094)* **3:10 - 3:40 - Coffee Break Outside Ballroom** 3:40 - S074: Rick Amasino - *Vernalization: remembering winter with an environmentally induced epigenetic switch (Plant Cell 11: 949–956)* 4:10 - S075: Rich Jorgensen - *Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. (Plant Cell 2: 279–289)*	Kalalaua Ballroom B & C
2:00 PM	4:00 PM	**PSA Plenary 3 - Coral Reef Ecology** 2:00 - P03006: Laurence McCook - *Coral reef resilience, degradation and climate change; the role of algal ecology* 3:00 - P03005: Mark Hay - *Killer seaweeds: variance in allelopathic impacts across coral species, seaweed species, and between the Caribbean and tropical Pacific* 3:30 - P03007: Guillermo Diaz-Pulido - *Climate change effects on coral reef algae: the missing piece in the future of coral reefs*	Ni'ihau 312
4:40 PM	6:10 PM	**PSA Talks** **Algal Phylogenetics & Taxonomy-IV** 4:40 - P05011: Ed Theriot - *A preliminary multigene phylogeny of the diatoms* 4:55 - P05025: Elizabeth Ruck - *Comparing chloroplast, nuclear, and mitochondrial phylogenies in the Surirellales (Bacillariophyta)* 5:10 - P05024: Matt Ashworth - *Holes and poles: a molecular approach to the phylogeny of the ocellate and pseudocellate diatoms* 5:25 - P05028: Teofil Nakov - *Preliminary molecular phylogeny of the Cymbellales (Bacillaryophyceae)* 5:40 - P05012: Cheong Xin Chan - *Rampant gene transfer in dinoflagellates and its implications to the tree of life* 5:55 - P05007: Sung Mi Cho - *Phylogenetic relationships of Heterokontophyta based on six genes data*	Ni'ihau 312

4:40 PM	5:55 PM	**PSA Talks** **Algal Ecology & Population Biology II** 4:40 - P04010: Michael Stekoll - *Competition between and co-existence of algal crusts and subtidal kelps* 4:55 - P04014: Charles Amsler - *Filamentous algal endophytes in macrophytic Antarctic algae: prevalence in hosts and palatability to mesoherbivores* 5:10 - P04022: Bruce Parker - *Shrinkage and disappearance of Mountain Lake, Virginia, USA* 5:25 - P05001: Poonam Sharma - *Studies on the taxonomy and biodiversity of microalgae occurring in fresh water habitats of Shivalik Himalayas of Jammu and Kashmir, India: Applications in aquaculture, pollution and bioremediation.* 5:40 - P04001: Nathan Smucker - *Acid mine drainage and remediation impacts on lotic biofilm structure and extracellular enzyme activities during succession*	Maui 316B
4:40 PM	6:20 PM	**Minisymposium 13: Brassinosteroids** - Chair: Steven Clouse 4:40 - M1301: Yanhai Yin -*Network and mechanism of brassinosteroid regulated gene expression and responses in arabidopsis thaliana* 5:05 - M1302: Tae-Wuk Kim - *Brassinosteroid signal transduction from cell surface receptor kinases to nuclear transcription factors* 5:30 - M1303: Xuelu Wang - *The primary signaling outputs of brassinosteroids are regulated by abscisic acid signaling* 5:55 - M1304: Steven D. Clouse - *Receptor kinases involved in brassinosteroid signal transduction phosphorylate protein translation initiation factors*	Lana'I 314
4:40 PM	6:20 PM	**Minisymposium 14: Cytoskeletal Dynamics** – Co-chairs: Valerian Dolja & Magdalena Bezanilla 4:40 - M1401: William R. Eisinger - *Reduction in guard cell microtubule stability correlates with stomatal closure in Arabidopsis* 5:05 - M1402: Chris Staiger - *Stochastic dynamics of actin filaments in the cortical array of Arabidopsis epidermal cells* 5:30 - M1403: Luis Vidali - *Class II formins and myosin XIs are required for tip growth* 5:55 - M1404: Valerian V. Dolja - *Myosin functions in organelle trafficking, F-actin organization, and plant development*	Kaua'I 311
4:40 PM	6:20 PM	**Minisymposium 15: Plant Pathogen Interactions** - Chair: Elizabeth Fontes 4:40 - M1501: Elizabeth P.B. Fontes - *NIK-mediated antiviral signaling, a novel layer of innate plant defenses suppressed by the geminivirus nuclear shuttle protein* 5:05 - M1502: Birgit Schulze - *The BAK1-FLS2 receptor complex: Dynamics of heteromerization and phosphorylation in response to flagellin perception* 5:30 - M1503: Ping He - *Bacterial Effectors Target A Common Signaling Partner To Impede Host Immunity and Development* 5:55 - M1504: Anneke Prins - *The role of protease inhibitors in the hypersensitive response*	Moloka'I 315
4:40 PM	6:20 PM	**Minisymposium 16: Tropisms** - Chair: Gabriele Monshausen 4:40 - M1601: Miyo T. Morita - *SHOOT GRAVITROPISM 9, a novel RING finger protein, is involved in statolith dynamics by modulating interaction between F-actin and amyloplasts.* 5:05 - M1602: Takeshi Yoshihara - *LAZY1 belongs to a novel class of genes involved in gravitropic signal transduction in monocot and dicot plants* 5:30 - M1603: Gabriele B. Monshausen - *Basipetal migration of Ca2+ and pH waves during the gravireponse of Arabidopsis roots* 5:55 - M1604: Yutaka Miyazawa - *Identification of genes responsible for root hydrotropism in Arabidopsis roots*	Maui 316A
4:40 PM	6:20 PM	**Minisymposium 17: Mineral Nutrition** - Chair: Li Li 4:40 - M1701: Li Li - *A broccoli COQ5 methyltransferase involved in ubiquinone biosynthesis mediates selenium volatilization* 5:05 - M1702: Jason D. Gillman - *Identification of the molecular basis of the seed low phytic acid phenotype in soybean line CX1834* 5:30 - M1703: Yi-Fang Tsay - *Mutation of the Arabidopsis NRT1.5 nitrate transporter causes defective root-to-shoot nitrate transport.* 5:55 - M1704: Narayanan N. Narayanan - *Functional Characterization of a Novel Iron Transporter, FEA1, from Chlamydomonas reinhardtii and its Application for Iron-Specific Metal Uptake in Plants*	Maui 316C
4:40 PM	6:20 PM	**Minisymposium 18: Photosynthesis** - Chair: Thomas Brutnell 4:40 - M1801: Sungsoon Park - *REP27, a thylakoid membrane protein functioning in the D1/32 kD reaction center protein turnover and PSII repair from photodamage* 5:05 - M1802: Shizue Matsubara - *Acclimation and adaptation of leaf carotenoid composition and biosynthesis in tropical plant species* 5:30 - M1803: Tammy L. Sage - *The functional anatomy of rice leaves: implications for refixation of photorespiratory CO2 and efforts to engineer C4 photosynthesis into rice.* 5:55 - M1804: Thomas P. Brutnell - *A systems approach to understanding C4 photosynthetic differentiation in maize*	313A
6:30 PM	8:30 PM	**PSA Banquet** (ticket required) Busses will pick up from the convention center at 6:00 pm and at 6:15 pm and return to the center at 9:15 pm and 9:30 pm.	Waikiki Aquarium
7:30 PM	10:00 PM	**Final Party**	Hilton Lagoon

Plant Biology 2009 Daily Schedule WEDNESDAY, July 22

Start Time	End Time	Event/Item	Location
7:00 AM	2:00 PM	**Poster Open –** remove posters by 2 PM, posters left will be discarded	Exhibit Hall II & III
8:00 AM	12:00 PM	**Registration Open**	HaExhibit Hall II & III
8:30 AM	12:30 PM	**Symposium VIII: JOINT ASPB-PSA-NSF RCN PORPHYRA - *PORPHYRA:* A CROP OF THE SEA** Organizer: Susan Brawley, University of Maine 8:30 - S081: Susan Brawley, University of Maine, Orono - *The crop and the organism* 9:00 - S082: Juliet Brodie, Natural History Museum, London - *Porphyra: modern systematics interprets an ancient lineage* 9:40 - S083: Mariana Cabral de Oliveira, University of São Paulo, São Paulo - *Mobil'omics in Rhodophyta: what can we learn from Porphyra genome* **10:15 – 10:40 Coffee Break Outside Ballroom** 10:40 - S084: Koji Mikami, University of Hokkaido, Hakodate - *Development and use of transient gene expression systems in Porphyra yezoensis* 11:15 - S085: Arthur Grossman, The Carnegie Institution of Washington, Stanford - *Using molecular and genomic tools to probe acclimation and developmental processes in algae* 11:50 - S086: John Stiller, East Carolina University, Greenville - *Porphyra genomics: Unraveling mysteries of ancient developmental evolution*	Kalakaua Ballroom B & C
8:30 AM	10:10 AM	**Minisymposium 19: Jasmonates** - Chair: Juergen Engelberth 8:30 - M1901: Juergen Engelberth - *Activity profiling of green leafy volatiles* 8:55 - M1902: Tayana V. Savchenko - *Role of fatty acid-based signaling in coordinating plant stress responses* 9:20 - M1903: Abraham J.K. Koo - *Wound-induced systemic synthesis of bioactive jasmonates in arabidopsis* 9:45 - M1904: John C. Withers - *Structure-function analysis of coronatine-mediated formation of the COI1:JAZ receptor complexes and their contribution to the pathogenicity of pseudomonas syringae*	Lana'I 314
8:30 AM	10:10 AM	**Minisymposium 20: Crop Improvement** - Chair: David Christopher 8:30 - M2001: Mark E. Westgate - *Elemental processes controlling soybean seed composition* 8:55 - M2002: Kelly M. Gillespie - *Elevated carbon dioxide and ozone concentrations alter soybean antioxidant metabolism* 9:20 - M2003: Abul K. Mandal - *Development of a new variety of rice for effective prevention of people and their environment from arsenic contamination* 9:45 - M2004: Kristie O. Matsumoto - *An extended AE-rich N-terminal trunk in secreted pineapple cystatin enhances inhibition of bromelain and is post-translationally removed during fruit ripening*	Kaua'I 311
8:30 AM	10:10 AM	**Minisymposium 21: Non-coding Regulatory RNAs** - Chair: Lila Vodkin 8:30 - M2101: Liang Song - *Characterization of pri-miRNA structures important for efficient miRNA processing in Arabidopsis thaliana* 8:55 - M2102: Lorenz Buelow - *Bioinformatic prediction of target genes for proposed small activating RNAs in Arabidopsis thaliana* 9:20 - M2103: Melissa D. Lehti-Shiu - *Abundant novel small protein and non-coding RNA genes in the Arabidopsis thaliana genome* 9:45 - M2104: Lila Vodkin - *Flux in the coding and small RNA transcriptomes during soybean seed and seedling development*	Moloka'I 315
8:30 AM	10:10 AM	**Minisymposium 22: Intracellular Signalling** - Chair: Cheolmin Yoo 8:30 - M2201: Xing-guo Lan - *A J domain protein that physically interacted with ARC1 is involved in pollination response in Brassica stigma* 8:55 - M2202: Yan Zhang - *Lipid raft-mediated internalization of arabidopsis pollen-specific receptor kinase PRK2a regulates polarized growth of pollen tubes through spatiotemporal activation of small GTPase ROP* 9:20 - M2203: Gregory L. Richter - *Mechanically induced Ca2+transients may play a role in curve-associated lateral root initiation.* 9:45 - M2204: Cheolmin Yoo - *Altered root hair polarity of the arabidopsis thaliana agd1 mutant is associated with defects in various components of the tip growth machinery*	Maui 316A

8:30 AM	10:10 AM	**Minisymposium 23: Vegetative Development** - Chair: Kathy Barton 8:30 - M2301: Kathryn Barton - *Using oppositely acting transcription factors to identify components of the ad/abaxial network of Arabidopsis* 8:55 - M2302: Derek W.R. White - *PEAPOD limits and coordinates vascular procambium activity and stomatal density in Arabidopsis.* 9:20 - M2303: Maureen C. McCann - *Functions of rhamnogalacturonan-I in plant growth* 9:45 - M2304: Eric Engstrom - *Arabidopsis orthologs of the Petunia HAM mutant regulate meristem indeterminacy, organ generation and growth in both the shoot and the root.*	Maui 316C
8:30 AM	10:10 AM	**Minisymposium 24: Cell Walls** - Chair: Allan Showalter 8:30 - M2401: Markus Pauly - *The substitution pattern of plant cell wall cross-linking glycans is determined by apoplastic glycosidases* 8:55 - M2402: Allan M. Showalter - *Identification and characterization of hydroxyproline β-galactosyltransferase activity involved in arabinogalactan-protein biosynthesis in tobacco and Arabidopsis* 9:20 - M2403: Patrick T. Martone - *'Lignified' seaweeds: mechanical consequences of cell wall elaboration in a red alga* 9:45 - M2404: Daniel L. Mullendore - *A new method to investigate the cell wall of living cells by high-resolution scanning electron microscopy*	O'ahu 313A
10:10 AM	10:40 AM	**Coffee Break**	Ala Halawai Concourse
10:40 AM	12:20 PM	**Minisymposium 25: Cell to Cell Communication** - Chair: David Jackson 10:40 - M2501: David P. Jackson - *Regulation of KNOTTED1 cell-to-cell trafficking by a chaperonin protein* 11:05 - M2502: Jae-Yean Kim - *Dof transcription factors: to move or not to move, that is the question* 11:30 - M2503: Linqu Han - *New insights into the CLAVATA signal transduction pathway* 11:55 - M2504: Gad Miller - *Reactive oxygen species mediate a rapid systemic signal in Arabidopsis thaliana.*	Lana'I 314
10:40 AM	12:20 PM	**Minisymposium 26: Transcriptional and Post-transcriptional Regulation** - Chair: Christoph Peterhansel 10:40 - M2601: Wen-hui Shen - *Histone methylation and histone ubiquitylation in regulation of gene transcription, plant growth and development* 11:05 - M2602: Christoph Peterhansel - *Signal integration on chromatin: The histone language of photosynthetic gene expression in maize* 11:30 - M2603: Yoo-Sun Noh - *The RNA-Binding Protein ELF9 Directly Reduces SOC1 Transcript Levels through Nonsense-Mediated mRNA Decay in Arabidopsis* 11:55 - M2604: Q. Quinn Li - *Calcium signaling and the role of a polyadenylation factor in plants response to environment*	Kaua'I 311
10:40 AM	12:20 PM	**Minisymposium 27: Bioenergy Crops** - Chair: Frank Dohleman, ASPB Ambassador 10:40 - M2701: Leyla T. Hernandez-Gomez - *Development of Dunaliella strains for enhanced biofuel feedstock production.* 11:05 - M2702: Rui Zhou - *A functional genomics approach to understanding and remodeling plant cell walls of bioenergy crops* 11:30 - M2703: Jaemo Yang - *Controlled silencing of 4-Coumarate:Coenzyme A Ligase alters lignocellulose composition.* 11:55 - M2704: Frank G. Dohleman - *Sixty percent more productive than maize in the Midwest! How does Miscanthus do it?*	Moloka'I 315
10:40 AM	12:20 PM	**Minisymposium 28: Rhythms** - Chair: Shu-Hsing Wu 10:40 - M2801: Shu-Hsing Wu - *Two new clock proteins, LWD1 and LWD2, regulate Arabidopsis photoperiodic flowering* 11:05 - M2802: Norihito Nakamichi - *Pseudo-response Regulator 9, 7 and 5 are repressors of CCA1 and LHY transcription in arabidopsis circadian clock* 11:30 - M2803: Sergei A. Filichkin - *Diurnal and circadian transcript profiling defines functionally conserved key clock regulated genes among arabidopsis, rice, and poplar* 11:55 - M2804: C. Robertson McClung - *Natural allelic variation in circadian clock function in brassica rapa*	Maui 316A
10:40 AM	12:20 PM	**Minisymposium 29: Protein Trafficking** - Chair: Bonnie Bartel 10:40 - M2901: Tishiaki Mitsui - *Plastid-targeting of rice alpha-amylase glycoprotein from the Golgi apparatus through the secretory pathway* 11:05 - M2902: Naxhiely Martinez - *Interdependence of the PEX5 and PEX7 peroxisome-targeting receptors in Arabidopsis thaliana* 11:30 - M2903: Sundaram Kuppu - *The Arabidopsis ankyrin repeat-containing protein 2A is an essential molecular chaperone for the biogenesis of a class of membrane-bound proteins and it plays an important role in plant growth and development* 11:55 - M2904: Rosa Lopez-Marques - *Lipid pumps required for endocytosis and formation of secretory vesicles*	Maui 316C

Plant Biology 2009 Daily Schedule WEDNESDAY, July 22 (continued)

10:40 AM	12:20 PM	**Minisymposium 30: Programmed Cell Death & Senescence** - Chair: Julie Stone 10:40 - M3001: Julie M. Stone - *Sphingolipids and programmed cell death in Arabidopsis thaliana* 11:05 - M3002: Gerald A. Berkowitz - *Leaf senescence signaling: Ca2+ accumulation mediated by Arabidopsis cyclic nucleotide gated channel2 acts through nitric oxide to repress senescence programming* 11:30 - M3003: Judy A. Brusslan - *A bioinformatics/genetic approach to identify chloroplast proteases that degrade chloroplast proteins during leaf senescence in Arabidopsis* 11:55 - M3004: Susheng Gan - *Plant senescence: a paradigm of translational plant sciences*	O'ahu 313A
12:30 PM	1:30 PM	**ASPB Annual Business Meeting**	Lana'l 314
12:30 PM	1:30 PM	**PSA Annual Business Meeting**	Ni'ihau 312
2:00 PM	5:05 PM	**Symposium IX: PRESIDENT'S SYMPOSIUM - Biological Networks** Organizer: Sarah Assmann, Penn State University 2:00 Sarah Assmann - *Introduction* 2:05 - S091: Reka Albert, Ph.D. and Sarah M. Assmann, Ph.D., Pennsylvania State University - *Boolean modeling of microarray data reveals modes of heterotrimeric G protein action* 2:45 - S092: Nicholas J. Provart, Ph.D., University of Toronto, Canada - *Biological networks for hypothesis generation in plant biology using large-scale data sets* **3:25 – 3:55 Coffee Break Outside Ballroom** 3:55 - S093: Joel S. Bader, Ph.D. Johns Hopkins University, - *Surfing the web of biological interactions* 4:30 - S094: Elena R. Alvarez-Buylla, Ph.D., UNAM-Instituto de Ecologia, Mexico - *Flower evo-devo: a conserved theme and an exception from the Mexican tropics*	Kalakaua Ballroom B & C

Plant Biology 2009 Daily Schedule THURSDAY, July 23

Start Time	End Time	Event/Item	Location
8:00 AM	8:00 PM	**Bridging the Roadmap to C4- a one day workshop dedicated to engineering C4 photosynthesis in C3 plants** *(NOTE: This event is separate from PB 09 and requires separate pre-registration)*	Kalia Conference Center – Hilton Hibiscus Suite
8:00 AM		**PSA Marine Algae Field Trip** *(Requires a pre-purchased ticket)* Field trip participants will be picked up in front of the convention center at 8:00 am & will return to the Univ. of HI at 3:00 pm for processing collections, shuttles will return attendees to the convention center.	
8:00 AM		**PSA Fresh Water/Terrestrial Algae Field Trip** *(Requires a pre-purchased ticket)* Field trip participants will be picked up in front of the convention center at 8:30 am & will return to the Univ. of HI at 3:30 pm for processing collections, shuttles will return attendees to the convention center.	

SPECIAL EVENTS

SATURDAY, JULY 18

Undergraduate Networking Poster Session
11:00 am – 12:00 pm at the Hawaii Convention Center – Maui 316B
Undergraduate Poster session welcomes all individuals. Undergraduate attendees are encouraged to attend and display their posters, mingle with each other, various Society leaders, and other participants to share their research, network and learn how to get the most out of the meeting. Undergraduate students can move their posters to the exhibit hall poster area after the event.

Opening Address & ASPB Awards Ceremony
1:00 – 2:00 pm at the Hawaii Convention Center – Kalakaua Ballroom B&C
All attendees are invited to attend this prominent annual ceremony, which recognizes meritorious research and service in plant biology by the presentation of awards to deserving individuals. Awards to be given this year are the ASPB-Pioneer Hi-Bred International Graduate Student Prize, the Early Career Award, the Charles Albert Shull Award, the Charles Reid Barnes Life Membership Award, the Corresponding Membership Award, the Fellow of ASPB Award, the Martin Gibbs Medal, the Dennis R. Hoagland Award, and the Stephen Hales Prize. The ceremony is immediately followed by the ASPB Opening Symposium, which will feature the 2008 Charles Albert Shull Award Winner, Sheng Luan from the University of California.

Perspectives of Science Leaders
7:00 – 8:20 pm at the Hawaii Convention Center – Kalakaua Ballroom B&C
Speaker: Dr. William H. Danforth II – 2009 ASPB Leadership in Science Public Service Award
Dr. William H. Danforth II has received the 2009 ASPB Leadership in Science Public Service Award. Danforth is Chancellor Emeritus of Washington University in St. Louis, serving as the university's thirteenth chancellor from 1971 until his retirement in 1995. Initially trained as a medical doctor and biochemist, Danforth has nevertheless maintained a lifelong interest in food, agriculture and sustainability. Indeed, following his retirement as Chancellor, Danforth became the driving force behind establishment of the Donald Danforth Plant Science Center which, since its establishment, has vigorously pursued its mission to improve the human condition through plant science research. In 2003, Danforth was appointed by then Secretary of Agriculture, Ann M. Veneman, to chair the Research, Education and Economics Task Force of the USDA, which recommended that the US establish the National Institute of Food and Agriculture within the USDA. The mission of this institute, since authorized by Congress, is to encourage technological innovations in and enhancements to American agriculture. Danforth is a member of the Institute of Medicine, is a director on the Board of Trustees of the Danforth Foundation, and is a trustee of the American Youth Foundation.

Opening Reception/Mixer
8:30 - 10:00 pm at the Hawaii Convention Center – Exhibit Hall II & III
Come and see old friends and colleagues, meet new friends and colleagues and enjoy a relaxing evening while having a snack. Guest tickets available.

SUNDAY, JULY 19

Small Colleges/PUI Networking Breakfast
7:00 – 8:30 am at the Hawaii Convention Center – Maui 316B (pre-purchased ticket required)
This annual event serves as an opportunity to bring people from PUIs together to network, share information on strategies for teaching and research in plant biology, and explore other opportunities.

Exclusive Poster & Exhibit Session and Lunch
12:30 – 3:00 pm at the Hawaii Convention Center – Exhibit Hall II & III
1:00 – 2:00 pm Even Poster Numbers
2:00 – 3:00 pm Odd Poster Numbers
*Boxed lunch provided

PSA Endowment Auction –
7:00 – 9:30 pm at the Hawaii Convention Center – O'ahu 313A
The purpose of the Auction is to raise funds for the Hoshaw Travel Award Fund, which are used to provide travel grants for students to attend the PSA annual meeting. The Auction also provides an opportunity for phycologists to exchange items of phycological interest. Past auction items have included: algal jewelry (pins, earrings, tie tacks, necklaces, belt buckles), books about algae including newly published books, and phycological classics, kelp baskets, seaweed wreaths, photographs of algae, photographs of phycologists, sculptures of algae, dinoflagellate prints, boxer shorts decorated in an algal motif, diatom pillows, note cards decorated with pressed algae, etc. All members and friends of PSA are invited to attend and bid on their favorite items.

NSF Awardee Workshop – Collaborating with Chinese Scientists
7:00 – 9:00 pm at the Hawaii Convention Center – Moloka'I 315
Representatives from the U.S. and China National Science Foundation will provide information about programs at each agency that support various types of collaborations between U.S. and Chinese scientists. Collaborations that foster and enhance research and education will be included. This workshop may be expanded to include collaborative opportunities with other Asian countries.

USDA Reception
7:00 - 8:00 pm at the Hawaii Convention Center – 325B
All employees of the U.S. Department of Agriculture are invited to attend this annual reception. Enjoy the opportunity to share a beverage and mingle with this diverse group.

Career Workshops I & II (pre-purchase ticket required - $12 fee includes dinner!)
7:00 – 10:00 pm concurrently at the Hawaii Convention Center – 318A&B and 319A&B
This year, the ASPB Women in Plant Biology Committee will present two career workshops at Plant Biology 2009. The workshops will not only focus on careers in the U.S. but also on international careers.

Career Workshop 1: "How to Survive A New Job" - 318A&B
This program for early-career individuals will highlight maximizing performance, becoming a manager, the importance of mentoring, networking skills (internal & external), and recognizing discrimination/bias. Following presentations from panelists with experience in these topics, participants will review two to three case studies and have the opportunity to ask questions and engage in discussion with the following panelists: **Time Management to Maximize Output/Performance:** Judy Callis, University of California, Davis; **Becoming a Manager:** Simon Gilroy, University of Wisconsin, Madison; **Mentoring:**Winslow Briggs, Carnegie Institution of Washington; **Networking Skills:** Rob McClung, Dartmouth College; **Discrimination/Bias:**Marta Laskowki, Oberlin College

Career Workshop II: "Alternate Careers" – 319A&B
This program will include presentations from individuals with experience in non-academic careers for plant biologists. Careers will include industry, science diplomacy, non-profit research, publishing/journalism, commercialization/patents, federal government, international companies, and R&D in commercial companies. Following the presentations, participants will have the opportunity to ask questions and engage speakers in discussion within small groups. Panelists include: **Industry:** Frederick Perlak, Monsanto; **Science Diplomacy:**Flo Paoli, USAID in the Israeli Programs Office, Office of Agriculture; **Nonprofit Research Institutes:** Rick Dixon, Samuel Roberts Noble Foundation; **Publishing:** Susanne Brink, Trends in Plant Science; **Government Agencies:** Sharlene Weatherwax, US Department of Energy; **International Jobs:** Huishan Guo, Institute of Microbiology, Chinese Academy of Sciences; **Research and Development in Companies:** Patrick Morgan, LiCor

Education Workshop: Talking Science in Public: Evolution, GMOs, and Other Challenging Issues
7:30 – 9:30 pm at the Hawaii Convention Center – Maui 316B
(Free - pre-reserved ticket is required)
Do you sometimes avoid talking about controversial issues in science? Do you prefer to just "stick to the facts" because it's too hard to gauge and respond to the needs or interests of your conversation partner(s)? Then come to the Education Workshop and learn how to test the waters and then wade confidently into talking about GMOs, evolution, and other challenging issues of science. Discover research and resources for teaching about controversial topics in a variety of school and outreach settings.

Guidelines for Preparing Digital Art — Beginner
7:30 – 9:30 pm at the Hawaii Convention Center – Lana'I 314
Speaker: Michael Hepp of The Sheridan Group
There are a lot of variables when creating digital art. What software are you using? What size should the figure be? In which format should it be saved? What color mode should be used? We can help you create publication-ready figures from the beginning so you won't have to spend time fixing problems later. If you've never created digital figures on your own, or you know only the basics, this is the best place to start. This presentation will include demonstrations in Adobe Photoshop and Illustrator, along with information on how to prepare figures that will reproduce the finest detail and most accurate color both online and in print. Topics will include color space, resolution, fonts, and file type. The presenter will be Michael Hepp, Technology Strategist for The Sheridan Group, the company that produces *Plant Physiology®* and *The Plant Cell*. There will be ample time for Q & A at the end of the session.

MONDAY, JULY 20

ASPB Women in Plant Biology Committee Sponsored Speaker and Luncheon
12:20 – 2:30 pm at the Hawaii Convention Center – Maui 316B (pre-purchased ticket required)
Our speaker this year is Mary Lou Guerinot, Dartmouth College. Attendees will enjoy a lovely lunch, networking with colleages at all levels of experience, and a dynamic speaker.

TAIR Workshop I: How to effectively use the tools and resources at TAIR to enhance your research
12:20 – 2:00 pm at the Hawaii Convention Center – 318 A&B
Speakers: Philippe Lamesch, Donghui Li, A.S. Karthikeyan
TAIR (www.arabidopsis.org) is a community database for *Arabidopsis thaliana*. This workshop is designed for users who wish to more effectively utilize the curated data and software resources provided by TAIR. We will address curation of three major data types: gene structure, gene function and metabolic pathway. We will also teach effective search strategies and highlight some important data sets available at TAIR. Both beginning and experienced users will undoubtedly learn new tricks for getting the information they need and are likely to discover new types of data housed at TAIR that can enhance their research efforts. In the Gene Structure Annotation section, we will give details on the recent TAIR9 genome release. We will explain how we used a variety of experimental data types to update gene structures. We will talk about upcoming projects aimed to further improve existing annotations and add missing genes. An overview of how to search for gene structure related data in TAIR will also be given. In the Gene Function section, we will describe the process of annotating from the literature using GO and PO controlled vocabularies, and then demonstrate how controlled vocabularies allow for standardization of annotation, assist in comparative genomics and can be used to classify large data sets. The Metabolic Pathway section will explain the process of pathway curation and methods of assigning genes to pathways. We will demonstrate how to query and retrieve information on pathways, enzymes, genes and metabolites. We will also discuss the use of tools for displaying and analyzing microarray, proteomic and metabolomic data. Finally we will also explain how the user community can actively participate in the ongoing process of improving database contents at TAIR.

Guidelines for Preparing Digital Art — Advanced
2:30 – 4:10 pm at the Hawaii Convention Center – 319 A&B
Speaker: Michael Hepp of The Sheridan Group
If you are familiar with the basic concepts related to creating digital art, this session will cover more advanced topics such as color management and using ICC profiles, working with MS Office documents and PDFs, and more. It will include demonstrations of advanced techniques for creating publication-ready figures. The presenter will be Michael Hepp, Technology Strategist for The Sheridan Group, the company that produces *Plant Physiology®* and *The Plant Cell*. There will be ample time for Q & A at the end of the session.

ASPB Minority Affairs Committee Sponsored Speaker and Dinner
6:30 – 8:30 pm at the Hawaii Convention Center – Maui 316B (pre-purchased ticket required)
Speaker: Clifton Poodry, Director, Division of Minority Opportunities and Research, National Institute of General Medical Sciences, NIH.
Clif will give a talk entitled "Developing Plant Scientists for 2020". Attendees will enjoy a pleasant dinner, the opportunity to network with colleagues at all levels of experience, and a dynamic and very well informed speaker.

TAIR Workshop II: TAIR, PMN, Gramene and SGN workshop: focus on comparative genomics and new tools
7:00 – 8:30 pm at the Hawaii Convention Center – 318A&B
Speakers: Philippe Lamesch, A.S. Karthikeyan, Lukas Mueller, Pankaj Jaiswal
In this workshop, four plant genome databases will give an overview of new tools available on their websites, including those focusing on comparative genomics. We will present our vision of the future in plant genome databases, and how they impact plant biology research.
TAIR (www.arabidopsis.org) is the central database for the plant model organism *Arabidopsis thaliana* which serves as an important resource and potential benchmark for the annotation of other plant genomes. New datasets and tools have recently been added to TAIR that should help non-Arabidopsis researchers analyze their data. For example, a track has been added to the TAIR genome browser GBrowse that allows the user to easily find orthologs of Arabidopsis genes in over forty different species. Another GBrowse track displays VISTA plots, which visualize genome sequence similarity between Arabidopsis and other plant genomes. Other Arabidopsis-specific tools recently added to TAIR will also be demonstrated.
The **Plant Metabolic Network** (PMN, www.plantcyc.org) is a collaborative project among databases and biochemists with a common goal to build a broad network of plant metabolic pathway databases. We will present an overview of the PMN resources and discuss how researchers can use this information to identify missing enzymes and to study metabolism in their species of interest.
The **Sol Genomics Network** (SGN, http://sgn.cornell.edu) is a clade-oriented, community driven database for the Solanaceae, such as tomato, potato and pepper, and closely related Asterids such as coffee and snapdragon. The SGN workshop aims to inform and demonstrate on how to employ (1) SGN comparative tools, such as the comparative map viewer, the alignment viewer, and tree browser; (2) SGN community annotation tools, which allow researchers from the community to contribute and share data. The workshop will also offer an overview of how to access the constantly emerging tomato genome data, and quantitative trait loci (QTL) analysis functionalities.
The **Gramene** (www.gramene.org) is a comparative plant genomics platform and a systems biology database. It hosts several annotated genomes, genetic maps from over 30 intra- and/or inter-specific crosses, storage of genetic markers and plant nucleotide sequences from GenBank and more. In this workshop we will demonstrate how researchers can use these resources (1) to carryout online data analysis, hypothetical modeling or confirmation of lab based findings using both forward and reverse genetics approaches to find genes, proteins, phenotypes (mutants and QTLs), function(s), expression, gene-gene interaction(s), metabolic pathways and polymorphisms in a genomic region of interest, (2) to make comparisons across genetic maps, genomes, genes and gene families and (3) to find candidate genes associated with phenotype and/or functional characteristics by way of whole genome alignments and synteny views.

Grantsmanship Workshop
7:00 – 8:30 pm at the Hawaii Convention Center – 319 A&B
Representatives from Federal agencies will provide overviews of funding opportunities. This is a session on federal plant research and education programs for attendees to learn about new directions in funding, new priorities within current programs, and how to get good ideas into well-written, compelling proposals. Both research- and education-centered opportunities will be explored. Tips and perspectives on grantsmanship will be shared. The workshop will be set up as an open forum facilitating discussion rather than a primarily formal presentation.

Exclusive Poster & Exhibit Session
8:30 – 11:00 pm at the Hawaii Convention Center – Exhibit Hall II & III
9:00 – 10:00 pm Odd Poster Numbers
10:00 – 11:00 pm Even Poster Numbers

TUESDAY, JULY 21

Exclusive Poster & Exhibit Session
12:00 – 1:30 pm at the Hawaii Convention Center – Exhibit Hall II & III
All Poster Open

PSA Banquet
6:30 – 8:30 pm – Waikiki Aquarium (pre-purchased ticket required)
The Phycological Society of America's 2009 banquet will be held on the evening of July 21 on the oceanside grounds of the beautiful Waikiki Aquarium. The evening will include a buffet-style Pacific-fusion dinner and the annual PSA awards ceremony, set within a Hawaiian theme. The Aquarium exhibits will be available for viewing during the evening. Transportation will be provided to and from the Convention Center, although you may also enjoy the 1.9 mile walk along the shores of Waikiki beach. Buses will pick up in front of the Hawaii Convention Center at 6:00 pm and at 6:15 pm. They will also return people to the convention center at about 9:15 pm and 9:30 pm.

Final Party
7:30 – 10:00 pm at the Hilton Lagoon
Come enjoy a beautiful lagoon side party, where you can enjoy one last night of lovely Hawaiian air, music, food and great conversation. Guest tickets available.

THURSDAY, JULY 23

Bridging the Roadmap to C_4
8:00 am to 8:00 pm– Kalia Conference Center Hilton Hibiscus Suite
(this event is separate from Plant Biology 2009 and requires separate pre-registration)
A one day workshop dedicated to engineering C_4 photosynthesis in C_3 plants.

PSA Marine Algae Field Trip
8:00 am (pre-purchased ticket required)
Field trip participants will be picked up in front of the convention center at 8:00 am and will return to the University of Hawaii at 3:00 pm for processing collections. Shuttles will return attendees to the convention center.

PSA Fresh Water/ Terrestrial Algae Field Trip
8:30 am (pre-purchased ticket required)
Field trip participants will be picked up in front of the convention center at 8:30 am and will return to the University of Hawaii at 3:30 pm for processing collections. Shuttles will return attendees to the convention center.

COMMITTEE MEETINGS

The following events are for ASPB and PSA Committee members only unless otherwise noted.

FRIDAY, July 17

ASPB Executive Committee Meeting	8:00 am – 6:00 pm Hilton – Rainbow Suite and Patio
ASPB *Plant Cell* Editorial Board Meeting	8:30 am – 5:00 pm Garden Room at the Halekulani Hotel
PSA Executive Committee Meeting	8:00 am – 5:00 pm Hilton – Hilton Kahili 1

SATURDAY, July 18

ASPB Education Committee	8:00 am – 10:00 pm Hawaii Convention Center – 325A
ASPB *Plant Physiology®* Editorial Board Meeting	8:00 am – 12:00 pm Hawaii Convention Center – 325B
ASPB Program Committee Meeting	11:00 am – 1:00 pm Hawaii Convention Center – 325A

SUNDAY, JULY 19

ASPB International Affairs Committee Meeting	7:00 am – 8:30 am Hawaii Convention Center – 325A
ASPB Education Foundation Board Meeting	7:00 am – 8:30 am Hawaii Convention Center – 325B
ASPB Minority Affairs Committee Meeting	7:00 pm – 8:30 pm Hawaii Convention Center – 325A

MONDAY, July 20

ASPB Women in Plant Biology Committee Meeting	7:00 am - 8:30 am Hawaii Convention Center – 325A
ASPB Fellows Reception	7:00 pm – 8:30 pm Hawaii Convention Center – 325A

TUESDAY, July 21

ASPB Public Affairs Committee Meeting	7:00 am – 8:30 am Hawaii Convention Center – 325A
ASPB Membership Committee Meeting	7:00 am – 8:30 am Hawaii Convention Center – 325B
ASPB Executive Committee Meeting/Luncheon	12:00 pm – 1:30 pm Hawaii Convention Center – 325A
PSA *Journal of Phycology* Editorial Board Luncheon	12:00 pm – 1:30 pm Hawaii Convention Center – 325B

WEDNESDAY, July 22

ASPB Annual Business Meeting ***ALL ASPB MEMBERS WELCOME***	12:30 pm – 1:30 pm Hawaii Convention Center – Lana'l 314
PSA Annual Business Meeting ***ALL PSA MEMBERS WELCOME***	12:30 pm – 1:30 pm Hawaii Convention Center – Ni'ihau 312

GENERAL INFORMATION

Location of Events and Exhibits
Most Plant Biology 2009 events will be held in the Hawaii Convention Center, although some meetings may take place at other locations. Please refer to the daily schedule for locations of specific events.

Registration
The registration desk will be available for questions and registration during the hours below. Tickets for available events may be purchased at the on-site registration desk, and t-shirts and abstract books/CDs will have a separate pick-up area. The registration desk will be at the Hawaii Convention Center in Exhibit Hall II & III. There are no Aloha shirts available for purchase onsite and no exchanges of sizes.

Registration Hours

Saturday, July 18 – 9:00 am – 9:00 pm
Sunday, July 19 – 7:00 am – 6:00 pm
Monday, July 20 – 8:00 am – 5:00 pm
Tuesday, July 21 – 8:00 am – 5:00 pm
Wednesday, July 22 – 8:00 am – 12:00pm

Housing
Any questions or problems concerning roommate matching will be handled by the housing agency, Hachero-Hill, which will have a representative at the Registration Desk. Individual housing concerns should be addressed with the respective hotel directly. Stop by the Registration Desk if you have any questions.

Speaker Ready Room
Room 321A at the Hawaii Convention Center willl be available for PowerPoint presentation testing. Speakers should test their presentations at least an hour prior to their presentation and be at their sessions at least 10 minutes before they are scheduled to begin.

Exhibits & Posters
Exhibits and posters are open during the exhibit hours as listed on your schedule. Please refrain from entering exhibits when they are closed. The posters will be organized by category. Exclusive poster session times are listed in the schedule. Please attend your posters during the time slots based on your final poster number. Poster abstracts will be available in the printed abstract book, CD or online. Late submissions are online only.

Job Board
The Job Board posting area is located near the Registration Desk at the convention center. Placement notices will be available for viewing during the conference. Placement Center procedures will be the same as in prior years; information sheets will be available to employers and candidates at the Registration Desk. Tables will also be available to conduct interviews on-site if the employer chooses to. Scheduling of interviews is the responsibility of the employer and job seekers. Employers may pick up their position notices and resumes during the last day of the meeting. Those not picked up will be mailed to the employers.

Internet Café
Plant Biology 2009 will feature an Internet Café in the Exhibit Hall. It will be open the same hours as the posters. Please limit your online time to 10 minutes or less if others are waiting.

(Shhhh! Dig it -- Only for student attendees)
Meet friends, network, play games, relax!
The ASPB Membership Committee invites you to visit a private lounge. Find the "DIG" lounge in our exhibit hall.

Message Board
Plant Biology 2009 will provide the traditional message boards near the Registration Desk at the convention center for attendees to send and receive messages. An online message board is also available on the ASPB website this year. Please do not use the Job Placement Service boards for messages.

Badge Scanning
Attendee badges have RFID cards attached to them. The cards are on your badges to allow exhibitors to scan your badge to contact you after the meeting if you'd like them to. The RFID cards contain your name, institution, email, and phone number and no additional information. Scanning requires direct contact to gather the information on them. Attendees are welcome to not allow exhibitors to scan their badges if they so choose. Exhibitors will receive a post-meeting mailing list that does not contain emails and phone numbers. Plant Biology will honor any attendee who wishes to not be placed on the mailing list.

We will also be testing paperless ticketing at some ticketed events and may ask to scan your badge at events you have a paper ticket for. We appreciate you assistance while we test this new technology for possible use in future years, which will allow us to use less paper by not having paper tickets.

Cameras/Video/Audio Recording
Taking photos, video, or audio recording of any kind of the posters and sessions unless will be **PROHIBITED** an author/speaker provides specific permission. This prohibition includes but is not limited to the use of camera phones and any other digital recording devices.

Visiting Hawaii – What to See and Do
Enjoy the warmth of the islands' timeless Polynesian hospitality and you'll discover that you have truly found paradise. If you would like assistance on touring please contact **http://www.activitysaleshawaii.com/aspb2009/**. Mention that you are attending ASPB's meetings in Mérida. They will work with you to create a specific tour or assist you in joining a group. Confirmation on all tour arrangements will be from them not ASPB.

Parking at the Hawaii Convention Center
The cost is $5 per exit. There are parking stalls on the 2nd floor of the facility. The entrance to the garage is located on Kalakaua Avenue. Parking is allowed during event hours only. Overnight parking is not allowed.

Safety Notice
While enjoying the beautiful island of Oahu, please remember to keep your personal belongings secured. Traveling in groups is a safe way to visit the many attractions of Oahu.

Childcare Reimbursement Funds Available
Through the ASPB Women in Plant Biology Committee, ASPB will provide a partial reimbursement for child care or babysitting services for children under 12 years of age whose ASPB-member parents are attending Plant Biology 2009. Funds come from the bequest of Eli Romanoff, a long-time member of ASPP/ASPB and winner of the Adolph E. Gude Award in 1995. This is a continuation of a program that was originally funded with a donation from John Radin.

The reimbursement is available to ASPB members on a sliding scale. Our intention is to reimburse the following amounts per family: Graduate Students - up to $400; Post-docs - up to $300; and Faculty - up to $200. However, please note that there are limited funds available for this program, so reimbursement amounts may be smaller in the event that many requests are received and approved.

The subsidy is for ASPB member parents who would otherwise be unable to attend sessions or networking activities. Parents should make their own arrangements via their hotel concierge or other services in the area. Childcare arrangements need to be made by the parents, not ASPB. Reimbursement forms will be available at the conference registration desk to those that pre-apply by submitting the following Childcare Reimbursement Application form. Please provide receipts to the conference registration desk at the meeting or by fax to 301-251-6740 afterwards. Reimbursements will be processed at ASPB headquarters following the meeting; no reimbursement claims can be honored if they are received more than 30 days after the meeting closes. A paid local service must be used; the reimbursement is not intended to cover costs for bringing individuals with you to provide childcare. ASPB assumes no responsibility with respect to child care services and accepts no liabilities relative to the services.

Policy On Bringing Children To The Meeting
Plant Biology is family friendly and we welcome parents of young children to attend the meeting. To ensure the safety and care of them, we have the following policy for attendees.

1. Children should be supervised by their caregiver at all times in the Plant Biology 2009 meeting facilities.
2. Children under the age of 18 are not allowed in the session rooms unless they are fully registered attendees. All individuals over the age of 18 must be registered to attend the sessions.
3. Children are not allowed in the exhibit hall during setup or breakdown for their safety. There are no exceptions.
4. Do not leave your child unattended in the public areas of the meeting facilities, including restrooms, empty meeting rooms, hallways, lobbies, at registration, or sitting in hallways outside of meeting rooms.
5. ASPB, partnering societies, meeting facilities, and meeting staff are not responsible for your children at any time.

Plant Biology 2009 Smoking Policy
Smoking is not allowed at any Plant Biology 2009 annual meeting functions or areas.

Meeting Survey
A meeting survey will be e-mailed to attendees immediately following the meeting. Your response is considered valuable by the society Program Committees. Thank you for your cooperation.

Additional Information/Questions
The conference Registration Desk will be able to assist you with any additional questions regarding the meeting.

PSA FIELD TRIPS

Marine Algae Field Trip

Synopsis of field trip: Field trip participants will be picked up in front of the convention center at 8:00 am. Marine algal collecting locations will include intertidal benches and shallow subtidal sites. A packed lunch will be provided. We will return to the University of Hawaii by approximately 3:00 pm. Laboratory facilities and microscopes will be available for processing collections. Participants will be shuttled back to the Convention Center.

What to bring: Please bring reef walkers or booties for rocky intertidal sites and, if venturing into the water, please bring your own snorkel gear for subtidal sites. You may also want a towel, hat, sunscreen, and plenty of water. Some collecting supplies will be provided (e.g. whirlpak bags), although it would be appreciated if you could bring your own as much as possible.

Freshwater/ Terrestrial Algae Field Trip

Synopsis of Field Trip: Field trip participants will be picked up in front of the convention center at 8:30 am. We will visit a variety of freshwater and terrestrial algal collecting locations, including a reservoir (suitable for plankton tows), streams, a bog and several high rainfall areas with heavy growth of terrestrial algae. A packed lunch will be provided, and we will stop a a beach park on the windward side of Oahu to eat. We will return to the University of Hawaii by approximately 3:30 pm. Laboratory facilities and microscopes will be available for processing collections. Participants will be shuttled back to the Convention Center.

What to bring: Be prepared for a tropical day! Wear a hat and bring sunscreen, bug spray, and plenty of water. For stream collecting, wear old sneakers or tabis (waders not needed). Some collecting supplies will be provided (e.g. whirlpak bags), although it would be appreciated if you could bring your own as much as possible.

WHAT TO DO AFTER THE MEETING

ASPB has negotiated discounts at the following properties before and after the annual meeting.

Grand Wailea Resort Hotel & Spa
3850 Wailea Alanui Drive
Wailea, Hawaii 96753
Phone Number: 808-875-1234
Group Code: GASP
Check-in: 11-JUL-2009
Check-out: 28-JUL-2009

Overlooking the southern shores of Maui, the Grand Wailea Resort's 40-acre property provides open spaces for the active vacationer, beauty for romantic getaways and fun for the whole family. The resort is situated on one of America's top beaches, featuring luxury accommodations, the action-packed, 2,000-foot long Wailea Canyon Activity Pool, the unparalleled Spa Grande, the exquisite Seaside Chapel for weddings and vow renewals and an array of recreational activities including championship golf and more.

Hilton Kauai Beach Resort
4331 Kauai Beach Drive
Lihue, Hawaii 96766
Phone Number: 808-245-1955
Group Code: ASPB
Check-in: 11-JUL-2009
Check-out: 29-JUL-2009

The Hilton Kauai Beach Resort, a deluxe five-story oceanfront resort situated on 25 lushly landscaped acres of Kauai's longest exploring and strolling beach is located north of the town of Lihue, Hawaii and five minutes from the Lihue Airport. Surrounded by exotic lagoons, cascading waterfalls and lush tropical settings, the Hilton Kauai Beach Resort offers a central, convenient location close to the airport, shopping, championship golf and all of Kauai's great activities and adventures. 350 deluxe hotel guestrooms and spectacular oceanfront suites at Hilton Kauai Beach Resort in Lihue, Hawaii feature amenities and activity options.

Hilton Waikoloa Village®
69-425 Waikoloa Beach Drive
Waikoloa, Hawaii 96738
Phone Number: 808-886-2963
Group Code: SPG
Check-in: 11-JUL-2009
Check-out: 29-JUL-2009

Explore the Spirit of Aloha at Hilton Waikoloa Village®. Located on the Kohala Coast of the Big Island of Hawaii in the midst of Waikoloa Beach Resort, the extraordinary property offers an unforgettable experience shrouded in breathtaking gardens, rich wildlife, and tranquil waterways. Discover a Big Island hotel like no other - Hilton Waikoloa Village. A destination in itself, this impressive property is nestled within 62 oceanfront acres, offering breathtaking tropical gardens and abundant wildlife. Explore our waterfront resort by air-conditioned trams. Or take a leisurely stroll along flagstone walkways flanked by Polynesian and Asian artwork. Located on the Kohala Coast of the Big Island of Hawaii, Hilton Waikoloa Village features 1,240 guest rooms and suites - perfectly suited to any taste. Experience award-winning dining, world-class shopping, and an array of activities ranging from golf, tennis and the only interactive dolphin program on the island. Delight in all this exceptional Kohala Coast hotel has to offer for even the most discriminating traveler.

Hawaii Tours and Activities

Enjoy the warmth of the islands' timeless Polynesian hospitality and you'll discover that you have truly found paradise. Make your reservations now for siteseeing activites: http://www.activitysaleshawaii.com/aspb2009/.

GOING GREEN!

This year ASPB is making every effort to ensure that our 2009 meeting is as environmentally friendly as possible. Plant Biology 2009. While the unique location does present a few challenges in holding an entirely green meeting, we hope the steps we are taking this year will increase awareness of the carbon footprint created by our meetings.

Logistical steps for greening our 2009 meeting

Printing
Materials printed for the meetings were mostly produced in Hawaii and on the western coast to avoid shipping and to provide income for the local economy. Whenever possible, printing was done with natural or recycled materials.

Shipping
We cut our shipping weight by leaving behind our large ASPB booth and eliminating many other materials. We encouraged our exhibitors to do the same. We've made sure to ship only what will be used to minimize waste and the necessity to ship back to headquarters.

Local Food and Supplies
We will serve mostly local food at all food events during the meeting. Materials, logistical items, and hired staff are all sourced out to local vendors.

Recycling
Recycling bins will be provided throughout the meeting space, and we have requested participating hotels to provide recycling and linen and towel reuse programs in the hotel rooms.

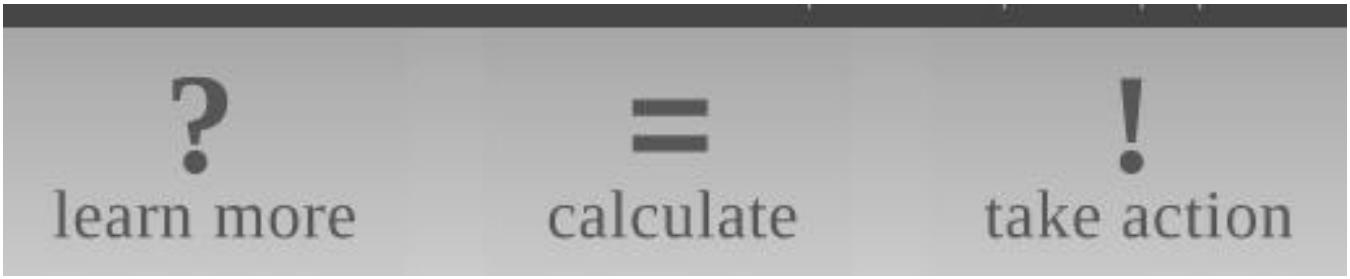
Name Badge
Name badge collection bins will be provided at the end of the meeting so that badges can be reused for future meetings.

Paperless
As in previous years, registration, housing and abstract submission forms were all online. ASPB has eliminated the mailing of meeting flyers and instead is using electronic marketing methods. We are taking steps to minimize waste on site.

Save Energy
We will conserve energy in meeting rooms that are not in use.

What can you do as an attendee?

1. Make a conscious effort to recycle, not only while in the meeting venues but also while touring or enjoying the local area.
2. Take advantage of the linen and towel reuse program in the hotel rooms.
3. Be conscious of your water consumption by turning off the faucet while brushing your teeth and limiting your shower. Use the water bottles provided at the meeting rather than purchasing water bottles.
4. Patronize the local shops and vendors.
5. Reduce your annual air travel, use carbon offset programs and make the ASPB meeting a high priority in the future!



Offset Your Carbon Emissions

Evolution Sage is proud to be the carbon offset provider for Plant Biology 2009! Provided online is a quick way to calculate the global warming pollution you've created by attending this event or by your personal home use.

The money that is received from your carbon offsets purchase will help install solar panels on rooftops spread throughout Hawaii in a partnership with Hawaii Energy Connection. The average Hawaii roof absorbs the equivalent of 15 gallons of gasoline every day in energy from the sun. By offsetting your footprint from this conference, you've helped capture some of that energy and prevented literally tons of global warming pollution from going into Hawaii's environment and our earth's atmosphere.

Energy efficiency and renewable energy offsets are the most expensive but currently most quantifiable means of offsetting.

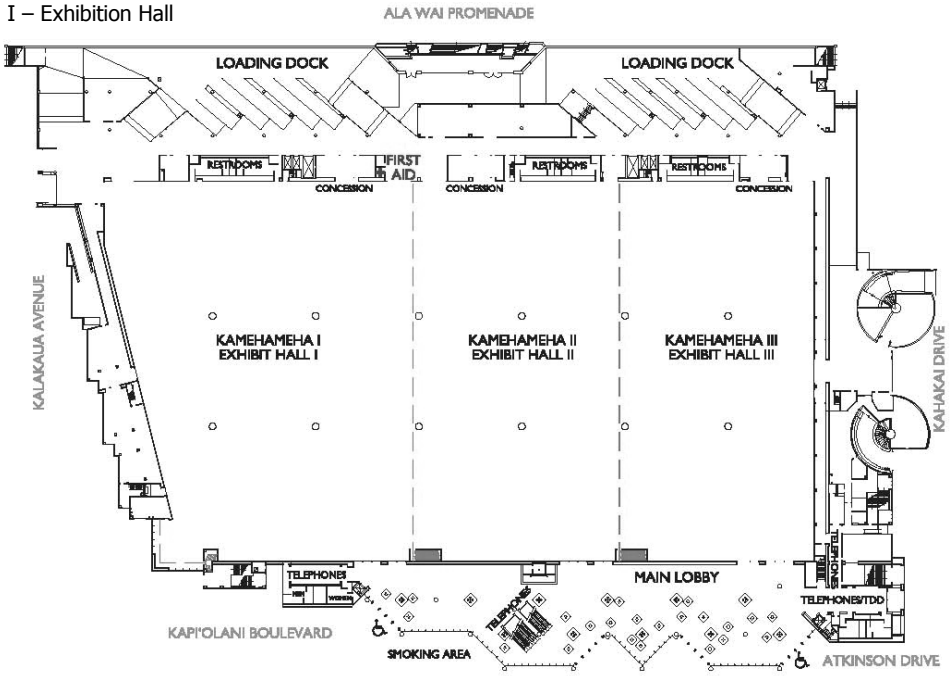
Visit the carbon calculators online at

http://www.evolutionsage.com/index.php?id=111

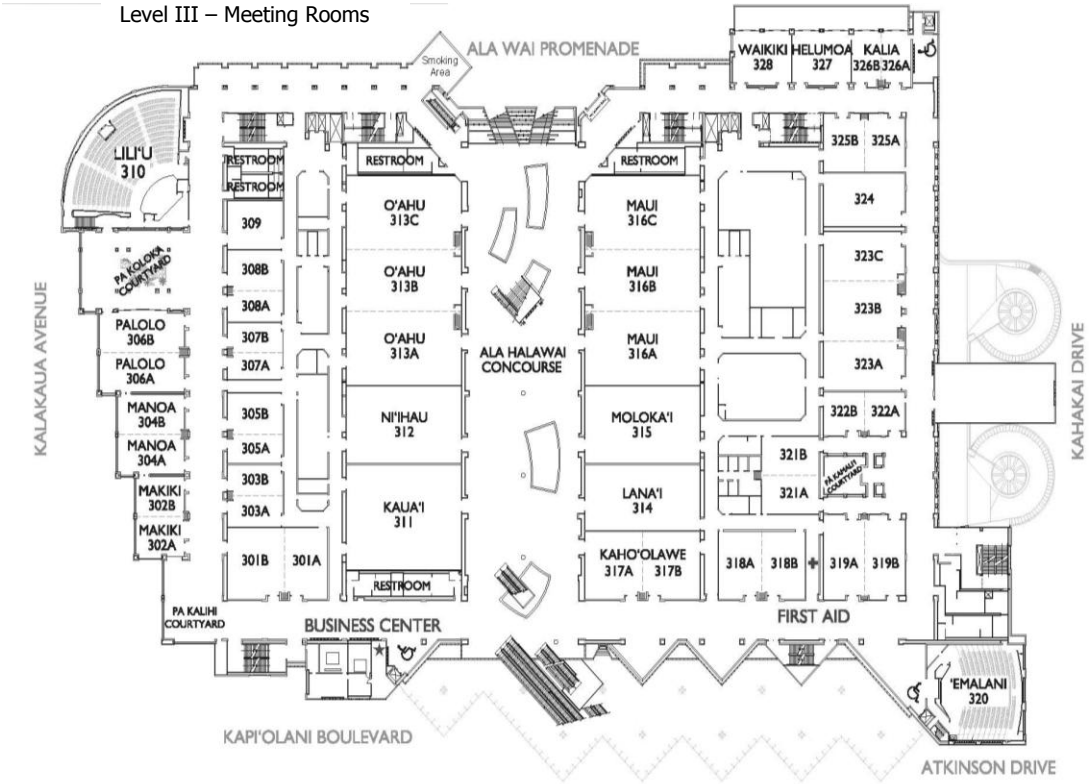
evolution sage
the new green

HAWAII CONVENTION CENTER FLOORPLAN

Level I – Exhibition Hall

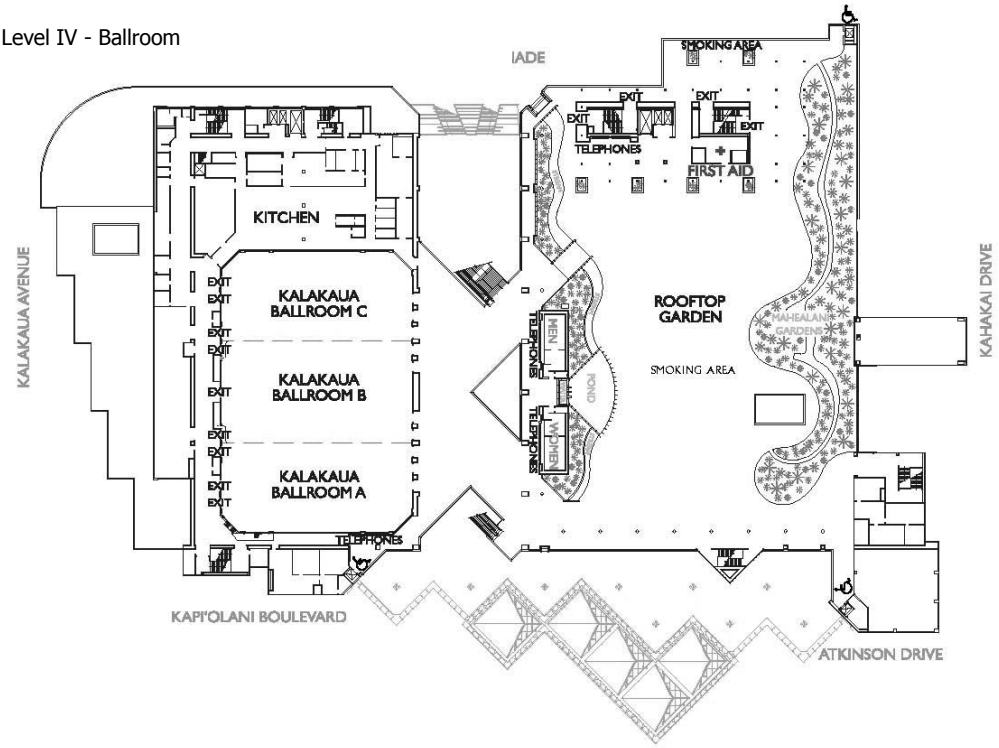


Level III – Meeting Rooms



HAWAII CONVENTION CENTER FLOORPLAN (continued)

Level IV - Ballroom



EXHIBITORS

American Society of Plant Biologists (ASPB) Booth 301
15501 Monona Drive
Rockville, MD 20855
Phone Number: 301-251-0560
www.aspb.org
Stop by the ASPB booth to learn about the many activities of our Society and meet the staff. Get the latest information about *ASPB publications including Plant Physiology®* and *The Plant Cell,* membership, education, Plant Biology 2010, and public affairs.

ASPB Education Committee Booth 507
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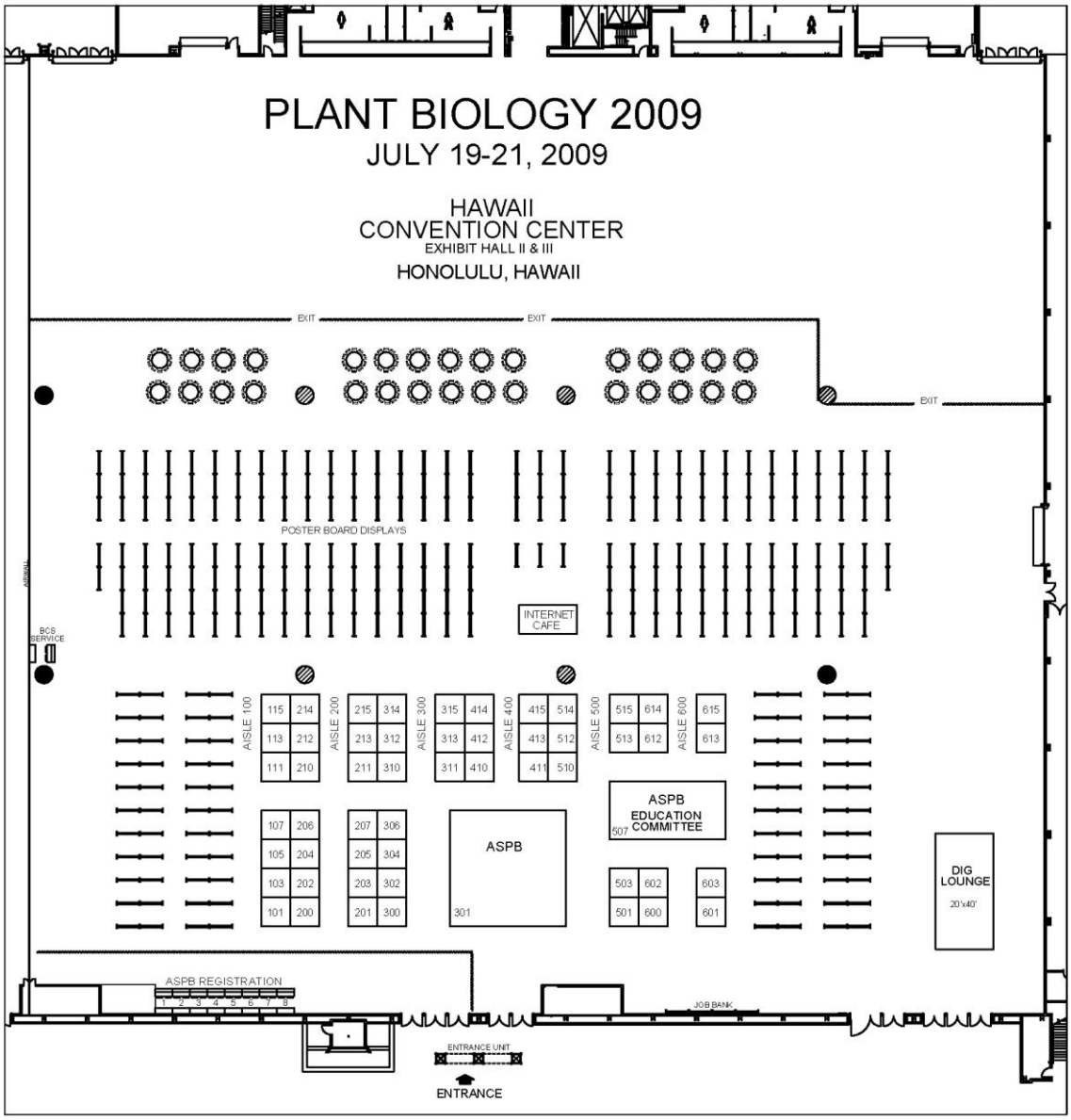

SYMPOSIA/PLENARIES – SATURDAY, JULY 18

Start	End	Event	Location
2:00 PM	2:40 PM	**Symposium I: Charles Albert Shull Award Winner** S011: Sheng Luan, University of California, Berkeley - *The CBL-CIPK calcium signaling network in plants*	Kalakaua Ballroom B&C

S011 The CBL-CIPK calcium signaling network in plants
Luan, Sheng-presenter sluan@nature.berkeley.edu(a)
"Plants sense their environment by signaling mechanisms involving calcium. Calcium signals are encoded by a complex set of parameters and decoded by a large number of proteins including the more recently discovered CBL-CIPK network. The calcium-binding CBL proteins specifically interact with a family of protein kinases CIPKs and regulate the activity and subcellular localization of these kinases, leading to the modification of kinase substrates. This represents a paradigm shift as compared to a calcium signaling mechanism from yeast and animals. One example of CBL-CIPK signaling pathways is the low-potassium response of Arabidopsis roots. When grown in low-K medium, plants develop stronger K-uptake capacity adapting to the low-K condition. Recent studies show that the increased K-uptake is caused by activation of a specific K-channel by the CBL-CIPK network. A working model for this regulatory pathway will be discussed in the context of calcium coding and decoding processes."
(a) UC Berkeley

Start	End	Event	Location
3:10 PM	7:00 PM	**Symposium II: ILLUMINATING PLANT PHOTOMORPHOGENESIS** Organizer: Richard Vierstra, Univ. Wisconsin 3:10 - S021: Winslow Briggs – *Carnegie Institute of Washington - The LOV domains of the phototropins: In loyal service to photoreceptors in bacteria, fungi, algae, and higher plants* 3:50 - S022: Mannie Liscum, University of Missouri - *Musings and cogitations on phototropic signaling* **4:30 - 5:00 - COFFEE BREAK OUTSIDE BALLROOM** 5:00 - S023: Richard D. Vierstra, University of Wisconsin - *Atomic perspectives on phytochrome photochemistry* 5:40 - S024: Peter H. Quail, USDA-Plant Gene Expression Center, UC Berkeley - *Phytochrome signaling networks* 6:20 - S025: Xing-Wang Deng, Yale University - *The roles of two CUL4-based E3 ligases and COP9 signalosome in light control of plant development*	Kalakaua Ballroom B&C

S021 "The LOV domains of the phototropins: In loyal service to photoreceptors in bacteria, fungi, algae, and higher plants"
Briggs, Winslow R.-presenter briggs@stanford.edu(a) Tseng, Tong-seung (a)
"The Arabidopsis blue-light receptors phototropin1 and phototropin 2 (phot1 and phot2) each one contains two highly similar chromophore domains designated LOV1 and LOV2. Both use a flavin molecule (FMN and/or FAD) as chromophore. The LOV domain is composed of five beta sheets plus alpha helices to form a structure tightly enclosing the ring structure of the flavins. Studied in isolation, LOV domains are still photoactive: blue light first causes formation of an excited singlet that decays in nanoseconds to a triplet. The triplet then decays in microseconds to form a covalent adduct between a nearby cysteine and the C(4A) carbon of the flavin. The consequent protein conformational change leads to massive downstream phosphorylation and the initiation of signaling. This cysteinyl adduct than decays over seconds or minutes as the C-S bond breaks and the domain returns to the dark state. The dark decay requires a) breakage of the C-S covalent bond, b) dephosphorylation, and c) protein refolding to return to the dark conformation. We have identified a phosphatase that dephosphorylates phot2 (but not phot1). We have demonstrated its role in down-regulating two physiological responses: phototropism and stomatal opening. We also report phot2 interaction with the lambda isoform from the 14-3-3 protein family. This protein is required for phot2-mediated (but not phot1-mediated) stomatal opening in response to blue light. It is not required for phot2-mediated phototropism. We then survey a wide range of LOV-domain-containing proteins in other organisms including algae, fungi, and bacteria. These LOV-domain proteins are a diverse group with many different functions, and include histidine kinases, cyclase/phosphatases, and different kinds of transcription factors. We demonstrate a role for a LOV-histidine kinase in the pathogenesis of an animal pathogen, *Brucella* sp. "
(a) Department of Plant Biology, Carnegie Institution for Science

S022 Musings and cogitations on phototropic signaling
Liscum, Mannie-presenter liscume@missouri.edu(a,b) Pedmale, Ullas (a,b) Roberts, Diana (a,b) Celaya, Brandon (a,b) Holland, Jennifer (a,b) Morrow, Johanna (a,b)
http://www.biosci.missouri.edu/liscum/liscumlabpage.html
"Plants have evolved adaptive strategies that involve dramatic, rapid, and reversible changes in morphology can result in differential growth, or unequal cellular elongation in one position of an organ relative to an opposing position, in order to respond to changes in their environment. One such response is phototropism, or organ bending in response to directional blue light (BL). Over the last decade or so tremendous progress has been made in the elucidation of the biochemical and molecular mechanism(s) underlying phototropism, most notably the identification and characterization of the primary photoreceptors, the phototropins (phot1 and phot2), and the role of the plant hormone auxin in modulation of responsiveness. In contrast, the NPH3 protein, known to be essential for phototropism for some time, has remained largely enigmatic. However, recent results suggest that NPH3 functions the substrate-adapter/cullin-interacting component of a CULLIN3 (CUL3)-based E3 ubiquitin ligase, designated $BCR3^{NPH3}$. It appears that NPH3 targets phot1 for mono-ubiquitylation, a response stimulated by BL. However, phot1 mono-ubiquitylation does not appear functionally equivalent under different light conditions. For example, under high fleunce rate conditions, mono-ubiquitylation stimulates degradation of phot1, while under low fluence rate conditions it does not. NPH3 function as a substrate adapter for $BCR3^{NPH3}$ may be modulated by reversible protein phosphorylation, which itself requires phot1 for proper regulation. Together these recent finding are suggesting a model in which proper phototropic signaling requires a dynamic feedback interaction and relationship between phot1 and NPH3, and that mono-ubiquitylation may be an

crucial signal in determining signal output."
(a) C.S. Bond Life Sciences Center, University of Missouri (b) Division of Biological Sciences, University of Missouri

S023 Atomic Perspectives on Phytochrome Photochemistry
Vierstra, Richard D-presenter vierstra@wisc.edu(a) Ulijasz, Andrew T (a) Li, Huilin (d) Wagner, Jeremiah R (a) Zhang, Junrui (a) Forest, Katrina T (a) Cornilescu, Gabriel (b) Markley, John L (a,b) von Stetten, David (c) Hildebrandt, Peter (c) Li, Hua (d)
"A complex array of photoreceptors coordinates the response of both prokaryotes and eukaryotes to their ambient light environment. One of the most influential is the phytochrome (Phy) superfamily, a diverse group of photochromic photoreceptors that use a bilin chromophore for light detection. These biliproteins sense red (R) and far-red light (FR) through two relatively stable conformations, a R-absorbing Pr form and a FR-absorbing Pfr form. By photointerconverting between Pr and Pfr, Phys act as light-regulated switches in various signaling cascades. Despite their agricultural importance, we still do not fully understand at the atomic level how Phys photoconvert between Pr and Pfr and how Pfr is then perceived. In the past few years, great strides have been made in determining how Phys function at the molecular level. Key was solving the first 3-D structures of the chromophore-binding domain (CBD) as Pr. These structures conclusively determined the conformation of the bilin linked to the apoprotein, revealed how the bilin is deeply buried in the CBD, showed that the CBD is uniquely folded into a rare figure-of-eight knot, identified a heretofore unknown dimerization contact in the CBD, and provided important clues for how plant Phys arose from their microbial progenitors. Current studies are using x-ray crystallography, NMR spectroscopy, resonance Raman, and Single-Particle EM combined with structure-guided mutagenesis to decipher the events required to generate Pfr from Pr and how Pfr then triggers associated signaling cascades. Especially useful was the discovery of novel thermostable Phys amenable to solving the solution structure of the Pr and Pfr forms. Eventually this work will provide a framework to redesign Phys for agricultural benefit. "
(a) University of Wisconsin (b) NMR Facility at Madison (c) Technische Universitat-Berlin (d) Brookhaven National Laboratory

S024 Phytochrome signaling networks
Quail, Peter H.-presenter quail@nature.berkeley.edu(a,b)
"A central goal of current phytochrome (phy) research is to define the cellular, molecular and biochemical mechanisms involved in the primary steps of the light-triggered, intra-cellular transduction process utilized by this photoreceptor family (phyA to phyE). The present paradigm charting this signaling pathway asserts that transduction involves rapid translocation of the light-activated photoreceptor molecule from the cytoplasm to the nucleus, where it interacts physically with a subset of members of the bHLH transcription factor family, termed phytochrome-interacting factors (PIFs), inducing transcriptional responses in target genes. Recently, we have provided evidence that a quadruple *pif* mutant (*pif1pif3pif4pif5* abbreviated *pifq*) exhibits a constitutively photomorphogenic (*cop*)-like phenotype in completely dark-grown seedlings, indicating that these transcription factors collectively repress photomorphogenesis in post-germinative darkness, and that photoactivated phy reverses this repression by inducing rapid degradation of the PIF molecules upon initial exposure to light. This process involves rapid, phy-induced phosphorylation of the interacting bHLH protein, followed by degradation via the ubiquitin proteasome system. Using random mutagenesis, we have identified a surface-exposed binding site for the PIFs in the light-sensing knot region of the photoreceptor, that is necessary for normal phy signaling in inducing seedling deetiolation, suggesting that this site is integral to the biochemical signal transfer process from the activated photoreceptor to target proteins in the cell. Microscopic examination of the *pifq* mutant indicates accelerated oil body mobilization and chloroplast differentiation in dark-grown seedlings. Genome-wide expression profiling of these seedlings shows robust derepression of a broad array of nuclear genes encoding chloroplast-targeted proteins in sustained darkness, consistent with the visible and cellular *cop*-like phenotypes. Collectively, the data support the notion that the PIF subfamily of bHLH transcription factors function constitutively to promote skotomorphogenic development in seedlings emerging from buried seed, repressing premature photomorphogenic development in subterranean darkness, until this repression is relieved by proteolytic degradation of the PIFs upon photoactivation of the phy system by initial exposure to light at the soil surface."
(a) Plant and Microbial Biology, UC Berkeley (b) Plant Gene Expression Center, ARS-USDA, Albany CA

S025 Light Control of Plant Development: a role of proteolysis
Deng, Xing Wang-presenter xingwang.deng@Yale.edu(a) Chen, Haodong(a) Zhu, Danmeng(a) Huang, Xi(b)
During our dissection of the genetic network involved in light control of Arabidopsis development, ten pleiotropic COP/DET/FUS loci have been identified and revealed to be responsible for mediating light control of *Arabidopsis* seedling developmental program switch. Among them, COP1 is the master repressor of photomorphogenic development and acts within the nucleus as an E3 ligase by directly targeting photomorphogenesis-promoting transcription factors' degradation by the 26S proteasome in darkness. Light inactivates COP1 and causes a reduction in its nuclear abundance. COP10, DET1, and DDB1 form the second complex, the CDD complex, which associates with CUL4 and RBX1 to form an E3 ligase. While most remaining genes encode subunits of a highly conserved protein complex, the COP9 signalosome (CSN), which responsible for the de-conjugation of NEDD8/RUB1 from the cullin-based E3 ligases. It has been suggested that the four partially redundant SPA (suppressor of *phyA*) proteins work in concert with COP1 to repress photomorphogenic development in Arabidopsis. In a collaboration with Hoecker lab, we biochemical characterized the SPA-COP1 complexes. The four endogenous SPA proteins exhibit distinct expression profiles in different tissue types and light treatments. All four SPA proteins can form stable complexes with COP1 *in vivo* regardless of light conditions. The SPA proteins can either self associate or interact with each other, forming a heterogeneous group of SPA-COP1 complexes in which the exact SPA protein compositions vary among the individual complexes. The relative abundance of individual SPA-COP1 complexes depends on the abundance of the individual SPA proteins in a given tissue type under a defined light condition. Loss-of-function mutations in a predominant SPA protein may cause a significant reduction in the overall SPA-COP1 E3 ligase activity, resulting in partial constitutive photomorphogenic phenotype. In a separate study, we demonstrated that those SPA-COP1 complexes could associate with CUL4-RBX1 to form a new group of CUL4-based E3 ligases as well. How this group of CUL4-based E3 ligases and the CUL4-CDD E3 ligase work together in mediating light control of plant development will be a subject of further investigation.
(a) Yale University (b) National Institute of Biological Sciences, Beijing

SYMPOSIA/PLENARIES – SUNDAY, JULY 19

Start	End	Event	Location
8:30 AM	12:20 PM	**Symposium III: ASPB-PSA JOINT SYMPOSIUM: GENOMICS APPROACHES FOR SYSTEMATICS, ENERGY METABOLISM AND ACCLIMATION IN ALGAE** Organizers: Sabeeha Merchant, UCLA Alison Sherwood, Univ. Hawaii 8:30 - S031: Debashish Bhattacharya, University of Iowa - *The evolution of photosynthesis on the tree of life* 9:10 - Abstract S032: Sabeeha Merchant, UCLA - *Functional analysis of trace nutrient homeostasis in chlamydomonas using next generation sequencers* **9:50 - 10:20 - COFFEE BREAK OUTSIDE BALLROOM** 10:20 - S033: Chris Bowler, CNRS, Molecular Plant Biology, Paris - *Genomics-enabled approaches for revealing the molecular secrets of marine diatoms* 11:00 - S034: Simon Prochnik - *Comparative genomic analysis of Chlamydomonas and Volvox sheds light on the evolution of developmental complexity* 11:40 - S035: Mary Rumpho, University of Maine - *Sea slug-algal chloroplast symbiosis: is horizontal gene transfer driving the evolution of photosynthesis in an animal?*	Kalakaua Ballroom B&C

S031 The evolution of photosynthesis on the tree of life
Bhattacharya, Debashish-presenter debashi-bhattacharya@uiowa.edu(a) Reyes-Prieto, Adrian (a) Moustafa, Ahmed (a) Yoon, Hwan Su (b) Andersen, Robert A (b) Chan, Cheong Xin (a) Gross, Jeferson (a)
http://cyanophora.biology.uiowa.edu/home
"The establishment of the photosynthetic organelle (plastid) in eukaryotes and the diversification of algae and plants were landmark events in the evolution of our planet. Recent genomic and phylogenomic approaches have significantly clarified the frequency of plastid endosymbioses, organelle genome evolution, time of plastid origins, and plastid distribution among eukaryotes. In this talk, I will present work from our lab on these topics with specific emphasis on the positions of algae in the eukaryotic tree of life, the evolution of the plastid proteome, and the remarkable impact of endosymbiotic gene transfer (EGT) on algal nuclear genome evolution. Finally, I will discuss a fundamental weakness in our understanding of plastid origin. Namely, everything we know about organelle (i.e., plastids and mitochondria) evolution has been gleaned from glancing backwards at events that occurred more then one billion years ago. The theory of endosymbiosis has therefore been erected in the absence of models in which this fundamental eukaryotic trait is currently at play. To address this issue I will present results of genome analyses aimed at understanding plastid endosymbiosis in action in the thecate, photosynthetic amoeba *Paulinella*. We use this singular model to address key questions ideas about endosymbiont genome evolution during the earlier, active phase of organellogenesis. These include: what is the order and tempo of organelle gene loss, are most genes shipped to the nucleus via EGT, and how dynamic is gene order when organelles are faced with the daunting forces of genome reduction?"
(a) Department of Ecology, Evolution and Natural Resources and Institute of Marine and Coastal Science, Rutgers University (b) Bigelow Laboratory for Ocean Sciences

S032 Functional analysis of trace nutrient homeostasis in chlamydomonas using next generation sequencers
Merchant, Sabeeha-presenter merchant@chem.ucla.edu(a,b) Castruita, Madeli (b) Casero, David (c) Kropat, Janette (b) Karpowicz, Steven (b) Urzica, Eugen (b) Cokus, Shawn (c) Pellegrini, Matteo (a,c)
"Chlamydomonas, a chlorophyte alga in the green plant lineage, is a choice model organism for the study of chloroplast-based photosynthesis and cilia-based motility. The 121 Mb draft genome sequence, determined at 13X coverage is estimated to encode approximately 15,000 protein coding genes. Besides the pathways for oxygen evolving photosynthesis, dark respiration of acetate and hydrogen production, the gene repertoire reveals less-studied pathways for fermentative metabolism, suggestive of extraordinary metabolic flexibility. The operation of these bioenergetic pathways is dependent on metal cofactors like copper, iron, manganese and zinc, and accordingly these elements are essential nutrients for Chlamydomonas. In a copper-deficient environment, Chlamydomonas will modify the photosynthetic apparatus by substituting a heme protein - Cyt c_6 - for an abundant copper protein - plastocyanin - that accounts for about half of the intracellular copper. This modification is viewed as a copper sparing mechanism and is dependent on a plant specific transcription factor CRR1. Four stages of iron-nutrition (excess, replete, deficient and limited) are defined for Chlamydomonas based on phenotypic and gene expression profiles. We have used Illumina-based RNA-Seq methodology to characterize the Chlamydomonas transcriptome under steady conditions of various degrees of iron- and copper-deficiency and in a bloom situation where cells deplete nutrients as they divide. Both methods are quantitative and show excellent correlation with real time PCR indicative of a large dynamic range relative to microarrays. Direct vs. indirect responses to copper-deficiency are distinguished by comparison of the *crr1* transcriptome to that of wild-type cells. The analyses indicate previously unknown modifications of the photosynthetic apparatus in copper-deficient cells and the potential for modification of redox pathways. "
(a) The Institute for Genomics and Proteomics (b) Department of Chemistry and Biochemistry, UCLA (c) Department of Molecular, Cell and Developmental Biology, UCLA

S033 Genomics-enabled approaches for revealing the molecular secrets of marine diatoms
Bowler, Chris-presenter cbowler@biologie.ens.fr(a)
"Diatoms are eukaryotic photosynthetic microorganisms found throughout marine and freshwater ecosystems that are responsible for around 20% of global primary productivity. A defining feature of diatoms is their ornately patterned silicified cell wall, which display species-specific nanoscale structures. These organisms therefore play major roles in global carbon and silicon biogeochemical cycles. The marine pennate diatom Phaeodactylum tricornutum is the second diatom for which a whole genome sequence has been generated. It was chosen primarily because of the superior genetic resources available for this diatom (eg, genetic transformation, RNAi, 130,000 ESTs), and because it has been used in laboratory-based studies of diatom physiology for several decades. The sequence is 27 mega base pairs and, together with the sequence from the centric diatom Thalassiosira pseudonana (34 Mbp; the first diatom whole genome sequence), it provides the basis for comparative and functional genomics studies of diatoms with other eukaryotes and provides a foundation for interpreting the ecological success of these organisms. In spite of the fact that the pennate and centric lineages have only been diverging for 90 million years, their genome structures are dramatically different and a substantial fraction of genes (~40%) are species specific. Analysis of molecular divergence compared with yeasts and metazoans reveals rapid rates

of gene diversification in diatoms. Unlike in other eukaryotes, genome duplication events do not appear to have contributed to diatom evolution. On the contrary, evidence has been found for selective gene family expansions, differential losses and gains of genes and introns, and differential mobilization of transposable elements. Even more significant is the unprecedented presence of hundreds of genes from bacteria. The ancient origins of these gene transfers are testified by the finding that more than 300 are found in both diatoms, and many are likely to provide novel possibilities for metabolite management and for perception of environmental signals. These findings go a long way toward explaining the incredible diversity and success of the diatoms in contemporary oceans. As a case in point I will show how genome-enabled resources can reveal how genes from different origins have been recruited to ensure diatom survival in chronically iron-limited regions of the ocean."
(a) Dept of Biology, Ecole Normale Superieure

S034 Comparative genomic analysis of *Chlamydomonas* and *Volvox* sheds light on the evolution of developmental complexity
Prochnik, Simon-presenter prochnik@gmail.com(a) Umen, James (b) Hallmann, Armin (c) Nedelcu, Aurora (d) Miller, Stephen (e) Nishii, Ichiro (f) Fritz-Laylin, Lillian (g) Schmutz, Jeremy (h,a) Grimwood, Jane (h,a) Rokhsar, Daniel (a,i)
"The single-celled chlorophyte *Chlamydomonas reinhardtii*, a model system for studying photosynthesis and eukaryotic flagella, diverged a little over 200 million years ago from the Volvocales, a lineage comprising species exhibiting simple colonial forms through intermediates of increasing complexity to multicellular species with germ-soma differentiation exemplified by *Volvox carteri*. The genomes of *Volvox* and *Chlamydomonas* each encode around 14,500 genes and their comparison sheds light on the genetic basis of differences in morphology, development and ecology. At the nucleotide level, there is rapid rearrangement at several scales, yet 80% of identifiable protein families contain a single *Volvox* and a single *Chlamydomonas* ortholog. Only a few dozen of approximately 7,000 protein familes show notable size differences. Of these the most striking are larger extracellular matrix protein families (VMP and pherophorin) in Volvox. We found a subtle *Volvox*-specific expansion in the cyclin D gene family. Cyclin D is involved in asymmetric cell division, which is needed to determine the *Volvox* germ line. Further, no new protein domain combinations arose in *Volvox* relative to *Chlamydomonas*, nor are there large numbers of novel genes in either lineage. The genome of *Chlamydomonas* previously revealed surprising complexity in this single-celled organism. Evidence from the genome of *Volvox* suggests the substantial increases in *Volvox*'s developmental complexity may have involved rather minor genetic changes."
(a) DOE Joint Genome Institute (b) Plant Biology Lab., Salk Institute (c) Dept. Cell and Devel. Biol. of Plants, University of Bielefeld (d) Dept. of Biology, University of New Brunswick (e) Dept. Biological Sciences, University of Maryland (f) FRS, RIKEN Institute (g) Dept. of Mol. and Cell Biol., UC Berkeley (h) HudonAlpha Institute for Biotech. (i) Center For Integrative Genomics, Dept. of Mol. and Cell Biol., UC Berkeley

S035 Sea slug-algal chloroplast symbiosis: is horizontal gene transfer driving the evolution of photosynthesis in an animal?
Rumpho, Mary E.-presenter mrumpho@umit.maine.edu(a) Worful, Jared M. (a) Pelletreau, Karen N. (a) Soule, Kara M. (a) Bhattacharya, Debashish (b) Moustafa, Ahmed (b) Devine, Susan (a) Mattsson, Helen (a) Manhart, James R. (c)
http://www.umaine.edu/bmmb/faculty/index.php/profile/mary_rumphokennedy
"The sea slug *Elysia chlorotica* has fascinated scientists for years because of its ability to retain algal plastids and carry out photosynthesis. These emerald green molluscs feed early in their life cycle by sucking out the cellular contents of their algal prey *Vaucheria litorea*. As a result of retaining the plastids in cells lining their digestive gut, they survive for months on only sunlight and air by carrying out photosynthesis as if they were a plant. This is perplexing because sustained plastid activity is highly dependent on the nuclear genome to encode most of the plastid proteins and there is no evidence supporting the presence of algal nuclei in the sea slug. Sequencing of the algal plastid genome has led us to rule out the possibility that the plastids support photosynthesis autonomously. Instead, we hypothesize that the animal provides the essential plastid proteins as a result of horizontal gene transfer (HGT: the exchange of DNA between unrelated organisms) from the algal nucleus to the sea slug. To test this hypothesis, we analyzed the Calvin Cycle enzyme phosphoribulokinase (PRK) gene and a subunit of the photosystem II oxygen generating complex, the manganese stabilizing protein (MSP, psbO gene) to determine if they had originated via HGT. Here we provide evidence for the presence and expression of both genes in adult sea slugs and in sea slug eggs. The source of these nuclear genes in the sea slug is the algal prey because the animal-derived sequences are nearly identical to those isolated from the prey genome. Because the *Elysia* mitochondrial genome does not contain foreign genes, we conclude that the algal genes have been integrated into the animal nuclear genome. We are currently employing high-throughput transcriptome and genome sequencing of *Elysia* to understand HGT on a global scale. This model system is allowing us to better study the role of the nucleo-cytoplasm in plastid function and dependency as well as the potential for evolution of photosynthesis in a normally heterotrophic organism. We are also exploring the possibility that the success of the symbiotic association is dependent on bacterial symbionts, and the evolution of mechanisms that enable the plastid to avoid detection by the host animal and attack by its innate immune system."
(a) University of Maine, Dept. of Biochemistry, Micro. and Mol. Biology (b) University of Iowa, Dept. of Biological Sciences (c) Texas A&M University, Dept. of Biology

Start	End	Event	Location
3:00 PM	6:50 PM	**Symposium IV: PLANT NATURAL PRODUCTS - CHEMICAL EVOLUTION IN TIME AND SPACE** Organizer: Robert Last, Michigan State University 3:00 - S041: Rick Dixon, Noble Foundation - *Changing the spatial accumulation of proanthocyanidins- what else do we need to know?* 3:40 - S042: Jonathan Gershenzon, Max Planck Institute for Chemical Ecology - *Glucosinolate hydrolysis products: Why they dare to be different* **4:20 - 4:50 - COFFEE BREAK OUTSIDE BALLROOM** 4:50 - S043: Rob Last, Michigan State University - *Integrated approaches to understanding tomato glandular trichome metabolism* 5:30 - S044: Joe Noel, Salk Institute - *Peeling Back the Layers of Time: Reconstructing the Evolutionary History of Nature's Biosynthetic Toolbox* 6:10 - S045: Anne Osbourn, John Innes Centre - *Metabolic diversification in plants*	Kalakaua Ballroom B&C

S041 Changing the spatial accumulation of proanthocyanidins- what else do we need to know?
Richard, Dixon A-presenter radixon@noble.org(a) Pang, Yongzhen (a) Zhao, Jian (a)
http://www.noble.org/PlantBio/Dixon/Personnel/index.html
"Proanthocyanidins (PAs, also known as condensed tannins) are flavonoid polymers that possess benefits for human health and are important quality factors for forage crops. In particular, the presence of modest levels of PAs in the leaves and stems of protein-rich forage crops is an important trait that helps prevent pasture bloat. Alfalfa (*Medicago sativa*), the most important forage legume in the world, lacks PAs in the tissues consumed by ruminant animals. The PAs in *Medicago* are composed primarily of epicatechin units and accumulate in the seed coat. The challenge for alfalfa,

therefore, is to introduce these complex compounds into tissues (leaf and stem) in which they do not naturally accumulate. Remarkably, in spite of significant advances in our understanding of both the genetics and chemistry of PAs, we still do not know how the polymerization process occurs, or even the exact nature of the polymerizing units. The availability of extensive genomic and genetic resources in the model legume *Medicago truncatula* provides a platform for gene discovery relevant to the improvement of the closely related target crop species alfalfa. Use of these resources has increased our understanding of transcriptional control, biosynthesis and transport of potential PA precursor units, in addition to providing new germplasms for alfalfa improvement. We summarize these new findings, and present hypotheses to explain how PA polymerization might occur and why moving the pathway under different spatial and temporal controls is still problematical. "
(a) Plant Biology Division, Samuel Roberts Noble Foundation

S042 Glucosinolate hydrolysis products: Why they dare to be different
Gershenzon, Jonathan-presenter gershenzon@ice.mpg.de(a)
"Plant natural products come in many colors and flavors, including the glucosinolates of the mustard family which are responsible for the taste and smell of mustard, cabbage, broccoli and other Brassica vegetables. Like other plant natural products, glucosinolates exhibit a bewildering amount of variation in content and composition among species, populations, organs, developmental stages and environmental conditions. Explaining this variation has long been a major challenge for plant biologists. Here I focus on the diversity of *Arabidopsis thaliana* glucosinolate hydrolysis products that form after plant tissue is damaged. First, the biochemical mechanisms by which such diversity is generated will be described. The formation of glucosinolate hydrolysis products appears to depend on the interaction of the glucohydrolase myrosinase and various associated proteins. Next, I will consider how diversity in hydrolysis products may provide defense against both generalist and specialist herbivores. The variability of glucosinolates and other defensive plant natural products may be explained by selection for defense against multiple herbivores, some of which have developed adaptations to circumvent these defenses. "
(a) Department of Biochemistry, Max Planck Institute for Chemical Ecology

S043 Integrated approaches to understanding tomato glandular trichome metabolism
Last, Robert L-presenter lastr@msu.edu(a) Schilmiller, Anthony L (a) Schauvinhold, Ines (b) Kim, Jeongwoon (a) Shi, Feng (a) Schmidt, Adam (b) Jones, Daniel (a) Pichersky, Eran (b)
http://www.trichome.msu.edu/
"Glandular secreting trichomes are structurally diverse chemical factories found on the surfaces of aerial organs of many plants[1]. Trichomes are implicated in a variety of adaptive processes including defense against herbivores and microorganisms as well as in ion homeostasis. They are excellent experimental systems for the discovery of the enzymes and pathways responsible for the synthesis of specialized metabolites. A multi-laboratory collaborative group is taking a cross-disciplinary approach to study these biosynthetic factories on the leaves and stems of several *Solanum* species, including cultivated tomato. These approaches have already yielded discoveries of new pathways and enzymes and genes As an example, evidence will be presented that monoterpenes in the trichomes are synthesized from neryl diphosphate (NPP) rather than geranyl diphosphate (GPP). Data from screening and mapping of mutants and introgression lines with altered chemistry, EST sequencing and proteomics, and biochemical assays will illustrate how these results and others are being obtained. [1] Schilmiller, Last and Pichersky, 2008. Harnessing plant trichome biochemistry for the production of useful compounds. Plant J. 54:702-711. This work is funded by NSF grants MCB-0604336 and DBI-0619489"
(a) Michigan State University (b) University of Michigan

S044 Peeling Back the Layers of Time: Reconstructing the Evolutionary History of Nature's Biosynthetic Toolbox
Noel, Joseph P.-presenter noel@salk.edu(a,b) O'Maille, Paul B. (a,b)
"I will describe the first quantitative characterization of a catalytic landscape underlying the evolution of sesquiterpene chemical diversity in nature. Based on our laboratory's previous discovery of a set of 9 naturally occurring amino acid substitutions that functionally inter- converted a pair of closely-related plant sesquiterpene synthases, we created a library of all possible residue combinations (2^9 = 512) in one parent. We characterized the product spectra of 418 active enzymes to reveal a rugged landscape where several minimal combinations of the 9 mutations encode convergent solutions to the inter-conversions of parental activities. Further, quantitative comparisons indicate context dependence for mutational effects - epistasis - in product specificity / promiscuity. Collectively, these results provide a measure of the mutational accessibility of phenotypic variability among a diverging lineage of terpene synthases of secondary metabolism. "
(a) Howard Hughes Medical Institute (b) The Salk Institute for Biological Studies

S045 Metabolic diversification in plants
Osbourn, Anne-presenter anne.osbourn@bbsrc.ac.uk(a) Mugford, Sam (a) Wegel, Eva (b) Kemen, Ariane (a) Owatworakit, Amorn (a) Melton, Rachel (a) Bakht, Saleha (a) Shaw, Peter (a)
http://www.jic.ac.uk/staff/anne-osbourn/
"Plants produce a huge array of natural products, many of which are specialised metabolites associated with particular species. These secondary metabolites often have important ecological roles, facilitating pollination and seed dispersal and/or providing protection against attack by pests and pathogens. Although the ability of plants to perform in vivo combinatorial chemistry by mixing, matching and evolving the genes required for different secondary metabolite biosynthetic pathways is likely to have been critical for survival and diversification of the Plant Kingdom we know very little about the mechanisms underpinning this process. This talk will focus on the function and synthesis of plant natural products and on the origins of metabolic diversity and will draw on our research on terpene synthesis in crop and model plants. "
(a) John Innes Centre, Norwich, UK (b) Dept. of Genetics, University of Cambridge, UK

SYMPOSIA/PLENARIES – MONDAY, JULY 20

Start	End	Event	Location
8:30 AM	12:20 PM	**Symposium V: ASPB-CSPP JOINT SYMPOSIUM: CROP FUNCTIONAL GENOMICS** Organizers: Jeffrey Bennetzen, Univ. Georgia Xiao-ya Chen, Shanghai Institute of Biological Sciences 8:30 - S051: Qifa Zhang, Huazhong Agricultural University, China - *Progress in rice functional genomics research in China* 9:10 - S052: Bin Han, National Center for Gene Research, and Beijing Institute of Genomics, Chinese Academy of Sciences - *High-throughput genotyping of rice recombinant inbred lines by whole genome re-sequencing* **9:50 -10:20 - COFFEE BREAK OUTSIDE BALLROOM** 10:20 - S053: Xiao-Ya Chen, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Science, Chinese Academy of Sciences - *Transcriptome and metabolic analysis of cotton* 11:00 - S054: Katherine VandenBosch, University of Minnesota - *Transcriptomics of legumes: Medicago truncatula as a case study* 11:40 - S055: Jeff Bennetzen, University of Georgia - *Comparative Genomic Approaches to Identify Novel Gene Functions, and Their Origins, in Cereals*	Kalakaua Ballroom B&C

S051 Progresses of Rice Functional Genomics Research in China
Zhang, Qifa-presenter qifazh@mail.hzau.edu.cn(a) Xue , Yongbiao (b) Han, Bin (c)
"A very large effort has been made in rice functional genomics research involving many institutions and multiple funding agencies in China with the long-term goal to identify the functions of all the genes in the rice genome together with joint efforts by the international community. Significant progresses have been made in recent years in the following areas: (1) development of technological platforms, (2) functional genomics of agriculturally important traits and, (3) molecular cloning of functional genes. The platforms aimed at enabling high throughput analyses and effective characterization of gene functions, which consist of three major parts: generation and characterization of a large mutant library by T-DNA insertion; global expression profiling of genes in the entire genome; and isolation of full length cDNAs of indica rice. The traits targeted for functional genomic studies in this program include grain quality, yield, stress tolerance, disease and insect resistance, and nutrient use efficiency. Totally 270,000 independent transformants have been generated for the T-DNA insertion mutant library and are now being screened for mutations affecting an array of traits. Over 40000 flanking sequences have been isolated, and their analyses identified a number of interesting features of nonrandom distributions of the T-DNA insertions in the rice genome. A large number of mutants have now been targeted for gene isolation. For genome-wide expression profiling, data have been collected from more than 40 tissues covering the whole life cycle of the rice plants and under various conditions. Map-based cloning has been applied to isolate genes of agronomic importance. Dozens of genes have been cloned using this approach including genes for yield traits, grain quality, fertility restoration, disease resistance and salt tolerance. Attempts have also been made to incorporate these genes into rice breeding programs."
(a) National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China (b) Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China (c) National Center for Gene Research, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200233, China

S052 High-throughput genotyping of rice recombinant inbred lines by whole genome re-sequencing
Han, Bin-presenter bhan@ncgr.ac.cn(a)
Not submitted.
(a) Beijing Institute of Genomics, CAS

S053 Transcriptome and metabolic analysis of cotton
Chen, Xiao-Ya-presenter xychen@sibs.ac.cn(a) Wang, Ling-Jian (a) Mao, Ying-Bo (a)
"Cotton provides the most important natural fiber for textile industry. We constructed a cotton fiber cDNA library and generated ~8,000 ESTs for Gossypium hirsutum L. These ESTs form 3,748 clusters with 2,496 singletons. Then 89 primer pairs were designed on the basis of EST-SSR and 123 polymorphisms were found. These markers were distributed over 20 chromosome and 6 linkage groups in the cotton genetic map. Cotton fibers elongate rapidly after initiation and deposit a large amount of cellulose after elongation. We compared transcriptomes and metabolite profiles of the two stages. Analysis with cDNA array identified 633 genes that were differentially regulated. The auxin signal, wall-loosening and lipid metabolism were highly active during fiber elongation, whereas the cellulose biosynthesis was predominant in the secondary cell wall synthesis stage. Profiling data demonstrated a specialization process of cotton fiber development toward cellulose synthesis. GhRDL1 is one of the genes that were up-regulated in developing fiber cells. RDL1 promoter contains a homeodomain binding L1 box and a MYB binding motif, both conferring trichome-specific expression in Arabidopsis. GaMYB2, a GL1-like gene of cotton, was able to activate GhRDL1 promoter; transgenic data suggest its participation in regulating cotton fiber development. Three cotton homeobox (HOX) genes were identified from ESTs and they may function differently in cotton fiber. Cotton plants accumulate gossypol and related secondary metabolites. We are also interested in gossypol biosynthesis and have isolated genes encoding farnesyl diphosphate synthase (FDS), (+)-δ-cadinene synthase (CAD1), (+)-δ-cadinene-8-hydroxylase (CYP706B1) and a WRKY transcription factor of the gossypol pathway. "
(a) National Key Lab of Plant Mol Genetics Institute of Plant Physiology and Ecology, SIBS Chinese Academy of Sciences

S054 Transcriptomics of legumes: *Medicago truncatula* as a case study
VandenBosch, Kathryn A-presenter vande102@umn.edu(a) Mesfin, Tesfaye (a) Silverstein, Kevin AT (a)
http://www.cbs.umn.edu/plantbio/faculty/VandenBoschKathryn/index.shtml
"The legume family (Fabaceae) contains many species important to human and animal nutrition, and to sustainable agricultural and environmental systems. To date, development of resources for genomics and molecular genetic analysis have focused on soybean (*Glycine max*) and the model legumes *Medicago truncatula* and *Lotus japonicus*. Here, we summarize transcriptomics approaches in legumes, with an emphasis on *M. truncatula* (Medicago), a model used to evaluate mutualistic interactions with microbes, cell wall biogenesis, seed development, and other aspects of legume biology. Transcriptional profiling in Medicago has used sequence- and hybridization-based approaches. Sequencing of cDNAs to produce Expressed

Sequence Tags (ESTs) enabled evaluation of condition-specific gene expression patterns in Medicago before microarrays were in common use. *In silico* analysis of ESTs has been instrumental in identifying small RNAs and novel protein-coding sequences. ESTs have also aided development of molecular markers for the physical map and have benefitted the annotation of the genome sequence of Medicago. Microarray studies of Medicago have used both cDNA and oligonucleotide probes. Worldwide esearch efforts with these arrays have enabled broad assessment of Medicago gene expression, recently resulting in a comprehensive gene atlas. Examples of Medicago transcriptomics that have advanced understanding of legume biology will be presented and contrasted with selected findings from other legumes. The presentation will also highlight the use of microarrays for discovery of expression and sequence polymorphisms among natural accessions, and design and use of a custom Affymetrix chip for expression analysis of the large gene family composed of defensin-like genes. "
(a) University of Minnesota, Department of Plant Biology

S055 "Comparative Genomic Approaches to Identify Novel Gene Functions, and Their Origins, in Cereals"
Bennetzen, Jeff-presenter maize@uga.edu(a)
http://www.genetics.uga.edu/jlblab/Index.html
"The average angiosperm genome (median size ~5500 Mb) contains a complex mixture of genes and transposable elements (TEs). TEs come in many different types, in many different relative abundances from single copy to highly repetitive, and with many different specificities for insertion, expression, chromosome rearrangement and effect on nearby genes. The genes inside TEs are often mistaken for normal organismal genes, but they also can evolve into genes with essential host functions. Both intra- and inter-species comparative analysis of the genomes of several grasses have shown the amazing interactions between TEs and genes, especially in the creation of species-specific genetic novelty. These comparative investigations have also shown how very rapidly, and by what mechanisms, unused genes are removed from plant genomes. Studies on maize, sorghum and rice will be presented that demonstrate the frequency, nature and possible outcomes of the creation of novel genetic functions in these highly dynamic plant genomes."
(a) Department of Genetics, University of Georgia

Start	End	Event	Location
2:30 PM	4:30 PM	**PSA Plenary 1 - Algal Biotechnology** 2:30 - P01001: David Chapman - *Algal biotechnology: an overview of past present and prognosis* 3:30 - P02020: Arthur Grossman - *Opening the floodgates: Comparative genomic analyses from a Chlamydomonas-centric perspective* 4:00 - P01002: Don Cheney - *Seaweed biotechnology: from phycocolloids to environmental bioremediation and renewable sources of biofuels*	Ni'ihau 312

P01001 Algal biotechnology: an overview of past present and prognosis
Chapman, David J-presenter chapman@lifesci.ucsb.edu(a)
"Algal biotechnology has come a long way since the early days of agronomy/mariculture for food use and the recovery of agars, carrageenans and alginates. Some current biotechnology applications of algae, for example, include, but are not limited to their use as a source of biologically useful compounds and removal of heavy metals from solution. Our increasing knowledge of algal physiology and biochemistry, combined with genetic engineering is opening up new vistas. For example there is the genetic engineering of Phaeodactylum by the Carnegie and Martek groups to enable heterotrophic growth. The application of the ability to grow microalgae to very high yields in bioreactors, to a percentage of a theoretical maximum, as opposed to ponds, holds great promise for the future of algal biotechnology. The uses of microalgae have generated a surge of activity in optimising bioreactor technology and design, including some applications of growing algae on foam plats. Example of applications currently in active development by many groups is the use of microalgae as a source of biofuel. There is the potential application of some microalgae that produce extracellular polysaccharides being used as a means of drag reduction for ship hulls. At the macroalgal side possibilities on the horizon involve their use as a source of antifoulants, the possible use of sulfated polysaccharides in biomedical applications and the possibility of genetically engineering macroalgae to provide deterrence to microbial pathogens in mariculture. In the final analysis the expanded future of applying algal biotechnology for good, new and imaginative use must lie in ensuring that good science is tied to good economics. "
(a) University of California

P02020 Opening the floodgates: Comparative genomic analyses from a *Chlamydomonas*-centric perspective
Grossman, Arthur R-presenter arthurg@stanford.edu(a) Merchant, Sabeeha (b) Karpowicz , Steven (b) Prochnik, Simon (c)
http://carnegiedpb.stanford.edu/grossman-lab
"The Volvocales, an order of the green algal class Chlorophyceae, and the Streptophyte algae (the lineage that evolved into land plants) shared a common ancestor about 1 billion years ago. *Chlamydomonas reinhardtii*, a unicellular member of the Volvocales, has traditionally been exploited as an organism associated with sophisticated physiological, genetic and molecular analyses, all of which have been used to elucidate numerous biological processes. Perhaps the most in-depth analyses of *Chlamydomonas* have been oriented toward elucidating the biogenesis and function of chloroplasts and the structure, assembly, and function of eukaryotic flagella (cilia); the latter were inherited from the common ancestor of animals and plants, but were lost during the evolution of land plants. The DOE - Joint Genome Institute has sequenced the ~120 Mb nuclear genome of *Chlamydomonas* and, with members of the community of researchers studying *Chlamydomonas*, has performed comparative genomic analyses. These analyses led to identification of genes encoding uncharacterized proteins that are phlyogenomically associated with the function and biogenesis of chloroplasts and eukaryotic flagella. I will emphasize how genomic technologies are providing insights into *Chlamydomonas* physiology and ecology, and discuss the ways in which comparative genomic analyses are providing new insights into chloroplast functions."
(a) carnegie institution for science, department of plant biology (b) university of california, los angeles, department of chemistry and biochemistry (c) doe-jgi

P01002 Seaweed biotechnology: from phycocolloids to environmental bioremediation and renewable sources of biofuels
Cheney, Donald P.-presenter d.cheney@neu.edu(a)
"This talk will review some key technological developments and successes made in seaweed biotechnology over the past 25 years. The 1980s and 90s were a period of great development in the production of new strains for the phycocolloid and food industries. The techniques of tissue culture and protoplast isolation and fusion were developed in several labs around the world and used to produce new strains of, for example, *Ulva*, *Porphyra*, *Eucheuma* and *Kappaphycus*. In the late 90s, attention switched to the development of genetic transformation techniques and to discovering new uses for seaweeds. In a study of PUFA content in *Porphyra yezoensis*, for example, we showed how the contents of omega-3 fatty acid and

arachidonic acid could be maximized and contribute to the use of *Porphyra* as a feed supplement for salmonid aquaculture. Seaweeds have also been shown to be exceptional natural bioremediation agents for removing nutrients produced in fish aquaculture and toxic man-made compounds like TNT and PCBs. In the latter case, we recently discovered a bloom of *Ulva* growing in a Massachusetts Superfund Site that is accumulating PCBs to levels as high as 100 ppm, and which may be reducing PCB transfer up the food chain. Finally, we are examining the use of seaweeds as a renewable source of biomass for biofuels. Instead of using seaweeds for methane production as has been done in the past, we intend to use them as a source of low-cellulosic, lignin-lacking polysaccharides that can be converted into hydroxymethylfurfural (HMF) using an approach based upon one recently reported for the conversion of fructose and glucose (Roman-Leshkov et al. 2006)."
(a) Northeastern University

Start	End	Event	Location
4:45 PM	6:45 PM	**PSA Plenary 2 - Algal Species Concept in Molecular Ecology** 4:45 P07006: Wayne Litaker - *Defining Algal Species in the 21st Century: Morphology vs. Genetics?* 5:45 P07005: Dale Casamatta - *Towards a method of untangling cyanobacterial systematics in the age of genomics.* 6:15 P07004: Robert Sheath - *What is a red algal species?*	Ni'ihau 312

P07006 Defining Algal Species in the 21st Century: Morphology vs. Genetics?
Litaker, Wayne-presenter wayne.litaker@noaa.gov(a)
"How the rapidly accumulating DNA sequence information should be employed in defining algal species is highly controversial. The International Code of Botanical Nomenclature's rules for defining species are based on morphological criteria and provide little guidance with respect to genetic information. Historically, as more powerful imaging techniques developed, the morphological details used to define algal species evolved, but could be accommodated by the ICBN rules. The recent deluge of DNA sequence data heralding the existence of many, heretofore, unrecognized species represents a data stream of even greater detail. However, the output is genetic rather than morphological, and not readily accommodated by the ICBN rules. Classically trained taxonomists tend to consider the genetic data of little consequence. Many molecular biologists advocate species definitions based primarily on the molecular data. This talk explores reconciliation of these divergent viewpoints using examples from ecologically and toxicologically important dinoflagellates. In brief, I propose that taxonomists and molecular biologists work to identify representative sets of morphologically defined species which can be systematically analyzed genetically. Once identified, specific genes (SSU, ITS, LSU, COB, COI, rbcL, cox2-3 spacer) from each relevant species can be sequenced to determine which genes exhibit consistently greater interspecific vs. intraspecific variation and hence represent robust characters for making species level determinations. This evaluation process must take into account whether or not sexual reproduction is occurring, whether or not the gene divergences are congruent with speciation events, and differing rates of evolution in specific lineages."
(a) National Oceanic and Atmospheric Administration

P07005 Towards a method of untangling cyanobacterial systematics in the age of genomics.
Casamatta, Dale A.-presenter dcasamat@unf.edu(a)
"Cyanobacteria are amongst the oldest, most abundant organisms on the planet, yet their systematic relationships within algal lineages remain poorly understood. Traditionally classified based on gross morphological features, classic taxonomic schemes have been proven erroneous or over-simplified. Consequently, an overwhelming inertia has blocked progress towards resolution of modern systematic assessments and comprehensive descriptions of alpha-level taxonomy. Thus, new methods of elucidating phylogenetic relationships are needed; fortunately, an abundance of new techniques have recently emerged which promote this end. Some of these approaches are reapplications and examinations of previous useful endeavors, such as the use of morphological analyses and attention to certain phenotypically stable characters, such as the patterns and type of cell division, presence of involution cells and thylakoid arrangements. Along with these microscopic techniques, traditional phylogenies are also being revised through molecular data, such as employing the 16S rDNA gene and 16S-23S internal transcribed spacer regions, leading to a resurgence in naming taxa and delineating fine and broad-scale phylogenetic relationships. While these efforts are considered state-of-the-art, much remains unknown. Thus, recent interest has turned toward genomic approaches for further insights. The genomes of more than 30 cyanobacteria have been sequenced so far, providing an excellent framework to reconstruct the evolutionary history of this phylum. This presentation will demonstrate the current state of cyanobacterial systematics and the role of genomics in phylogenetic reconstruction. In turn, this will hopefully provide the framework for meaningful research in the future."
(a) University of North Florida

P07004 What is a red algal species?
Sheath, Robert G-presenter rsheath@csusm.edu(a)
"The Rhodophyta is currently considered to be composed of seven classes and over 6,000 species, with a great range of criteria used to define species. The Cyanidiophyceae contains 3 genera with simple unicells but few distinguishing cellular features, necessitating sequence analyses of chloroplast and nuclear genes, but so far with mixed results. The Rhodellophyceae, with its 4 genera, has more cellular differentiation as well diversity of carbohydrate osmolytes but not all taxa have been subjected sequence analysis. The Porphyridiophyceae has 3 unicellular genera which are well separated in gene trees but species separation is still subject to debate, such as the use of pigmentation. The Stylonematophyceae, composed of unicells and pseudofilaments, contains 6 genera, well-separated morphologically and in phylogenetic trees, but exhibits limited species diversity. The Compsopogonophyceae shows a greater range of morphological diversity from prostrate discs, to saccate or bladed thalli to filaments in 8 genera, most of which are well separated in gene trees but not entirely at the species level. The 5 genera of the Bangiophyceae have either a filamentous or blade-like plant bodies and have long history of morphological and karyological study of species but the currently recognized genera are not monophyletic in chloroplast and nuclear gene trees. The Florideophyceae is the most diverse class with over 5,800 species with filamentous and pseudoparenchymatous thalli, some of which are well characterized by vegetative and reproductive features and well delineated in phylogenetic trees and others poorly studied in some or all of these features. "
(a) California State University San Marcos

SYMPOSIA/PLENARIES – TUESDAY, JULY 21

Start	End	Event	Location
8:00 AM	12:00 PM	**Symposium VI: DARWIN'S LEGACY: EVOLUTION AND PLANT BIOLOGY** Organizer: Barbara Schaal, Washington Univ, St. Louis 8:00 - S061: Michael Purugannan, NYU - *Adaptive Radiation and Regulatory Gene Evolution in the Hawaiian Silversword Alliance (Asteraceae)* 8:40 - S062: Kenneth Olsen, Washington University - *Clover cyanogenesis: evolution and ecology of an adaptive polymorphism* **9:20 - 10:00 - COFFEE BREAK OUTSIDE BALLROOM** 10:00 - S063: Brandon Gaut, UC, Irvine - *A mechanism of selection against transposable elements in Arabidopsis thaliana* 10:40 Abstract S064: Tzen-Yuh Chiang, Taiwan - *Ecological genomics of Miscanthus (Poaceae), a biofuel plant* 11:20 - S065: Leonie Moyle, Indiana University - *Insights into the origin of species from Solanum and other plant groups*	Kalakaua Ballroom B&C

S061 Adaptive Radiation and Regulatory Gene Evolution in the Hawaiian Silversword Alliance (Asteraceae)
Purugganan, Michael-presenter mp132@nyu.edu(a)
"The Hawaiian silversword alliance is a premier example of adaptive radiation, with the species exhibiting extensive and rapid diversification in reproductive and vegetative form. Molecular evolutionary analyses of the *ASAP1* and *ASAP3/TM6* floral regulatory genes indicate a significant acceleration in nonsynonymous relative to synonymous substitution rates in the rapidly evolving Hawaiian lineage. The analyses further indicate that the Hawaiian species are allotetraploids, deriving from an ancient interspecific hybridization event involving species in two lineages of North American tarweeds. Molecular population genetic analyses reveals a pattern of gene flow between recently-derived species in the radiation, and differences in the degree of morphological vs. molecular divergence. This species group provides a fascinating model for the study of plant adaptive radiation, but many of the species in the alliance are endangered; nevertheless, there have been some notable successes in the conservation of endangered species in this group, providing some hope that the risks of extinction within this island group can be mitigated."
(a) New York University

S062 Clover cyanogenesis: evolution and ecology of an adaptive polymorphism
Olsen, Kenneth M-presenter kolsen@wustl.edu(a)
http://biology4.wustl.edu/olsen/
"A major goal of modern evolutionary biology is to understand the genetic basis of adaptation. The natural polymorphism for cyanogenesis (HCN release upon tissue damage) that occurs in white clover offers an attractive ecological and genetic system for examining this question. This chemical defense polymorphism has been studied for over 90 years and is now considered a textbook example of natural variation maintained by opposing selective pressures. In this talk I describe recent work characterizing the molecular genetic basis and molecular evolution of the cyanogenesis polymorphism, and the relationship between this molecular variation and natural selection in wild populations. The clover cyanogenesis polymorphism is controlled by two independently segregating biochemical polymorphisms, where both compounds must be present for the cyanogenic phenotype. The gene *Ac/ac* controls the presence/absence of cyanogenic glucosides, stored in the vacuoles of photosynthetic tissue; *Li/li* controls the presence/absence of their hydrolyzing enzyme, linamarase, which occurs in the apoplast. Tissue damage that results in cell rupture brings the two components together, leading to the liberation of HCN. Ecological studies over the last half century have documented that cyanogenesis serves as a deterrent against herbivores, but that the production of the cyanogenic components is associated with fitness tradeoffs for vegetative growth and reproduction. Field studies have further shown that the frequencies of cyanogenic plants are closely correlated with winter climate, such that latitudinal and altitudinal clines have evolved in both the native and introduced species range. Our genetic studies indicate that the *Ac/ac* and *Li/li* biochemical polymorphisms arise through two unlinked gene presence/absence polymorphisms for the loci *CYP79D15* and *Li*, respectively; *CYP79D15* encodes the protein catalyzing the first step in cyanogenic glucoside biosynthesis, while *Li* encodes the linamarase enzyme. Patterns of molecular evolution at these loci and in flanking genomic regions are discussed, along with data on the occurrence of these polymorphisms in related *Trifolium* species and findings regarding the selective maintenance of the polymorphisms in North American clover populations. "
(a) Washington University in St. Louis

S063 A mechanism of selection against transposable elements in Arabidopsis thaliana
Hollister, Jesse (a) Gaut, Brandon S-presenter bgaut@uci.edu(a)
"Transposable Elements (TEs) comprise the majority of angiosperm DNA, but there is little understanding of the evolutionary forces that counter their proliferation. Here we propose a mechanism for selection against TEs, based on analysis of genomic, epigenetic, and population genetic data from *Arabidopsis thaliana* . We show that gene expression is a function of the proportion of methylated TEs close to genes. We also show that purifying selection acts on methylated TEs near genes but is not detectable on unmethylated TEs or TEs far from genes. We present a model in which host silencing of TEs near genes has deleterious effects on neighboring gene expression, which results in the preferential loss of methylated TEs from gene-rich chromosomal regions. This mechanism implies an evolutionary tradeoff in which the benefit of silencing TEs also imposes a fitness cost. "
(a) University of California, Irvine

S064 "Ecological genomics of *Miscanthus* (Poaceae), a biofuel plant"
Chiang, Tzen-Yuh -presenter tychiang@mail.ncku.edu.tw(a) Ho, Chuan-Wen (a) Wang, Wei-Kuang (a) Huang, Chi-Chun (a) Osada, Naoki (b)
"*Miscanthus*, a C4 plant phylogenetically affined to sugarcane, is one of the most important biofuel plants with characteristics of fast growth and high biomass. *Miscanthus* is native to East Asia and tropical Pacific islands. In contrast to the less diverged *M. floridulus*, *M. sinensis* is composed of morphologically distinct intraspecific taxa. *M. sinensis* var. *condensatus* grows at high-salinity seashore, var. *transmorrisonensis* is an alpine plant, and var. *glaber* can endure heavy metals and sulfur. De-novo transcriptome of a nonmodel species of *M. sinensis* was constructed using high-throughput sequencing techniques. Over 7G bases and 51M bases were obtained from Solexa and 454, respectively. In total, 5,216 contigs were assembled via both approaches. For examining the speciation mode between *M. floridulus* and *M. sinensis*, 40 nuclear loci were randomly selected from the transcriptome library. In the standard model of allopatric speciation, the ancestral population was split into two geographically regional populations with no subsequent gene flow. In the study, multi-locus population genetic analyses revealed frequent gene flow both between species

and among intraspecific taxa of *M. sinensis*. Furthermore, none of the nuclear loci supported the species phylogeny. Statistical analyses showed that such systematic inconsistency cannot be explained solely by the ancestral polymorphisms, while was caused by gene flow after speciation. Altogether, rampant historical and recurrent genetic exchanges suggest likely parapatric speciation in *Miscanthus*. Nevertheless, frequent gene flow usually leads to morphological homogeneity across populations, seemingly contradicting the high phenotypic diversity in *Miscanthus*. In contrast to most nuclear genes that were shaped by negative selection, Ka/Ks >1 detected at the Heat-Shock Protein 70 locus suggests a critical role of the gene in local adaptation and genetic diversification in *Miscanthus*. Genome sequencing of a nonmodel species is extremely challenging. Merging data from 454 and Solexa platforms would improve the accuracy and efficiency significantly. "
(a) Department of Life Sciences, National Cheng Kung University (b) Department of Biomedical Resources, National Institute of Biomedical Innovation

S065 Insights into the origin of species from Solanum and other plant groups
Moyle, Leonie C.-presenter lmoyle@indiana.edu(a)
http://www.bio.indiana.edu/facultyresearch/faculty/Moyle.html
"The last 150 years have confirmed Darwins 1859 argument that an evolutionary worldview best explains the origin and maintenance of biological diversity. Nonetheless, fundamental questions remain about the specific mechanisms involved in evolutionary diversification, including the formation of new species. Focusing on the genetic basis of species differences and isolating barriers, and drawing on data from Solanum and other plant groups, I discuss recent progress in our understanding of speciation. While many challenges remain, the integration of evolutionary, genomic, and molecular functional approaches to understanding species differences, promises to contribute to the growing demystification of Darwins *mystery of mysteries*. "
(a) Dept Biology, Indiana University, Bloomington

Start	End	Event	Location
1:30 PM	4:40 PM	**Symposium VII: THE PLANT CELL 20TH ANNIVERSARY** Organizer: Cathie Martin, John Innes Foundation 1:30 Cathie Martin - Introduction 1:40 - S071: Kazuo Shinozaki - *Transcriptional regulatory network in drought stress response and tolerance Plant Cell 10: 1391–1406* 2:10 - S072: Karin Schumacher - *pH in the endomembrane system: Moving on Plant Cell 18: 715–730* 2:40 - S073: John Ryals - *Systemic Acquired Resistance Plant Cell 3: 1085–1094.* **3:10 - 3:40 - COFFEE BREAK OUTSIDE BALLROOM** 3:40 - S074: Rick Amasino - *Vernalization: remembering winter with an environmentally induced epigenetic switch Plant Cell 11: 949–956* 4:10 - S075: Rich Jorgensen - *Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. Plant Cell 2: 279–289*	Kalakaua Ballroom B&C

S071 Transcriptional regulatory network in drought stress response and tolerance
Shinozaki, kazuo-presenter sinozaki@rtc.riken.jp(a) Yamaguchi-Shinozaki, Kazuko (b,c)
http://www.psc.riken.jp/english/index.html
"Plants respond to survive under drought conditions via a series of physiological, cellular, and molecular processes culminating in stress tolerance. Drought stress induces a variety of genes at transcriptional level. Their gene products function in drought stress tolerance and response. Many drought-inducible genes have been used to improve stress tolerance of plants by gene transfer. In this meeting, we present recent progress on global analysis of expression profiles of drought-responsive gene expression, and functional analyses of transcription factors involved in drought-inducible gene expression and their application to molecular breeding of drought tolerant transgenic plants and crops. We have analyzed expression profiles of the drought-inducible genes and identified several different regulatory systems in stress-responsive gene expression; one group is ABA-dependent and the other ABA-independent. In one of the ABA-independent pathways, a cis-acting element (DRE/CRT) and its binding proteins, DREB1/CBF and DREB2, are important cis- and trans-acting elements in stress-responsive gene expression, respectively. Based on microarray analysis, many DREB1A/CBF-target genes that function in stress tolerance have been identified. Overexpression of these genes improves stress tolerance in transgenics. DREB2 is also involved in heat stress response In the ABA-dependent pathways, bZIP transcription factors (AREB/ABF) function as major regulatory factors after the accumulation of ABA, and MYC/MYB and NAC transcription factors are involved in stress-inducible gene expression. We also identified genes for key enzymes, involved in ABA biosynthesis and metabolism, and signal transduction pathways upstream of the transcription factors in drought stress response. We will discuss complex regulatory networks in drought stress response and tolerance. (Yamaguchi-Shinozaki and Shinozaki: Annual Rev of Plant Biol. 57: 781-803 2006, Umezawa et al. : Current Opinion of Biotech. 17: 113-122 2006) "
(a) Gene Discovery Research Group, RIKEN Plant Science Center (b) Biological Resources Division, Japan International Research Center for Agricultural Sciences (JIRCAS) (c) Laboratory of Plant Molecular Physiology, The University of Tokyo

S072 pH in the endomembrane system: Moving on
Schumacher, Karin-presenter karin.schumacher@hip.uni-heidelberg.de(a)
"The endomembrane system that serves endocytic and secretory trafficking is a complex and dynamic network. Given the amount of exchange and maturation that takes place in this network, it is important to understand how the molecular characteristics of the individual compartments are established and maintained. We have shown that the VHA-a1 isoform of the vacuolar H^+-ATPase (V-ATPase) is localized in a compartment that, based on its ultrastructure, is defined as the trans-Golgi network (TGN), but by function also serves as an early endosome (EE). pH in this compartment is crucial for both endocytic and secretory trafficking leading us to suggest that the TGN/EE represents a central hub for protein sorting in plant cells. We will discuss our model in the light of recent results on trafficking of PM-proteins like the brassinosteroid receptor BRI1 and the cellulose-synthase complex CesA. Moreover, we will report on our attempts to further characterize this highly dynamic compartment. "
(a) Heidelberg Institute for Plant Sciences, University of Heidelberg

S073 Systemic Acquired Resistance
Ryals, John A.-presenter jryals@metabolon.com(a)
http://www.metabolon.com
"Innate immunity in plants was observed and documented as early as the 1890s as an induced resistance to pathogen infection caused by a previous

unsuccessful infection by an unrelated pathogen. Over the next 70 years, there were hundreds of publications describing various forms of this inducible resistance in plants. Frank Ross, in the early 1960s, undertook the first well characterized, physiological study of induced resistance using tobacco infected with tobacco mosaic virus and defined a response he called systemic acquired resistance (SAR). To study SAR at a molecular level, in the 1980s, we carried out a process of systematically isolating and characterizing proteins and genes from tobacco mosaic virus infected tobacco. In the paper by Ward, et al, (1991), we used these genes as probes to define a molecular SAR fingerprint that consisted of the coordinated expression of nine sets of genes. We showed that both salicylic acid and a synthetic inducer 2,6 dicholoroisonicotinic acid (INA) would also induce the same set of genes and pathogen resistance as biologically induced SAR. Finally, we proposed a model that would link the induction of a hypersensitive reaction to the production of salicylic acid (SA) as a signal for SAR in plants. In the following years, we extended those studies to show that SA was indeed involved in signal transduction, but it was not the translocated signal. We also demonstrated that SAR involved a potentiation or priming of the plant response which was analogous to viral priming of cytokine expression in innate immunity in humans suggesting that innate immunity in plants and animals are branches of an ancient pathogen defense mechanism. Over the past 12 years, my laboratories have been involved in developing a powerful new technology we originally called biochemical profiling, now called metabolomics. In a collaboration with Metraux lab at the University of Fribourg, we have used this technology to examine the biochemicals present in phloem exudates from cucumbers induced to resistance by tobacco necrosis virus. In collaboration with scientists at Syngenta, we have examined the biochemical basis of drought tolerance in corn plants under water stressed conditions. The results of these studies will be presented. "
(a) Metabolon, Inc.

S074 Vernalization: remembering winter with an environmentally induced epigenetic switch
Amasino, Richard-presenter amasino@biochem.wisc.edu(a)
"Certain plants, such as biennials or winter annuals, require relatively long periods of cold exposure during winter to initiate flowering the following spring. Cold exposure renders the meristem of such cold-requiring species competent to flower, and this acquisition of competence is known as vernalization. A vernalization requirement ensures that flowering does not occur prematurely before the onset of winter. Cold exposure is also involved in other developmental responses such as the release bud dormancy; in many species, bud dormancy is not broken until a the plant has counted a sufficient number of days of cold to ensure that any subsequent warm weather actually indicates that spring has arrived. Our studies have revealed that, in Arabidopsis, vernalization-mediated meristem competence is a function of the expression level of certain MADS-box genes such as FLOWERING LOCUS C (FLC). FLC is a repressor of flowering. Exposure to prolonged cold causes epigenetic silencing of FLC and some FLC relatives, thus rendering the shoot apical meristem competent to flower. During cold exposure, specific components of chromatin-remodeling complexes are induced, and these chromatin-remodeling complexes catalyze covalent modification of histones of the chromatin of the flowering repressors resulting in silencing of their expression. "
(a) Department of Biochemistry, University of Wisconsin

S075 Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. Plant Cell 2: 279-289
Jorgensen, Richard-presenter raj@ag.arizona.edu(a)
Not submitted.
(a) University Of Arizona

Start	End	Event	Location
2:00 PM	4:00 PM	**PSA Plenary 3 - Coral Reef Ecology** 2:00 P03006: Laurence McCook - *Coral reef resilience, degradation and climate change; the role of algal ecology* 3:00 P03005: Mark Hay - *Killer seaweeds: variance in allelopathic impacts across coral species, seaweed species, and between the Caribbean and tropical Pacific* 3:30 P03007: Guillermo Diaz-Pulido - *Climate change effects on coral reef algae: the missing piece in the future of coral reefs*	Ni'ihau 312

P03006 "Coral reef resilience, degradation and climate change; the role of algal ecology"
McCook, Laurence J-presenter L.McCook@gbrmpa.gov.au(a,b)
"Coral reefs are of enormous environmental, social and economic value, and places of exquisite beauty. However, those values are under serious, urgent threat from human activity, most critically from climate change. Unlike most shallow, hard-bottom marine habitats, typical coral reefs have low biomass of benthic algae. The most common outcome of reef degradation is a 'phase shift' from abundant corals to abundant turfing or fleshy benthic algae. Unravelling the ecological processes involved in those phase shifts has been a major focus for reef ecology over the last 20 years, but has been hampered by insufficient attention to the ecology and diversity of reef algae. Key aspects have included: Demonstration of the critical variability amongst algal groups in ecological processes; Distinguishing between nutrient and herbivore effects on algal growth biomass, and empirical evidence that, when present, herbivores usually control biomass; Recognition that competition between corals and algae is neither simple nor uniform, and provision of a conceptual framework for structuring this variability; Recognition that large-scale coral mortality is often the cause, not the consequence of algal abundance, and that algal colonisation is the near-universal outcome of coral mortality. This observation becomes critical in the context of climate change: increasing severity and frequency of mass bleaching of corals means algal dominance becomes the norm for reefs. The ecology of reef algae is therefore a major priority, including: the impacts of climate change on the algae themselves, and algal effects on coral regeneration and recruitment. Both aspects are critical to the capacity of reefs to maintain coral populations, and hence to the resilience of reefs and their management. "
(a) Great Barrier Reef Marine Park Authority (b) Pew Fellowship in Marine Conservation

P03005 "Killer seaweeds: variance in allelopathic impacts across coral species, seaweed species, and between the Caribbean and tropical Pacific"
Hay, Mark E.-presenter mark.hay@biology.gatech.edu(a) Rasher, Douglas B. (a)
"Coral reefs are in dramatic global decline, with seaweeds replacing corals. However, it is unclear whether seaweeds harm corals directly or colonize opportunistically following their demise from other causes. We show that 60-70% of the common seaweeds we tested in both the Pacific and Caribbean caused bleaching of coral tissues on contact and that their lipid-soluble extracts alone produced these same effects. More extensive experiments in the Pacific using numerous corals and seaweeds show considerable variance among seaweeds and corals in the outcome of seaweed-coral contacts, but abundant seaweeds like *Sargassum* and *Turbinaria* have minimal allelopathic effects on all corals while less abundant seaweeds

like *Chlorodesmis, Galaxaura*, and *Dictyota* have strong negative effects on most corals. Allelopathic seaweed-coral interactions may be common on modern reefs where herbivores no longer control seaweeds. In the field, chemically-rich seaweeds were rapidly consumed on a reef protected from fishing, but undamaged or consumed at low rates on an adjacent fished reef. Continuing harvest of reef herbivores will lead to more seaweed-coral contacts, increased allelopathic suppression of remaining corals, and continued loss of reef corals. . "
(a) Biology, Georgia Institute of Technology

P03007 Climate change effects on coral reef algae: the missing piece in the future of coral reefs
Diaz-Pulido, Guillermo-presenter g.diazpulido@uq.edu.au(a) Anthony, Kenneth R.N. (a) McCook, Laurence J. (b) Hoegh-Guldberg , Ove (a) Dove, Sophie (a)
"Climate change models suggest increasing coral reef degradation, causing increased dominance of reefs by benthic algae. However, little is known about the impacts of climate change on the algae themselves, despite the critical roles they play in coral reef ecology. This paper critically reviews the available literature and presents results of a series of experiments looking at the effects of ocean acidification and warming on reef algae and their interactions with corals. Literature review identifies potential climate vulnerabilities for reef algae, potential threats to reefs from the algae, and key knowledge gaps and strategic directions for research. New experimental studies on the Great Barrier Reef, Australia indicate that both crustose coralline algae and fleshy macroalgae are highly sensitive to climate change. Rates of calcification, photosynthesis, survivorship and recruitment of crustose coralline algae were severely affected with increasing CO_2 levels and temperature. Growth of the fleshy macroalga *Lobophora variegata* was also inhibited under high levels of CO_2 and temperature representative of levels predicted for year 2100. Some algae had very narrow physiological thresholds for temperature; others had large variability in the responses to ocean acidification. Upright macroalgae do not respond uniformly to increasing atmospheric CO_2, probably due to the variety of carbon concentrating mechanisms. Our results suggest that the coral reef macroalgae studied are at least as vulnerable to ocean acidification and warming as hard corals. The ecological consequences of these effects are likely to be complex, with major effects on ecosystem primary production, calcification and interactions with corals, and thus on the resilience of coral reefs."
(a) Centre for Marine Studies, The University of Queensland (b) Great Barrier Reef Marine Park Authority

SYMPOSIA/PLENARIES – WEDNESDAY, JULY 22

Start	End	Event	Location
8:30 AM	12:30 PM	**Symposium VIII: JOINT ASPB-PSA-NSF RCN PORPHYRA - PORPHYRA: A CROP OF THE SEA** Organizer: Susan Brawley, University of Maine 8:30 - S081: Susan Brawley, University of Maine, Orono - *The crop and the organism* 9:00 - Absract S082: Juliet Brodie, Natural History Museum, London - *Porphyra: modern systematics interprets an ancient lineage* 9:40 - S083: Mariana Cabral de Oliveira, University of São Paulo, São Paulo - *Mobil'omics in Rhodophyta: what can we learn from Porphyra genome* **10:15 - 10:40 - COFFEE BREAK OUTSIDE BALLROOM** 10:40 - S084: Koji Mikami, University of Hokkaido, Hakodate - *Development and use of transient gene expression systems in Porphyra yezoensis* 11:15 - S085: Arthur Grossman, The Carnegie Institution of Washington, Stanford - *Using molecular and genomic tools to probe acclimation and developmental processes in algae* 11:50 - S086: John Stiller, East Carolina University, Greenville - *Porphyra genomics: Unraveling mysteries of ancient developmental evolution*	Kalakaua Ballroom B&C

S081 The crop and the organism
Brawley, Susan H-presenter Brawley@maine.edu(a)
"Several species of the marine red alga *Porphyra* are grown commercially in Japan, China, and South Korea. The annual value (U.S. $) of this aquaculture crop is ~ $1.4 billion/year. *Porphyra* spp. are valued as human food because of their Vitamin B_{12}, Vitamin C, mineral, and protein content. Attempts are underway in the U.S. to develop other species of Porphyra for integrated aquaculture with finfish and shellfish. One of these species is *Porphyra umbilicalis* (~270 Mb), which is being sequenced by the Joint Genome Institute (Dept. of Energy). *Porphyra* spp. are part of an ancient eukaryotic lineage; have an alternation of generations between the commercially valuable blade (gametophyte) and a microscopic, filamentous sporophyte; demonstrate high tolerance to stressful temperatures, drying, and high light; and have a large array of light-harvesting and photoprotective pigments. This symposium explores these aspects of *Porphyra* biology in the context of the genomics project."
(a) School of Marine Sciences, University of Maine, Orono, ME 04469, USA

S082 *Porphyra*: modern systematics interprets an ancient lineage
Brodie, Juliet A-presenter J.Brodie@nhm.ac.uk(a)
"*Porphyra* sensu lato, a cosmopolitan genus, is one of the largest genera of red algae. Humans have used species as a source of food probably for thousands of years, and *Porphyra* may have been the first seaweed under cultivation. Fossil evidence suggests that bangiacean red algae occurred at least 1.2 billion years ago and this may be reflected in the remarkable diversity of life histories exhibited by species and an array of life history strategies. The diversity of species, global spread and antiquity of the genus suggest a remarkably successful group of organisms with the ability of both the blade and conchocelis phase of the heteromorphic life history to survive global environmental changes. Species of *Porphyra* are notoriously difficult to identify, and the complete number of species remains unknown. However, the application of molecular techniques, the ability to use these methods on type or historical material, plus a global taxonomic approach, is enabling a much better understanding of biodiversity and distribution, speciation and evidence of endemism within the group. At the same time, generic circumscriptions are under review because the genus is polyphyletic. In this talk, I will review the progress made in understanding the taxonomy and phylogenetics of *Porphyra* and discuss the impact of a molecular taxonomic approach. "
(a) Natural History Museum, Department of Botany

S083 Mobil'omics in Rhodophyta: what can we learn from *Porphyra* genome
Oliveira, Mariana C-presenter mcdolive@usp.br(a)
"The genome sequence of *Porphyra* will uncover the whole set of genes and functions required for the survival of this organism. Genes related to basic functions such as generation of metabolic energy and flow of genetic information form the core 'stable' genome, through which it is possible to trace the evolutionary history of an organism. But, survival depends also on the ability of the organism to adapt to environmental changes; organisms can adapt as they acquire foreign DNA by horizontal gene transfer (HGT). This occurs through integration of mobile genetic elements, such as transposable elements (TEs), self-splicing introns and associated genes, plasmids and viruses. Such elements can carry genes that may bring new features into the cell and be a source of genetic variability. These mobile genetic elements may be associated with chromosome breaks and rearrangements, and TEs are a major source of variation in genome size among the eukaryotes. There is not much information on mobile genetic elements yet in Rhodophyta. *Cyanidioschyzon merolae* has a low number of TEs, but whether this reflects *C. merolae*'s adaptation to extreme environments and is related to a small compact genome, or is a general tendency in the red algae lineage it is not clear. *Porphyra*'s 250+ Mbp genome should be much richer in TEs. The identification of HGT events in the *Porphyra* genome will be achieved by different strategies, such as GC content and codon usage, identification of coding frames for tranposases, recombinases, integrases, reverse transcriptases etc. The type and distribution of TEs will be an important general feature of the genome, and the presence of TE transcripts in the EST, will indicate the extent of transposition activity."
(a) Mariana C Oliveira

S084 Development and use of transient gene expression systems in *Porphyra yezoensis*
Koji, Mikami-presenter komikami@fish.hokudai.ac.jp(a)
"The red alga *Porphyra yezoensis* has been proposed as a model marine plant for physiological and genetic studies in marine algae because of its biological and economical importance. Especially, collection and analyses of expressed sequence tags (ESTs) enable us to identify genes of interests. However, functional analysis of genes in this organism has been inhibited by the lack of genetic transformation systems and the inability to express foreign genes in *Porphyra*. To eliminate this problem, we developed transient gene expression systems in *P. yezoensis* using particle bombardment. Critical factors required to achieve reproducible and efficient expression of the β-glucuronidase gene were optimization of codon usage (increased GC content in the coding sequence) in combination with use of the strong glyceraldehyde-3-phosphate dehydrogenase (*PyGAPDH*) or actin 1 (*PyAct1*)

promoters. Using this system, a regulatory region of the sporophyte-specific expression of the plasma membrane Na^+-ATPase gene has been identified. We further succeeded in the expression of fluorescently-labelled proteins in living *P. yezoensis* cells. Using this system, the Pleckstrin homology domains from human phospholipase Cδ1 and Akt1 were localized in the *P. yezoensis* plasma membrane, where they proposed to specifically bind to different kinds of phosphoinositides (PIs) involved in determination of cell polarity. Additionally, fluorescently-labelled transcription factors were localized in the nucleus. These successful applications of transient gene expression systems could contribute to understand regulatory mechanisms of the haplodiplontic life cycle and PI-based determination of cell polarity during development in *P. yezoensis*."
(a) Faculty of Fisheries Sciences, Hokkaido University

S085 Using molecular and genomic tools to probe acclimation and developmental processes in algae
Grossman, Arthur R.-presenter arthurg@stanford.edu(a) Pootakham, Wirulda (a,b) Gonzalez-Ballester, David (a)
http://carnegiedpb.stanford.edu/grossman-lab
"As the environment undergoes fluctuations, organisms in that environment can modulate both gene expression and the activities of proteins through post-translation modifications that adjust their physiology to better cope with changing extracellular and intracellular conditions. The model, unicellular green alga *Chlamydomonas reinhardtii* exhibits strong phenotypic and genotypic acclimation programs when the cells are transferred to a new environment (e.g. altered nutrient, oxic or light conditions). With the sequencing of the *Chlamydomonas* genome and the development of an array of emerging high throughput sequencing technologies, many acclimation and developmental processes are readily queried at the genome-wide level. Furthermore, because DNA sequencing has become very high throughput and its cost has dropped precipitously, there is renewed interest in generating genome sequences for algae that perform novel biological processes, that undergo intricate developmental programs, that are positioned at key evolutionary branch points, and that have the potential to produce oils and other commercially valuable products. *Porphyra* represents a group of macroalgae that satisfy more than one of these criteria. While my talk will emphasize the use of genomic information and genome-wide approaches to examine how *Chlamydomonas* copes with changing environmental conditions, I will also discuss in more general terms how many of these genomic approaches can be applied to novel aspects of *Porphyra* development and physiology. "
(a) Carnegie Institution for Science, Department of Plant Biology (b) Stanford University, Department of Biology

S086 *Porphyra* genomics: Unraveling mysteries of ancient developmental evolution.
Stiller, John W-presenter stillerj@ecu.edu(a)
http://www.ecu.edu/cs-cas/biology/stiller_john.cfm
"It generally is accepted that major groups of multicellular organisms evolved independently, each from its own unique protistan ancestor. Many genetic components involved in the evolution of developmental complexity appear to be ancient, predating the split between animals and plants; however, this common genetic toolbox was used quite differently during the respective evolution of these two groups. As the putative sister group to the Viridiplantae, red algae offer a compelling system for investigating yet another independent foray into multicellular development, presumably beginning with comparable building blocks to those present in the ancestor of the green plant lineage. One enduring and particularly intriguing question is why rhodophytes never acquired the levels of cell and tissue differentiation found in green plants? Rather, the most complicated morphologies in the most developmentally complex red algae are achieved through elaboration of filamentous structures. *Porphyra* occupies a pivotal place in the evolution of red algal development, representing the lineage that diverged just before the emergence of more diverse and structurally complicated florideophyte red algae. Moreover, well-preserved fossils suggest that *Porphyra*'s basic life history and developmental plan have not changed substantially for the last 1.2 billion years. Thus, *Porphyra* offers a unique window into perhaps the earliest and among the most successful experiments in multicellular development. Combined with existing genomic resources from both simple and complex green plants, a unicellular red alga (*Cyanidioschyzon merolae*), and concurrent development of a florideophyte genome (*Chondrus crispus*), the complete *Porphyra* genome will foster direct comparative analyses of the genetic bases of development, both within and between these two major photosynthetic lineages."
(a) East Carolina University

Start	End	Event	Location
2:00 PM	5:05 PM	**Symposium IX: PRESIDENT'S SYMPOSIUM - Biological Networks** Organizer: Sarah Assmann, Penn State University 2:00 Sarah Assmann - *Introduction* 2:05 - S091: Reka Albert, Ph.D. and Sarah M. Assmann, Ph.D., Pennsylvania State University - *Boolean modeling of microarray data reveals modes of heterotrimeric G protein action* 2:45 - S092: Nicholas J. Provart, Ph.D., University of Toronto, Canada - *Biological networks for hypothesis generation in plant biology using large-scale data sets* **3:25 - 3:55 - COFFEE BREAK OUTSIDE BALLROOM** 3:55 - S093: Joel S. Bader, Ph.D. Johns Hopkins University, - *Surfing the web of biological interactions* 4:30 - S094: Elena R. Alvarez-Buylla, Ph.D., UNAM-Instituto de Ecologia, Mexico - *Flower evo-devo: a conserved theme and an exception from the Mexican tropics*	Kalakaua Ballroom B&C

S091 Boolean modeling of microarray data reveals modes of heterotrimeric G-protein action
Albert, Reka-presenter ralbert@phys.psu.edu(a) Assmann, Sarah (b)
"Heterotrimeric G proteins, composed of Gα and Gβγ dimers, regulate numerous cellular and developmental responses. In metazoan systems, it is well known that some responses depend on Gα, some on Gβγ, and some on interaction of both Gα and Gβγ with downstream effectors. Plants also exhibit G-protein control of numerous hormonal and developmental pathways, but the underlying mechanisms are less well established. In Arabidopsis, T-DNA mutants of the sole Gα subunit gene, *GPA1*, and of the sole Gβ subunit gene, *AGB1*, exhibit abscisic acid (ABA) hyposensitivity of some guard cell responses, and ABA hypersensitivity in some seed and seedling responses, suggesting cell/tissue specificity of G-protein based signaling cascades. To further elucidate the relationships between ABA and G-protein signaling, we used microarray technology to profile the transcriptomes of guard cells and mature leaves of wild-type plants, *gpa1* mutants, *agb1* mutants, and *gpa1 agb1* double mutants, with and without ABA treatment. Microarray data were then assessed in terms of Boolean functions representing different modes of G-protein operation. We found that guard cells and leaves appear to prioritize different modes of G-protein operation. For example, in guard cells in the absence of ABA there was strong evidence both for gene expression dependent on the presence of both Gα and Gβ (Boolean rule: GPA1 AND AGB1) and for expression dependent only on the presence of Gβ (Boolean rule: AGB1), whereas in leaves the mechanism requiring both Gα and Gβ for expression predominated. Many more genes were co-regulated by ABA and G-protein subunits in leaves than in guard cells; only 3 genes in leaves showed the same mode of G-protein regulation in both the absence and presence of ABA. Thus, comprehensive transcriptomic studies of G-protein mutants are

yielding clues as to the nature of cell/tissue specific G-protein signaling cascades."
(a) Physics Department, Penn State University (b) Biology Department, Penn State University

S092 Biological Networks for Hypothesis Generation in Plant Biology Using Large-scale Data Sets
Provart, Nicholas J.-presenter nicholas.provart@utoronto.ca(a) Geisler, Matt (b) Geisler-Lee, Jane (b) Lan, Hui (c) Bonner, Anthony (c) Bassel, George (d) Morris, Quaid (e)
http://www.csb.utoronto.ca/faculty/provart-nicholas
"We have entered the post-genomic era, where technological advances have made the generation of data about the levels and states of all biological molecules - transcripts, proteins, metabolites - in a cell or organism increasingly high-throughput and cost-effective. These data can provide a wealth of information to lab-based researchers, if the data are 'mined' appropriately. The ability to perform so-called 'electronic Northerns', i.e. querying expression data sets with a gene of interest to see how it is responding across all treatments in the database, has proved to be of enormous utility, especially in the context of non-redundancy within gene families. In this vein, the international AtGenExpress Project and individual researchers have generated gene expression data sets from representative experiments in Arabidopsis and has made them available to the community. We have developed tools, available as part of the Bio-Array Resource at http://bar.utoronto.ca, for exploring these and other data, to allow deeper insights into biological questions and to help guide lab-based research. An emerging theme in plant biology is that interactions, be they regulatory or protein-protein, create networks. In the former instance, coexpression networks can provide more robust support for inferred biological involvement than simple coexpression analyses alone. Coexpression networks developed using publicly-available gene expression data sets from dormant and germinating seeds have provided high-quality candidates for genes involved in regulating these two important processes (joint work with George Bassel, Hui Lan and Anthony Bonner). In the latter instance, the complex cellular functions of an organism frequently rely on physical interactions between proteins. A map of all protein-protein interactions, an interactome, is thus an invaluable tool. An interactome for Arabidopsis thaliana predicted from interacting orthologs in 7 organisms will be presented (joint work with Matt Geisler and Jane Geisler-Lee). These predictions can aid researchers by extending known complexes and pathways with candidate proteins. Finally, methods for integrating networks of coexpression, protein-protein interaction, and of other high-throughput data, can provide additional levels of support for novel function identification. An algorithm for doing so, called GeneMANIA, will be presented and discussed (joint work with Quaid Morris). "
(a) Department of Cell & Systems Biology / Centre for the Analysis of Genome Evolution and Function, University of Toronto, CANADA (b) Department of Plant Biology, Southern Illinois University Carbondale, IL., USA (c) Department of Computer Science, University of Toronto, CANADA (d) Department of Horticulture, Oregon State University, OR., USA (e) Centre for Cellular and Biomolecular Research, University of Toronto, CANADA

S093 Surfing the web of biological interactions
Bader, Joel S.-presenter joel.bader@jhu.edu(a)
http://www.baderzone.org
"Networks are a powerful metaphor for biological systems because they provide an interpretable model for the functional organization of genes and proteins. Networks are practical models because modern technologies make it feasible to measure underlying gene and protein interactions systematically and at high throughput. Paralleling the growth of molecular network data has been the explosion of social network data collection, with rapid cross-fertilization of ideas for analyzing networks at these two length scales. We will describe effective strategies for two dominant uses of network data: searching a network for functional neighbors of genes of interest, and clustering a network to automatically detect gene and protein communities. We will discuss how methods have been adapted for different classes of biological interactions: physical interactions between proteins, epistatic interactions between genes, and metabolic correlations. We will also discuss methods that draw information from multiple types of network data. Finally, we will discuss the use of networks as a lens for interpreting genome-wide screens for quantitative trait loci."
(a) Department of Biomedical Engineering, Johns Hopkins University

S094 Flower evo-devo: a conserved theme and an exception from the Mexican tropics
Alvarez-Buylla, Elena-presenter eabuylla@gmail.com(a)
"Evolutionary developmental biology (evo-devo) addresses an important neglect of the 'modern synthesis' of evolutionary biology: development. Evo-devo aims at explaining the origins of organismal form mechanistically. Flowers are complex structures, with an overall conserved body plan in all, except one, angiosperm species. In this talk I summarize our efforts to mechanistically explain such conserved theme in flower patterning and the unique inside-out floral arrangement of *Lacandonia schismatica*, a Mexican species alone among the 250,000 species of flowering plants, with central stamens (male organs) instead of central carpels (female organs). A gene network model for floral organ cell specification grounded on experimental data for *Arabidopsis thaliana* shows that precise signaling pathways are not required to restrain cell types of floral primordial organs; these and their temporal morphogenetic pattern, are rather determined by overall gene network architecture in a robust dynamic fashion, that resists minor modifications in gene interactions and duplications present in other model species with the stereotypical floral plan. In contrast, *L. schismatica* bears central stamens and third whorl carpels. Spatio-temporal patterns of *L. schismatica* orthologues of the A, B and C-type floral identity genes characterized in model systems, suggest that the B function necessary for stamen specification is displaced to the centre of the *L. schismatica* flower. Thus, this is an example of a fixed morphological saltation in extant natural populations, that may have resulted from a small genetic modification. We discuss this exceptional discovery in the context of the uncovered gene regulatory network for floral organ specification."
(a) Departamento de Ecologia Funcional, Instituto de Ecologia, UNAM, Mexico

MINISYMPOSIA/ TALKS – SATURDAY, JULY 18

Start	End	Event	Location
12:30 PM	5:00 PM	**PSA Bold Talks (15-20 min poster talks)** 12:30 - P04002: Craig Aumack - *Impacts of filamentous algal epiphytes/endophytes on macroalgal productivity in the Western Antarctic Peninsula* 12:45 - P02010: Stephanie Brunelle - *Post-transcriptional regulation of the DNA Replication Fork Proteins in the Florida Red Tide dinoflagellate, Karenia brevis* 1:00 - P05018: Amy Lynn Carlile - *Molecular systematics of North Pacific Ceramiaceae species, with a focus on the tribe Ceramieae* 1:15 - Abstract P02009: Jillian Lynch - *Metacaspase activity in aging Karenia brevis cultures: preliminary insight into cellular mechanisms regulating bloom termination.* 1:30 - P05021: Kimberly Peyton - *Exotic threats to biodiversity: A new species of Udotea forms meadows in the deep-water of Hawaii with an invasive alga bordering its edges* 1:45 - P04015: Rosemary Romero - *Recruitment strategies of the early colonizing macroalgae Ulva (Linnaeus) and Porphyra perforata in central California* 2:00 - P04012: Heather Spalding - *Discovering deepwater algal meadows in Hawaii: Home is where the Halimeda is* 2:15 - P03001: Cheryl Squair - *Feast or famine? Evaluating the significance of nutrient enrichment to crustose coralline algae on Ofu, American Samoa.* **2:30-3:00 Coffee Break In Ala Halawai Hallway** 3:00 - P04020: Daniel O'Doherty - *Genetic assessment of the pantropical alga Acanthophora spicifera (Rhodophyta) as revealed by DNA sequencing and microsatellite genotyping, with a focus on Hawaiian populations* 3:15 - P06001: Yen-Chun Liu - *Mechanism for Differential Desiccation Tolerance in Porphyra Species* 3:30 - P05019: Bridgette Clarkston - *A comparison of two DNA barcode markers for species discrimination in marine red algae* 3:45 - P05017: Katy Hind - *Identification of the genus Corallina (Corallinales, Rhodophyta) in Canada* 4:00 - P06005: Erin Cox - *Taking tropical stress in stride? An investigation of the impact of low tides on the brown alga Padina sanctae-crucis distribution and physiology.* 4:15 –P03002: Thomas Sauvage - *Assessing impacts of the non-indigenous red alga Gracilaria salicornia on Waikiki's reef health: Algal distribution changes over a 40 year period.* 4:30 –P04007: Selena McMillan - *Grazing effects of the turban snail, Chlorostoma brunnea, on the productivity of the giant kelp, Macrocystis pyrifera, in central California.* 4:45 –P04004: Megan Wehrenberg - *Alternative life history of Gracilariopsis sp. (Rhodophyta) within the dynamic substrate regime of a central Californian estuary*	Kaua'I 311

P04002 Impacts of filamentous algal epiphytes/endophytes on macroalgal productivity in the Western Antarctic Peninsula
Aumack, Craig F-presenter aumack@uab.edu(a) Amsler, Charles D (a) McClintock, James B (a) Baker, Bill J (b)
"It has been hypothesized that the extensive mesograzer community along the western Antarctic Peninsula controls epiphytic algae as well as emergent filaments from endophytic species. However, should grazing limit exterior fouling by potentially pathogenic microphytes, than the macrophyte population may actually benefit from the massive population of primary consumers. Although the idea may initially seem counter intuitive, the negative impacts of epi/endophyte fouling may outweigh the stresses caused by amphipod grazing. Epiphytic fouling, which can reduce light availability and interfere with gas exchange, may provide enough stress to significantly alter the infested tissue's photochemistry. To test this theory, a series of mesocosm experiments were conducted at Palmer Station, Antarctica. Three individuals from six different species of Antarctic macroalgae were placed in ten different flow-through unfiltered seawater mesocosms. Amphipods were added to five mesocosms while the other five remained herbivore free. At the end of six weeks, a PAM Flourometer and visual assessments were used to assess any differences between the photochemistry and epiphytic growth on individuals kept in a consumer free environment versus those in simulated natural conditions. Initial results indicate that some species of macroalgae, contained in an herbivore free environment, demonstrated decreased photo-efficiencies and noticeably higher instances of epiphyte presence and diatom colonization while other exhibited no change to differing regimes of grazing. These results, along with discussion on the exact nature of photochemical change in response to increased biofouling will also be addressed. "
(a) The University of Alabama at Birmingham (b) The University of South Florida

P02010 "Post-transcriptional regulation of the DNA Replication Fork Proteins in the Florida Red Tide dinoflagellate, *Karenia brevis*"
Brunelle, Stephanie A-presenter brunell@musc.edu(a,b) Van Dolah, Frances M (b,a)
"The dinoflagellate, *Karenia brevis*, is responsible for harmful algal blooms in the Gulf of Mexico that cause extensive marine mortalities and human illness. The molecular mechanisms controlling the cell cycle in this dinoflagellate are important because bloom development occurs through vegetative cell division. Microarray and qPCR studies have demonstrated that, unlike typical eukaryotes, dinoflagellate cell cycle genes are not regulated at the transcriptional level, including replication fork proteins that are typically activated by transcription upon S-phase entry. Post-transcriptional control of these genes is further suggested by the presence of a common trans-spliced leader sequence on their transcripts. The absence of transcriptional control raises the question of what mechanism(s) limit replication fork activity to S-phase. Here we demonstrate by immunolocalization that *K. brevis* PCNA is constitutively present in the nucleus throughout the cell cycle. However, its distribution within the nucleus changes, with prominent staining of chromatin-bound PCNA during S-phase, whereas it is present in a peripheral pool during the rest of the cell cycle. A similar pattern is observed in the trypanosome, a protist that utilizes spliced leader mediated post-transcriptional control of gene expression. Two additional replication fork proteins, K. brevis RPA70 and RFC5, show similar nuclear localization and expression. qPCR analysis of polysome fractionated RNA is currently underway to determine if their translation is cell-cycle dependent. Further, localization-specific protein modification by Sumo or ubiquitin is being investigated. Together, these results will provide insight into how the dinoflagellate regulates S-phase in the absence of

transcriptional control. "
(a) Medical University of South Carolina (b) Center for Coastal Health and Environmental and Biomolecular Research, National Ocean Service, NOAA

P05018 "Molecular systematics of North Pacific Ceramiaceae species, with a focus on the tribe Ceramieae"
Carlile, Amy L-presenter acarlile@u.washington.edu(a) Waaland, J. Robert (a)
"The Ceramiaceae is a species rich family containing approximately 7% of all red algal species. Although this family has recently been the subject of taxonomic revision, some relationships among species remain unclear. This research infers the evolutionary history of North Pacific Ceramiaceae taxa using molecular sequence data from the nuclear encoded gene RPB1, a novel marker for red algal systematics that codes for the large subunit of RNA Polymerase II, and the chloroplast *rbc*L gene, which codes for the large subunit of RuBisCo. Resulting phylogenies are used to test the validity of current taxonomic groupings. These data are also compared to existing data sets to evaluate congruency between results. Emphasis will be on taxa within the tribe Ceramieae, which account for approximately half of the Ceramiaceae as currently defined. Within this tribe, the genus *Ceramium* has been found to be polyphyletic, and therefore warrants some revision. As phylogenies are inferred from molecular data, morphological traits will be mapped onto the molecular based topology to better understand their evolution. From these results, traits that define certain monophyletic groups will be deduced and considered as diagnostic for taxonomy. Determination of diagnostic characters will have positive implications for current taxonomic schemes and facilitate accurate identification."
(a) University of Washington

P02009 Metacaspase activity in aging *Karenia brevis* cultures: preliminary insight into cellular mechanisms regulating bloom termination.
Lynch, Jillian G-presenter lynchjg@musc.edu(a,b) Van Dolah, Frances M (a,b)
"*Karenia brevis*, a toxic dinoflagellate, is responsible for near annual harmful algal blooms in the Gulf of Mexico. These blooms cause extensive ecological and economic losses due to massive fish kills, marine mammal mortalities, and human illness caused by neurotoxic shellfish poisoning and respiratory irritation. The development of successful management strategies for *K. brevis* blooms is contingent upon understanding the molecular mechanisms that regulate bloom initiation, propagation, and termination. Mechanisms regulating *K. brevis* bloom demise, in particular, have remained largely uninvestigated, although recent studies have identified programmed cell death (PCD) pathways in several other bloom-forming phytoplankton species. We have identified in *K. brevis* putative metacaspases, known central mediators of PCD in plants, fungi, and protists. Immunoblot analysis of *K. brevis* over a growth curve revealed immunohybridization of multiple proteins to a polyclonal antibody raised against a recombinant *Emiliania huxleyi* metacaspase protein, which changed in prominence as cultures aged. Caspase-specific activities examined by in vitro caspase cleavage assays exhibited a significant increase during the transition from logarithmic to stationary phase, and remained elevated until late death stages. Identification of metacaspases from our EST libraries, cross reactivity with an *E. huxleyi* metacaspase polyclonal antibody, as well as caspase-specific activities in naturally aging cultures provide preliminary evidence that *K. brevis* contains PCD machinery and may utilize an apoptosis-like pathway during cell death. Further characterization of the function(s) of metacaspases in *K. brevis* may lead to the identification of molecular biomarkers for bloom termination. "
(a) Marine Biomedicine and Environmental Sciences, Medical University of South Carolina (b) Marine Biotoxins Program, NOAA Center for Coastal and Environmental Health and Biomolecular Research

P05021 Exotic threats to biodiversity: A new species of *Udotea* forms meadows in the deep-water of Hawaii with an invasive alga bordering its edges
Peyton, Kimberly A-presenter peyton@hawaii.edu(a) Ballantine, David L (b) Smith, Celia M (a)
"Although rarely explored, deep-water marine assemblages in the tropical photic zone can yield remarkable biodiversity including new species to science. Receiving even less attention in deep-water ecosystems is one of the primary global-scale threats to biodiversity - the human-mediated introduction of species. *Avrainvillea amadelpha*, a Bryopsidalean alga first discovered on the west and south shores of O`ahu, Hawai`i in the 1980s, is one of at least 20 non-indigenous macroalgae in the coastal areas of the Hawaiian Islands. Consistent with predictions that the siphonous green algae are exceptional in their ability to invade a broad range of habitats and substrates, *A. amadelpha* has established persistent populations on O`ahu in shallow water on tidal benches, coral reefs and seagrass meadows. An additional source of concern with Bryopsidalean invaders is their broad light requirements, and as a result, many of these algae can occur beyond the limits of conventional scuba diving. We completed baseline surveys in the deep-waters of O`ahu using manned submersibles, a remotely controlled vehicle and technical diving near the earliest collection site of *A. amadelpha*. Our objectives were to report on the presence/absence of *A. amadelpha* in deep-water and the native assemblages that may be at risk to invasion. During the course of this work, we discovered a previously unknown deep-water *Udotea* sp. forming vast meadows growing in sand and extending to at least 90 m. *Avrainvillea amadelpha* was found to 70 m where it co-occurred with the *Udotea* sp. at the shallower extent of the latter species' depth range. We propose a new species of *Udotea*, based on morphometric and molecular analyses."
(a) University of Hawaii at Manoa (b) University of Puerto Rico

P04015 Recruitment strategies of the early colonizing macroalgae *Ulva* (Linnaeus) and *Porphyra perforata* in central California
Romero, Rosemary-presenter rromero@mlml.calstate.edu(a)
"Ephemeral algae are early colonizers of the rocky intertidal after a disturbance, although the mechanism of early colonization (including benthic microscopic stages and waterborne propagules) is poorly known. Recruitment of the ephemeral *Ulva* spp. was studied in two types of disturbance manipulations (partial removal where all macroscopic organisms were removed v. complete removal where all macro-and microscopic organisms were removed) and an un-manipulated control in the mid-low intertidal (*Mazzaella* zone). Replicate disturbances were created in August 2007, November 2007, January 2008, and May 2008 and were monitored until August 2008 on a rocky bench north of Pigeon Point, CA. Recruitment into partial removals resulted from both waterborne propagules and microscopic remnants left on the substrate post-disturbance, while recruitment to complete removals did not result from fragments. *Ulva* recruitment peaked after two months in plots cleared in August 2007 and May 2008 but peaks differed among treatments indicating temporal differences in recruitment strategies. Among treatment differences in *Ulva* abundance following August 2007 and May 2008 disturbances indicated that remnants do not contribute a large portion to post-disturbance recruitment. *Ulva* failed to recruit into plots cleared in November 2007 and January 2008, although, *Porphyra perforata* (*Porphyra*) responded to both treatment disturbances and natural disturbances. *Porphyra* recruitment was 10 times greater in cleared plots than controls. Both *Ulva* and *Porphyra* responded to disturbances in unexpected ways; *Ulva* recruited seasonally and *Porphyra* recruited to a zone in which it usually is absent. "
(a) Moss Landing Marine Laboratories, SJSU

P04012 Discovering deepwater algal meadows in Hawaii: Home is where the *Halimeda* is
Spalding, Heather L-presenter hspaldin@hawaii.edu(a)

http://www.botany.hawaii.edu/gradstudentpages/Heather_Spalding.htm
"The green alga *Halimeda kanaloana* forms meadows in Hawaii over soft sediments, but little is known about its ecology or demography. We used technical diving, ROV surveys, and submersibles to describe spatial and temporal variation in distribution, abundance, demography, and growth of *H. kanaloana* across a broad depth gradient. We found *H. kanaloana* meadows occurred to 90 m and covered hundreds of kilometers of the ocean floor. The meadows formed a unique habitat for cryptic organisms, and were used as a hunting ground for large, predatory fish and Hawksbill sea turtles. *Halimeda* individuals were long-lived (> 27 months), but fluctuated greatly in segment number and height over time. Densities peaked at 20 m (342 +/- 13 SE individuals per m^2), but varied seasonally and among locations. *Halimeda* growth was rapid (9.8% +/- 1.4% SE new growth per plant per week) and generally decreased with increasing depth. Episodic abundances of other green algae (e.g., *Caulerpa filicoides*) and cyanobacteria (*Lyngbya majuscula*) overgrew *Halimeda*, negatively affecting growth. Manipulative clearing experiments (mimicking observed anchor scars) showed *Halimeda* could quickly regrow from the intact holdfast, but was slow (> 20 months) to recolonize areas cleared of both holdfast and thallus. The perennial nature and rapid growth rates of *H. kanaloana* appear to contribute toward the broad success of this species and serves to inform management of deeper reefs. As an example, disturbance removing entire individuals over a large area, e.g. repeated cruise ship anchoring, would require years for recovery. *Halimeda* meadows cover a substantial area of the ocean floor in Hawaii, and form a critical habitat linking soft sediments to coral reefs."
(a) University of Hawaii at Manoa, Botany Department

P03001 "Feast or famine? Evaluating the significance of nutrient enrichment to crustose coralline algae on Ofu, American Samoa."
Squair, Cheryl A-presenter squair@hawaii.edu(a)
"On the pristine, remote island of Ofu, American Samoa, crustose coralline algae (CCA) are the dominant functional group and comprise 27-45 % of the reef crest/ back reef area. In most studies to date, CCA in coral reef ecosystems have been evaluated by changes to percent cover or biomass. While valuable, these studies cannot address physiological processes that may underlie cover/ biomass outcomes. To test if elevated nutrients might impact the growth of CCA, a randomized block design nutrient enrichment experiment was conducted on a reef on Ofu. Because of the difficulties inherent in assessing growth of CCA *in situ*, a pulse amplitude modulated (PAM) fluorometer was used to measure the relative Electron Transport Rate (rETR) as a proxy for photosynthesis and to document changes in rates that might result from experimentally elevated nutrient levels. Across 10 sites, over a five-week period, there was no treatment effect, i.e., no increase in $rETR_{max}$ or alpha, suggesting that CCA are nutrient sufficient. Further, mean $rETR_{max}$ and alpha values from CCA were constant across the reef. These results are consistent with other studies that noted no changes in percent cover of CCA following nutrient enrichment. The reef on Ofu experiences high wave energies, which may drive tidal upwelling, episodic nutrient transport to reefs by internal waves, and/or other processes to provide a sufficient flux of nutrients such that CCA at Ofu have a favorable nutrient status."
(a) University of Hawaii - Manoa

P04020 "Genetic assessment of the pantropical alga *Acanthophora spicifera* (Rhodophyta) as revealed by DNA sequencing and microsatellite genotyping, with a focus on Hawaiian populations"
O'Doherty, Daniel C-presenter odoherty@hawaii.edu(a) Sherwood, Alison R (a)
"*Acanthophora spicifera* (Vahl) Borgesen is a common red alga in warm waters throughout the world and has been reported as a nuisance in several widely-separated Pacific ecosystems. We conducted a geographically broad investigation of the phylogeography and genetic population structure of *A. spicifera* using mtDNA COI sequences and microsatellite markers. Analyses revealed an unexpectedly high level of genetic structure and variation, especially relative to other macroalgae. Because *A. spicifera* in Hawaii was recently introduced, is seldom observed in a sexually reproductive state, frequently produces clonal recruits, and has broad dispersal capabilities, genetic analyses were expected to indicate uniformity both within and among populations. Microsatellite genotyping of Hawaiian samples confirmed the widespread occurrence of clonal reproduction but revealed an unexpected degree of population structure within the archipelago. For worldwide phylogeographic analyses, samples were collected throughout the Pacific Basin, Caribbean Sea, and West Australia. Sequence polymorphism was found to be conserved on a local geographic scale; in the majority of cases a single haplotype was identified from each oceanic island or along vast stretches of coastline. However, the occurrence of 14 biogeographically informative haplotypes with pairwise differences as high as 12 bases represents unusually high intraspecific polymorphism for COI in rhodophycean lineages. Genetic analyses indicated the presence of several intraspecific lineages and provided insight regarding the original sources of invasive populations in Hawaii, Mexico, and the Marshall Islands. "
(a) University of Hawaii at Manoa - Botany Department

P06001 Mechanism for Differential Desiccation Tolerance in *Porphyra* Species
Liu, Yen-Chun -presenter liu.yenchunliu@gmail.com(a) Lawton, Jamie (b) Miljkovic, Milos (b) Wong, Jonathan (a) Hennequart, Frank (c) Cheney, Donald (a)
"Intertidal algae provide an unique model system for the study of desiccation tolerance because they face a rapid change in water content twice a day. Our study on two intertidal algae, *P. umbilicalis* and *P. yezoensis*, show that both species lose about 95% of their water in the first two hours of desiccation and their final relative water content is virtually the same. Massive membrane leakage, reduced respiration and reduced oxygen evolution were observed in *P. yezoensis* after desiccation, but not in *P. umbilicalis*. TEM observation revealed extensive membrane disruption only in *P. yezoensis* after desiccation. Reactive oxygen species (ROS) defense, repression of membrane phase transition and formation of cellular glass are the three major desiccation tolerance mechanisms reported in land plants. ROS defense does not appear to be the key factor here, because neither species showed an increase in membrane peroxidation after desiccation. Repression of membrane phase transition cannot explain the different response because the membranes of both species remain in liquid crystalline when desiccated. Our data suggest that the cytoplasm of *P. umbilicalis* forms a more stable glass when the organism is desiccated, and that the molecular mobility is lower in the drying *P. umbilicalis*. A dehydrin-like protein was detected in great abundance in *P. umbilicalis* and could play a key role in the better desiccation tolerance of this species. "
(a) Department of Biology, Northeastern University (b) Department of Chemistry and Chemical Biology, Northeastern University (c) Department of Biochemistry, National Universtiy of Ireland

P05019 A comparison of two DNA barcode markers for species discrimination in marine red algae
Clarkston, Bridgette E.-presenter bridgette.clarkston@unb.ca(a,b) Saunders, Gary W. (a,b)
"The accurate identification of many red algae to the species level using traditional morphological characters can be frustrating and overwhelming. For example, members of the red algal family Kallymeniaceae (Gigartinales, Florideophyceae) are often challenging to identify due to high plasticity of the morphological and anatomical traits typically used to delimit species. The emerging field of 'molecular-assisted alpha taxonomy' can greatly alleviate this issue. In this approach a large number of specimens are sequenced for a standard DNA marker as a first step to genetic species assignment, followed by detailed morphological observations. Regions of the mitochondrial cytochrome c oxidase I gene (COI-5') and the plastid 23S rRNA gene (UPA) have been proposed as DNA barcode markers for rapid species identification in red algae. COI-5' is a more sensitive marker for

delimiting species, however, it can be difficult to acquire clean amplification products from many isolates of the Kallymeniaceae because of biological contamination. This problem can be overcome by using species specific primers. The UPA, on the other hand, has primers that are universal and work in diverse lineages (e.g. red algae, brown algae, green algae), however, lower interspecific sequence variation in this marker has the potential to underestimate species diversity. Here a comparison of COI-5' and UPA for resolving species of the Kallymeniaceae from British Columbia, Canada will be discussed."
(a) Center for Environmental & Molecular Algal Research (b) Biology Department, University of New Brunswick

P05017 "Identification of the genus *Corallina* (Corallinales, Rhodophyta) in Canada"
Hind, Katharine R-presenter katy.hind@unb.ca(a) Saunders, Gary W (a)
http://www.unb.ca/cemar/saunders/
"The process of identifying and classifying seaweed has largely been restricted to the study of morphological and anatomical traits. Species identification of seaweeds is problematic for many groups due to a high level of phenotypic plasticity and cryptic diversity. In the last decade, the use of genetic characters in the identification of red algal species has become an established tool for such challenging groups. This study examines the species diversity of the genus *Corallina* in Canada using an integrative taxonomic approach. The approach involves establishing genetic species groups using the mitochondrial gene cytochrome C oxidase subunit 1 (CO1) and then assessing the morphological characters that are ubiquitous within these groups. Current taxonomic classifications recognize five species in the genus *Corallina* in Canada, however my preliminary results suggest nine unique genetic species groups. This finding indicates that cryptic diversity may be present for this genus. My future research will be directed towards using molecular data in combination with a rigorous morphological examination to elucidate species diversity in this taxonomically challenging algal group. "
(a) University of New Brunswick, Department of Biology

P06005 Taking tropical stress in stride? An investigation of the impact of low tides on the brown alga *Padina sanctae-crucis* distribution and physiology.
Cox, T. Erin-presenter erincox@hawaii.edu(a) Smith, Celia M. (a)
"Lush macroalgal beds cover intertidal benches in Hawaii, however, tropical reefs characterized by high temperatures and irradiances are among the most stressful habitats worldwide for photosynthetic organisms. Yet, few studies examine responses of macroalgae in situ to such stresses. To address this gap, we have begun to investigate impacts of tropical micro-tidal regime on distribution and physiology of common intertidal macroalgae. We used point-intercept techniques at two intertidal sites on Oahu (Barber's Point and Diamond Head) to identify macroalgal spatial patterns. Sites were 57% similar in organism abundance and macroalgae occurred in discrete zoned bands. Physiological tolerances can contribute to zonation patterns. Thus photosynthetic physiology of dominant intertidal brown alga *Padina sanctae-crucis*, was evaluated across a week long series of spring low tides with rapid light curves (RLCs) via pulse amplitude fluorometry. Sampling began prior to low tide and continued to submersion of intertidal zone. Two to five individuals of *P. sanctae-crucis* were collected hourly from three locations (high, mid, and low) and RLCs performed to estimate the parameters $rETR_{max}$, E_k, α, and β. Preliminary results indicate that severe spring low tides actually enhance photosynthetic performance. On days with lower low tides, macroalgae exhibited increased $rETR_{max}$ values. In contrast, at time of maximum low tide, $rETR_{max}$ values were lower. Multiple regressions revealed that tide magnitude predict $rETR_{max}$ for *P. sanctae-crucis* for both sites. Shore location (seaward vs. landward) was an important predictor of $rETR_{max}$ values at one site. Additional analyses and manipulative experiments (in progress) will be used to investigate these patterns further."
(a) Botany Department, University of Hawai'i at Manoa

P03002 Assessing impacts of the non-indigenous red alga *Gracilaria salicornia* on Waikiki's reef health: Algal distribution changes over a 40 year period.
sauvage, thomas m-presenter sauvage@hawaii.edu(a) smith, celia m (a)
"Waikiki reef lies at the edge of a watershed that has experienced major urbanization and demographic growth over the past century. Anthropogenic pressures on this reef have included physical disturbances (e.g. dredging, sand-filling), over-fishing, and water quality fluctuations. An additional impact to near shore benthic communities has been the invasion by *Gracilaria salicornia* (C. Agardh) E.Y. Dawson, a non-indigenous marine alga (NIMA) introduced in Waikiki in 1971. The benthic changes that have occurred on Waikiki reef during the 20th century have not been characterized despite the availability of numerous ecological assessments starting as early as 1928. Maxwell Doty, whose research focused on algal productivity, conducted extensive field work on this reef in the late 1960s, prior to the introduction of *G. salicornia*. The largest data set reports individual algal species biomass at >100 geo-referenced quadrats on a reef surface of 3.36 hectares for each of the 6 collection periods conducted between March 1967 and April 1968. This multivariate spatio-temporal data set is re-analyzed using model-based geostatistics to describe past, reef-wide distribution of algal species and seasonal variation. Comparison with recent assessments demonstrates local extinction of *Sargassum echinocarpum* and *S. obtusifolium*, and major algal biomass distributional changes. The importance of historical data in understanding long-term impacts of NIMAs on coral reef ecosystems and overall coral reef degradation will be discussed."
(a) University of Hawaii at Manoa

P04007 "Grazing effects of the turban snail, *Chlorostoma brunnea*, on the productivity of the giant kelp, *Macrocystis pyrifera*, in central California."
McMillan, Selena-presenter smcmillan@mlml.calstate.edu(a)
"The purpose of this study was to evaluate how the most abundant kelp forest herbivore in central California, the turban snail genus *Chlorostoma* (formally *Tegula*), affects the productivity and survivorship of *Macrocystis pyrifera* within central Californian giant kelp forests. The effects of the most abundant turban snail species, *Chlorostoma brunnea*, were investigated using experimental field manipulations of *M. pyrifera* sporophytes and supplementary laboratory experiments. Ten *M. pyrifera* sporophytes were selected in Stillwater Cove, Carmel, CA at approximately 10m depth. These individuals were randomly chosen to be stocked with low to high densities (0-400 per sporophyte) of *C. brunnea*. One-meter-square copper cages were secured at the base of each stocked kelp sporophyte to reduce immigration and emigration of all turban snail species. Surveys of the *M. pyrifera* individuals were conducted bi-weekly to determine changes in growth. After six weeks, there were no significant trends in *M. pyrifera* growth rate as a function of *C. brunnea* density; however, a piece-wise regression indicated a significant threshold effect with lower growth occurring at extreme snail densities. Laboratory feeding experiments identified marine fungi growing on *M. pyrifera* as a potential primary food source for *C. brunnea*. Fungal biomass was significantly lowered by *C. brunnea* grazing at moderate densities. Higher densities of *C. brunnea*, however, grazed directly on *M. pyrifera* fronds and fungal biomass increased as sporophytes deteriorated. Therefore, *Chlorostoma* may feed mainly on fungi when associated with *M. pyrifera*, except at high snail densities when direct consumption on *M. pyrifera* can inhibit sporophyte growth. "
(a) Moss Landing Marine Laboratories

P04004 Alternative life history of *Gracilariopsis* sp. (Rhodophyta) within the dynamic substrate regime of a central Californian estuary

Wehrenberg, Megan L-presenter mwehrenberg@mlml.calstate.edu(a)
http://phycology.mlml.calstate.edu/wehrenberg

"Gracilaroids are one of the dominant groups of seaweeds found in CA estuaries. Their tolerance of physical stressors such as salinity, desiccation and their psammophytic nature make them well suited to these environments. These algae utilize a broad suite of reproductive strategies in a tri-phasic life-history including: sexual fertilization, release of tetraspores, apogamy, and vegetative fragmentation. The purpose of this project was to examine the mechanisms for growth and reproduction utilized by *Gracilariopsis* sp. in the Elkhorn Slough estuary (Moss Landing, CA). To investigate this, a year-long time series was initiated whereby monthly random samples were collected within a 100m x 30m plot to make estimates of biomass and reproductive capacity. In 12 months, the population exhibited little to no utilization of sexual fertilization, and peaked in biomass in the fall and winter months. Surprisingly, the majority of the population was found up to 45cm below the sediment surface during all winter months and much of the spring. The latter half of the year showed a marked shift to above-ground biomass as well as a net loss of sediment, suggesting that sediment movement may play an extremely important role in the growth and reproductive cycles of these algae. The capabilities of these thalli to survive underground for extended periods of time may be more consequential to the life-longevity and successful propagation of individuals than any other mechanism. "

(a) Moss Landing Marine Laboratories

Start	End	Event	Location
6:30 PM	8:00 PM	**PSA Talks -** **Algal Phylogenetics & Taxonomy-I** 6:30 –P05016: Lesleigh Kraft - *A study of Australian Ulva challenges notions of cosmopolitanism and the utility of anatomical species designations* 6:45 – P05023: Charles O'Kelly - *Molecular assessment of the species of Ulva (Ulvophyceae, Chlorophyta) in the Hawaiian Islands* 7:00 – P05015: Gerald Kraft - *The marine macroalgae of Lord Howe Island: 32 years on* 7:15 – P05013: Judith Broom - *Progress in documenting the common coralline algae of New Zealand* 7:30 – P05006: Haj Allali - *A biodiversity survey of subaerial algae from an African tropical Rainforest* 7:45 – P05002: Daryl Lam - *Epiphytic Biodiversity of the Raleighvallen Rainforest (Suriname, South America) Inferred from Environmental Sequencing*	Kaua'I 311

P05016 A study of Australian *Ulva* challenges notions of cosmopolitanism and the utility of anatomical species designations

Kraft, Lesleigh K-presenter lkraft@unimelb.edu.au(a) Kraft, Gerald T (a) Waller, Ross F (a)

"Appreciation of the true species diversity of the genus *Ulva* (Ulvales, Chlorophyta) in Australian waters has been constrained by the unproved assumption that its representatives there are largely cosmopolitan. *Ulva* species can be notoriously difficult to identify due to the few and often variable characters on which classical taxonomic studies focus, so that names of specimens in-hand, as well as names appearing in historical distribution records, are frequently difficult or impossible to verify. In this study of cool- to warm-temperate Australian populations, both morphological and molecular analyses have been undertaken. Although habit- and anatomy-based keys of standard taxonomic literature were adequate to assign species names to most taxa represented in Australian waters, these obscured several cryptic Australian taxa that were distinguishable only by molecular analyses. Some of the cryptic taxa occupy sufficiently distinct molecular clades as to warrant new-species designations. Furthermore, phylogenetic analyses that include publicly available *Ulva* GenBank accessions reveal a number of species designated there that imply polyphyly. Morphological data in these cases are essential to assessing monophyly, although in many cases matching sequences are not featured in GenBank or the literature. In total we have identified six cosmopolitan taxa in Australia, five cryptic taxa (four of which have been assigned new species names), and one new taxon that does not match any previous gene sequence or morphological description. This study also highlights the importance of recording both anatomical and molecular data for morphologically plastic algae with few distinguishing characteristics."

(a) University of Melbourne

P05023 "Molecular assessment of the species of *Ulva* (Ulvophyceae, Chlorophyta) in the Hawaiian Islands"

O'Kelly, Charles J.-presenter okelly@hawaii.edu(a) Kurihara, Akira (a) Chandrasekharan, Tara (a) Sherwood, Alison (a)

"At present, twelve species of *Ulva* (including *Enteromorpha*) are accepted in the flora of Hawaii, all of which are based on European and Middle Eastern types. To test whether these species assignments are accurate, we sequenced the nuclear-encoded ITS1 region and/or the chloroplast-encoded rbcL gene from 94 specimens collected from five of the eight main Hawaiian Islands. We placed these 94 specimens into 15 operational taxonomic units (OTUs), based on primary sequence data and comparisons of ITS1 secondary structure. Of the 15 OTUs, we assigned only three to named species (*Ulva fasciata* Delile; *U. ohnoi* Hiraoka & Shimada; *U. procera* (Ahlner) Hayden et al.); the latter two, which are potentially green tide species, are new records for Hawaii. Four additional OTUs match undescribed species recorded from other floras, mostly from East Asia or New Zealand. The remaining eight are unattested. On this evidence, only one (*U. fasciata*) of the twelve species names previously used in Hawaii is correctly applied. We think that *Ulva* populations in tropical and subtropical regions consist of species that are largely unique to the tropics, and for which the application of names based on types from temperate and boreal European and North American waters is inappropriate."

(a) Department of Botany, University of Hawaii at Manoa

P05015 The marine macroalgae of Lord Howe Island: 32 years on

Kraft, Gerald T.-presenter gtk@unimelb.edu.au(a)

"In 1977 the author gave his first conference talk on the marine algae of Lord Howe Island following a year'"s study there, and this presentation 32 years later will be his swansong recap of additional taxonomic research conducted with many colleagues over that long timespan. A macroalgal biodiversity hotspot located at the world'"s highest latitude of consolidated coral-reef formation, this small volcanic outcrop in the middle of the Tasman Sea has a unique flora with many endemic elements. Some aspects of the island''s physiognomy will be illustrated, and a few of the more interesting taxa will be cherry-picked for highlighting. Of particular richness are members of the order Dictyotales and several red-algal genera such as Ceramium. Sargassum presents particularly interesting problems dating back to the island''s first algal collections, which included four species described by Zanardini in 1874."

(a) University of Melbourne

P05013 Progress in documenting the common coralline algae of New Zealand
Broom, Judith E.-presenter judy.broom@otago.ac.nz(a) Farr, Tracy J. (b) Neill, Kate F. (b) Hart, Darren R. (a) Nelson, Wendy A. (b)
"Since 2002, we have been funded by the New Zealand Ministry of Fisheries to study the coralline algae flora of New Zealand. This program focused on encrusting Corallinales of the central New Zealand region from 2002 to 2005, and was extended to geniculate and encrusting Corallinales of northern New Zealand from 2005 to 2008. In the northern programme, more than 650 collections of coralline algae were made from 91 intertidal and subtidal sites throughout the study area. Collections were identified by anatomical and morphological characters where possible, and over 90% of collections were sampled for molecular analysis. Sequence data from the *psb*A gene was obtaining from nearly 500 specimens, allowing us to rapidly bin specimens into clusters with near-identical sequences. Nuclear small subunit ribosomal RNA (nSSU) sequences were obtained from representative specimens from each clade, allowing the direct comparison of New Zealand material with sequences from other regions. New records for New Zealand of two genera and several species were obtained. One specimen was not resolved within any of the three extant families within Corallinales by either gene. The major product of the research programme is an identification guide to the common coralline algae of northern New Zealand, which will be available electronically."
(a) Department of Biochemistry, University of Otago (b) National Institute of Water and Atmospheric Research

P05006 A biodiversity survey of subaerial algae from an African tropical Rainforest
Allali, Haj A-presenter allal001@ua.edu(a) Lopez-Bautista, Juan M (a)
"Although economically and ecologically important, subaerial algae from African tropical rainforests have been largely understudied compared to their marine and freshwater counterparts. Since rainforests have been shown to be centers of subaerial algal biodiversity in South America, India, and Australia, it is reasonable to expect that they also host a higher diversity in Africa. Algae living in these habitats, on surface above the soil, are constantly exposed to extreme conditions. These algae are found on a wide range of substrates, including rocks, walls, metal, bark, leaves of trees and even animals. Subaerial algae from African tropical rainforests include different algal lineages. Our work during the last two years has been centered in Gabon, a West African country with a large system of national parks. Findings and new records of trentepohlian taxa as well as other microchlorophytes are reported. A phylodiversity survey is also in effect utilizing cloning techniques to generate environmental DNA algal signatures. These approaches will provide with a better understanding of the biodiversity from these poorly known algal habitats. "
(a) The University of Alabama

P05002 "Epiphytic Biodiversity of the Raleighvallen Rainforest (Suriname, South America) Inferred from Environmental Sequencing"
Lam, Daryl W-presenter dwlam@ua.edu(a) Lopez-Bautista, Juan M (a)
http://bama.ua.edu/~dwlam
"Raleighvallen is nature preserve in the heart of Amazonian tropical rainforest. The region is one the most pristine and biologically diverse terrestrial habitats on the planet. This study seeks to evaluate the epiphytic algal diversity found on trees located within the preserve. Microbial epiphytes were collected from tree bark and extracted for total DNA under sterile conditions. A portion of the 23S ribosomal DNA from the plastid genome was amplified with universal primers (Sherwood and Presting 2007) via touchdown PCR. PCR products were ligated into cloning vectors. The newly generated plasmids were transformed into *E. coli* cells. Subsequently, colony PCR was performed and products were sequenced. Homologous sequences from GenBank were added to the dataset. A maximum likelihood phylogeny was inferred through the RAxML (Stamatakis 2006) software package. The dataset included the following taxonomic groups: Apicomplexa, Chlorophyta, Chlororachinophyceae, Cryptophyta, Cyanobacteria, Euglenoids, Glaucocystophyceae, Haptophyceae, Stramenopiles, Rhodophyta, and Streptophyta. Results indicated the Raleighvallen epiphytic community is remarkably diverse. Our environmental sequences were found from the following groups: nonvascular land plants (mosses and liverworts), diatoms, green algae and blue-green algae. An interesting side note involves the molecular evolution of the Apicomplexa, the longest branch of the phylogeny. Apicomplexans are parasitic organisms; their life cycles are dependent of vertebrate hosts and they no longer have functional plastids. Hence, their chloroplast DNA is free to evolve because of a lack of selective pressure. Future studies include culture-based observations of the epiphytes and the addition of more taxonomic groups to the phylogeny."
(a) University of Alabama

Start	End	**Event**	Location
6:30 PM	7:45 PM	**PSA Talks -** **Applied Phycology-I** 6:30 – P01007: Maria Ghirardi - *Hydrogen Fuel Production by Microalgae: Issues and Future Directions* 6:45 – P01003: Matthew Timmins - *High-efficiency hydrogen production from green microalgae* 7:00 – P01006: Takashi Yamamoto - *Elevation of the hydrogenase activity to produce hydrogen by Synechocystis sp. strain PCC6803* 7:15 – P02008: Yunyun Zhuang - *Regulatory network of cell cycle in marine phytoplankton* 7:30 – P01005: Makoto Wakayama - *Elevation of the production rate of the intracellular D-glucose in Synechococcus sp. strain PCC6301*	Lana'I 314

P01007 Hydrogen Fuel Production by Microalgae: Issues and Future Directions
Ghirardi, Maria L-presenter maria_ghirardi@nrel.gov(a)
"Some microalgae are able to photoproduce H_2 gas from water under anaerobic conditions, and they are being considered as potential sources of renewable, clean fuel. The H_2-producing property of green algae is a result of the coupling of photosynthetic reducing power generation to the catalytic activity of the algal hydrogenases. Two [FeFe]-hydrogenase genes have been reported in the green alga, *Chlamydomonas reinhardtii*. Their expression and activity are regulated by O_2, and they may both be involved in fermentative pathways as well. I will discuss recent gene expression microarray studies done in collaboration with Stanford University and the Colorado School of Mines and will present research directions at NREL aimed at identifying and solving major metabolic and rate-limiting steps in H_2 photoproduction, including the high sensitivity of these enzymes to O_2. Recent work will be presented on (a) structural and mechanistic aspects of [FeFe]-hydrogenases, (b) transcriptional regulation of algal [FeFe]-hydrogenases, and (c) issues related to application of green algae for alternative fuel production at high light conversion efficiencies. "
(a) National Renewable Energy Laboratory

P01003 High-efficiency hydrogen production from green microalgae
Timmins, Matthew-presenter m.timmins@uq.edu.au(a) Doebbe, Anja (b) Schenk, Peer (a) Zhou, Wenxu (c) Lim, Lyscha (a) Waudoo, Winnie (a) Smith, Steven (c) Marx, Ute (a) Mussgnug, Jan (b) Kruse, Olaf (b) Hankamer, Ben (a)
http://www.solarbiofuels.org
"Algae have the potential to meliorate global energy supply and environmental problems associated with rising CO_2 levels. This may be achieved by using algae to produce a range of biofuels, including hydrogen (H_2). Photosynthesis and endogenous energy supply are central to the production of H_2 from green microalgae. Under anaerobic circumstances, certain algae have the ability to direct electron flow from water oxidation and endogenous substrate oxidation through the photosynthetic apparatus to hydrogenase enzymes. Hydrogenase catalyses the reduction of protons to produce H_2. This talk will present work performed to determine the H_2-producing capacity of new algal specimens isolated from Australian waters and on metabolomic studies performed on *Chlamydomonas reinhardtii* during sulphur-deprived anaerobic H_2 production. Fundamental changes in metabolism upon the onset of anoxic H_2 are evident and new strategies to increase H_2 generation for commercial production are discussed. "
(a) The University of Queensland (b) University of Bielefeld (c) University of Western Australia

P01006 Elevation of the hydrogenase activity to produce hydrogen by *Synechocystis* sp. strain PCC6803
Yamamoto, Takashi-presenter yamamoto.t.af@m.titech.ac.jp(a) Asami, Kazuhiro (a) Ohtaguchi, Kazuhisa (a)
"Photosynthesis is the process that converts solar energy to chemical energy and stores it in the bond of sugars incorporating carbon dioxide (CO_2). The reserved sugars are important source for biological renewable energy. Biohydrogen (H_2) is the ideal form of future solar-converted renewable energy. The present study was undertaken to determine the participation of reaction conditions in the bidirectional hydrogenase-mediated H_2 formation mechanism of cyanobacteria, which are widely utilized in photosynthesis research. Bidirectional hydrogenase catalyzes the formation of H_2 from NAD(P)H and H^+. *Synechocystis* sp. strain PCC6803, which performs both photoautotrophic growth on CO_2 and photoheterotrophic growth on CO_2 and D-glucose, was utilized in experiments. Growth was performed at 307 K in 70 mL basal or modified BG-11 medium. Culture was aerated with 6% CO_2 in air at 70 mL/min, and illuminated at 100 μ;mol/m^2/s *PPFD* by fluorescent lamp. Cells were harvested at 24 h of cultivation. H_2 formation was performed at 307 K under the dark in 10 mL buffer solution with or without D-glucose. The initial cell mass concentration was fixed at 2.0 g/L. Hydrogenase activity, in terms of the rate of H_2 formation by unit cell mass, was highest at 0.62 U/g when cells were prepared in NO_3^- free medium. Such hydrogenase activity was unrelated to the attainable level of hydrogenase gene expression that was assayed in the presence of methyl viorogen. It turns out that H_2 formation was strongly related to the activity of reducing power supply, rather than the level of hydrogenase gene expression. Hydrogenase activity of cells with 4 g/L D-glucose was 2.9 times that without D-glucose. External D-glucose was found to offer additional reducing power for H_2 formation."
(a) Department Chemical Engineering,Tokyo institute of technology

P02008 Regulatory network of cell cycle in marine phytoplankton
Zhuang, Yunyun-presenter yunyun.zhuang@uconn.edu(a) Lin, Senjie (a)
http://www.phytoplankton.uconn.edu
"Cell cycle regulation is the key to growth, development, and differentiation of an organism, and is of particularly relevance to phytoplankton population dynamics since cell division in these unicellular organisms directly lead to population growth. Knowledge on the molecular regulatory network of the cell cycle of marine phytoplankton will be important in order for us to understand how they adapt to the environment and respond to its changes. While the network is well studied in model systems such as yeast and humans, there has been no systematic analysis of such network for marine phytoplankton. In this study, we analyzed currently available phytoplankton genomic and EST data, searched and annotated cell cycle related genes in these algae. Core regulatory genes, including Cdc2 and other Cdks, cyclin, and proliferating cell nuclear antigen, were identified and mapped to KEGG cell cycle pathway. Similarity and uniqueness in the cell cycle engine among marine phytoplankton species is being characterized and compared to counterparts in other eukaryotes to look for an evolutionary trend of this critical cellular machinery. "
(a) Department of Marine Sciences, University of Connecticut

P01005 Elevation of the production rate of the intracellular D-glucose in *Synechococcus* sp. strain PCC6301
Wakayama, Makoto-presenter mwakayam@chemeng.titech.ac.jp(a) Ohtaguchi, Kazuhisa (a)
"Cyanobacteria are evolutionary ancestors of the chloroplasts in green plants and provide an excellent host to express the light-regulated genes coding for high value compounds. Taking into account that many of them are produced intracellularly, it can be assumed that high-cell density cultivation is the key for the efficient production of these compounds. Photosynthesis of cyanobacteria in a reactor appears to be effected by availability of light energy for chlorophylls and that of inorganic ions. Fundamental parameters for the light availability involve the level of photosynthetic photon flux density (*PPFD*), the action spectrum of incident light, and the light path length of the reactor. This study was designed to evaluate the effects of these parameters upon the growth of unicellular cyanobacterium *Synechococcus* sp. strain PCC6301. First, cells were grown in the photobioreactor (light path length, 1 mm) under the illumination of not high *PPFD*. Second, white fluorescent lump or red LED lump was used as light source to investigate the effect of light source on the production of intracellular D-glucose. When cultures were illuminated by white fluorescent lump with the incident *PPFD* of 150 μ;mol/m^2/s, cell mass concentration at 120 h reached 24.5 g/L with sufficient supply of inorganic ions. Under the premise of short light path length, such not high *PPFD* was found to be sufficient to perform high-cell density cultivation. D-glucose content per unit cell mass reached 0.455 g/g in nitrogen limited condition under the *PPFD* of 300 μ;mol/m^2/s of red LED lump. The cultivation in nitrogen limited medium in the reactor that was illuminated by red LED lump was found to be preferred for accumulating intracellular D-glucose."
(a) Department of Chemical Engineering, Tokyo Institute of Technology

Start	End	**Event**	Location
6:30 PM	8:00 PM	**PSA Talks -** **Algal Ecology & Population Biology-I** 6:30 –P04013: Peter Thompson - *The phytoplankton ecology of Western Australia.* 6:45 –P04018: Dennis Hanisak - *Water quality in the Indian River Lagoon, Florida: Relationship to the macroalgal community* 7:00 –P04009: Jennifer Ress - *Bryophytic algal communites from Nu'uanu Pali, O'ahu, (Hawai'i, U.S.A.)* 7:15 –P04017: Hugh Forehead - *Effects of reduced physical disturbance and nutrient enrichment on the ecology of subtidal benthic microalgae in Western Australia*	Moloka'I 315

		7:30 –P04003: Rex Lowe - *Distribution and morphological variability of Cosmioneis (Bacillariophyceae) in Hawaii* 7:45 –P04024: Alan Millar - *Threatened seaweeds and how we could protect them?*	

P04013 The phytoplankton ecology of Western Australia.
Thompson, Peter A-presenter peter.a.thompson@csiro.au(a) Waite, Anya M (b) Bonham, Pru (a)
"General trends in phytoplankton distribution along ~ 2000 km of the west Australian coast are derived from research cruises spanning 1995 to 2007. Pigments showed statistically significant differences in their spatial patterns. For example, there was a shallow distribution of zeaxanthin while 19'-hexanoyloxyfucoxanthin and 19'-butanoyloxyfucoxanthin were deep but they were all offshore relative to neoxanthin, violaxanthin, prasinoxanthin and alloxanthin. Temporal variation in biomass (remotely sensed chlorophyll *a*)showed a seasonal pattern that varied with latitude. At 32S biomass peaked in midwinter and was positively correlated with the Southern Oscillation Index (SOI) at monthly and annual time scales. The southward flow of the dominant Leeuwin Current strengthens and spins off more mesoscale eddies during winter in high SOI years. Intensive studies of these mesoscale, warm-core eddies shows them to have increased biomass, more large cells and fewer prochlorophytes than outside the eddy or in nearby cold core eddies. The mature 2003 warm-core eddy had a very deep mixed layer and high rates of vertical mixing that favoured an increase in diatom abundance. High SOI years were also associated with more diatoms near-shore during summer. It is hypothesized that this response is due to a change in the prevailing summer wind pattern to favour upwelling and a near-shore counter current. "
(a) CSIRO Marine and Atmospheric Research (b) University of Western Australia

P04018 "Water quality in the Indian River Lagoon, Florida: Relationship to the macroalgal community"
Hanisak, M Dennis-presenter dhanisak@hboi.fau.edu(a)
"The Indian River Lagoon system (IRL), the longest barrier island/tidal inlet system in the continental United States, spans more than one third of Florida's east coast. Water quality in the IRL has changed significantly over the past eight decades due to watershed alteration and land drainage patterns. High-frequency water quality monitoring was conducted for three years at four sites in the central lagoon near Vero Beach and Fort Pierce. While there was considerable interannual variability in absolute values of key water quality parameters (related to varying precipitation and associated freshwater runoff), the water quality gradient was consistent throughout the study. From north to south, salinity increased, while turbidity, color, suspended solids, and chlorophyll a (all attenuators of light) decreased, as did nutrients and K. Pulses of inorganic nitrogen were associated with the initial onset of the wet season and following tropical storms/hurricanes and other major periods of freshwater discharge. A series of in situ measurements of tissue nitrogen of the dominant macroalgal species in the IRL and their in situ growth rates indicated that the growth rate of the drift algal macroalgal community was strongly related to the measured nitrogen gradient in this part of the lagoon. These findings suggests that, if proposed reductions in anthropogenic loadings into the lagoon become a reality, macroalgal productivity and abundance should significantly decline and lead to a reduced competition of macroalgae with seagrasses in the lagoon. "
(a) Harbor Branch Oceanographic Institute

P04009 "Bryophytic algal communites from Nu'uanu Pali, O'ahu, (Hawai'i, U.S.A.)"
Ress, Jennifer-presenter jar569@hotmail.com(a) Lowe, Rex (a) Waite, Mashuri (b)
"A diverse bryophyte flora inhabits the exposed rocks on Nu'uanu Pali, on Ko'olau Mountain, in southeastern O'ahu (Hawai'i, U.S.A.). The structures of bryophytes are known to provide habitat for algal communities and are important in aerial habitats, such as that of Nu'uanu Pali, due to the ability of bryophytes to retain moisture which can be used by their algal associates. We predicted that different bryophyte species would support distinct algal communities due to the variability of microhabitats among bryophyte species. Differing bryophyte morphologies will influence their ability to retain moisture which will ultimately influence the algal community composition. Twenty-eight samples were collected from Nu'uanu Pali in February and March, 2008. Physical and chemical factors, including aspect, moisture levels, light levels, and pH were measured at each sampling location. Bryophytes and associated algal communities were identified and relative biovolumes were calculated for algal species within each community. Patterns were explored via non-metric multidimensional scaling and analysis of similarities on a Bray-Curtis similarity matrix. The analysis demonstrated an association between bryophyte species and aspect, north versus south, with algal community composition. This is most likely due to the relationship between sun exposure and moisture loss from the bryophytes and associated algal communities. "
(a) Bowling Green State University (b) University of Hawai'i at Mānoa

P04017 Effects of reduced physical disturbance and nutrient enrichment on the ecology of subtidal benthic microalgae in Western Australia
Forehead, Hugh I.-presenter hugh.forehead@gmail.com(a,b) Kendrick, Gary A. (c) Thompson, Peter A. (a)
"An experiment was designed to test whether the benthic microalgae (BMA) of subtidal sediments in oligotrophic waters of Western Australia would respond to decreased physical disturbance by increasing biomass or changing community composition; and whether decreasing the N:P ratio of the water column would stimulate N_2 fixation. Concentrations of fatty acids, neutral lipids and pigments in the sediment were measured, and intact sediment cores were incubated to measure fluxes of oxygen, inorganic nutrients and N_2 fixation at the start and end of the experiment. Shelter resulted in a 30% increase in the biomass of BMA, and there was no evidence of an effect of enrichment on biomass. There was an increase in diatoms as a fraction of the BMA community, but only a very small increase of cyanophytes; the shift in the N:P ratio did not increase N_2 fixation over the 10 day period. The remaining biomass, bacteria and other heterotrophs, also increased in response to enrichment and shelter. Levels of gross primary production (GPP) were high for oligotrophic sediments, up to 3.67 mmol O_2 m^{-2} h^{-1} and community respiration (CR) ranged up to 3.06 mmol O_2 m^{-2} h^{-1}. Lipid biomarkers suggested shifts in the composition of the community of grazers. There were small changes in hourly rates of sediment-water fluxes of PO_4 and Si in response to shelter."
(a) Cellana LLC (b) CSIRO Marine and Atmospheric Research (c) University of Western Australia, School of Plant Biology

P04003 Distribution and morphological variability of *Cosmioneis* (Bacillariophyceae) in Hawaii
Lowe, Rex L-presenter lowe@bgsu.edu(a) Sherwood, Alison R (b,b)
"*Cosmioneis* Mann & Stickle, is a pennate diatom in the family Cosmiodeidaceae and order Naviculales. A single specimen of this genus (*Navicula pusilla* W. Sm., now *Cosmioneis pusilla* (W. Sm.) Mann & Stickle) had been previously reported from Oahu. In extensive surveys of algae of subaerial habitats on Maui and Oahu, Hawaii we collected populations of three species of *Cosmioneis*. Reports from other habitats describe *C. pusilla* as morphologically variable with valves ends ranging from subrostrate to strongly capitate. Our populations of *C.* cf. *pusilla* showed little variability and were either strongly capitate or rostrate and are probably two separate species. In addition we found populations of a third putatively undescribed

species of *Cosmioneis*. Here we will discuss the morphology and ecology of this genus in Hawaii."
(a) Department of Biological Sciences (b) Department of Botany

P04024 Threatened seaweeds and how we could protect them?
Millar, Alan J.K.-presenter alan.millar@rbgsyd.nsw.gov.au(a)
http://www.aussiealgae.org
"When it comes to threatened species on this planet, most would not consider macroalgae/seaweeds to be included in this category of organisms facing extinction. Recent research within Australian waters has brought to light several macroalgae that have not only become extinct, but are potentially threatened with a range of anthropogenic influences. Protection and conservation of these algae will include both *in situ* and *ex situ* physical conservation, as well as the protection of the law. Evidence also suggests that some anthropogenic influences such as global warming may be beyond our control, but are already having an effect on seaweeds."
(a) Royal Botanic Gardens Sydney

MINISYMPOSIA/ TALKS – MONDAY, JULY 20

Start	End	Event	Location
8:30 AM	10:15 AM	**PSA Talks** - **Algal Phylogenetics & Taxonomy-II** 8:30 - P05008: Juan Lopez-Bautista - *New insights in the systematics of Trentepohliales based on rbcL and morphological analyses* 8:45 - P05027: Gabrielle Rocap - *The chloroplast genome of the marine picoplankter Pinguiococcus pyrenoidosus* 9:00 - P05026: Jeffrey Johansen - *Using secondary structure of the 16S rRNA molecule and associated 16S-23S ITS to examine phylogenetic relationships of Aulosira (Nostocaceae, Cyanobacteria).* 9:15 - P04019: Michael Jacobs - *Differential rate of divergence in mitochondrial and chloroplast genome sequences from geographically separate strains of Heterosigma akashiwo.* 9:30 - P05004: Michael Wynne - *The recognition of Caulerpa integerrima (Zanardini) comb. et stat. nov. (Bryopsidales, Chlorophyta) from the Red Sea* 9:45 - P04021: Chi Chiu Cheang - *The phylogeography of Sargassum fusiforme (Fucales, Heterokontophyta) in the northwestern Pacific* 10:00 - P04008: Kyle Demes - *Phenotypic plasticity reconciles incongruous molecular and morphological taxonomies: Macrocystis is a monospecific genus*	Ni'ihau 312

P05008 New insights in the systematics of Trentepohliales based on *rbc*L and morphological analyses
Lopez-Bautista, Juan M-presenter jlopez@ua.edu(a) Rindi, Fabio (a) Lam, Daryl W (a)
http://bama.ua.edu/~jlopez/
"The order Trentepohliales comprises one family and five genera: *Cephaleuros, Phycopeltis, Stomatochroon, Trentepohlia* and *Printzina*. Representatives of these taxa are found solely in subaerial environments. They occur on natural and artificial substrates. Although more abundant and diversified in tropical rainforests they can also be found in temperate regions. Our previous work has demonstrated the phylogenetic position of this monophyletic group of subaerial green algae inside the class Ulvophyceae and as a sister group to the marine green algal orders Dasycladales, Cladophorales/Siphonocladales complex and Bryopsidales. Recent collections from tropical rainforests have yielded an expanded database of Trentepohliales. Newly developed primers for the chloroplast-encoded *rbc*L have resulted in new insights on the relationship of trentepohlialean genera and species. Implications for the classification of the species-rich *Trentepholia* and *Printzina* will be discussed based on *rbc*L and morphological analyses. These results also clarify the taxonomic identity of several species, in particular *T. flava vis-a-vis T. aurea*."
(a) The University of Alabama

P05027 The chloroplast genome of the marine picoplankter *Pinguiococcus pyrenoidosus*
Rocap, Gabrielle-presenter rocap@ocean.washington.edu(a) Jacobs, Michael J (b) McKay, John (a) Frischkorn, Kyle (a) Ong, Han (c) Cattolico, Rose Ann (d,a)
http://chloroplast.ocean.washington.edu/
"The recently described stramenopile class Pinguiophyceae consists of 5 genera characterized by a large percentage of long-chain polyunsaturated fatty acids (PUFA), particularly eicosapentaenoic acid (EPA). Though phylogenetic analysis using both 18S rRNA and rbcL confirms the monophyly of the class, the position of the Pinguiophyceae remains unresolved within the stramenopiles. As part of an Assembling the Tree of Life (ATOL) project the plastid genome of *Pinguiococcus pyrenoidosus* strain CCMP2188 was sequenced from total cellular DNA using a fosmid cloning approach. Chloroplast clones were identified from a fosmid library using end sequencing and then subject to multiple complete digestion (MCD) restriction fragment mapping. A minimal tiling path of five fosmids was determined and the chosen fosmids were shotgun sequenced, assembled and finished individually. Thus, the genome sequence was completed without the need for specific isolation of chloroplasts or chloroplast DNA, a critical advantage since these cells are small (4-6 µ;m in size) and contain a single chloroplast per cell. The chloroplast genome of *P. pyrenoidosus* is 114,670 bp and contains ~130 protein coding genes, including the 92 genes shared by the 7 stramenopile chloroplasts sequenced to date. The genome has a small inverted repeat containing only the ribosomal RNA operon. Of interest is an open reading frame of 583 amino acids with no homology to sequences in public databases. The chloroplast genome sequence provides numerous additional genetic markers to help resolve the phylogenetic relationships of the Pinguiophyceae within the stramenopile lineage. Both single gene and genome-based (gene order, rearrangement and loss) approaches to phylogenetic position of the Pinguiophyceae will be presented."
(a) School of Oceanography, University of Washington (b) Department of Immunology, University of Washington (c) Department of Biology, Lyon College (d) Department of Biology, University of Washington

P05026 "Using secondary structure of the 16S rRNA molecule and associated 16S-23S ITS to examine phylogenetic relationships of *Aulosira* (Nostocaceae, Cyanobacteria)."
Johansen, Jeffrey R.-presenter johansen@jcu.edu(a) Martin, Michael P. (a) Rehakova, Klara (b) Casamatta, Dale A. (c)
" *Aulosira bohemensis* Lukesova et al., a recently described taxon in the Nostocaceae, has an uncertain phylogenetic placement in the Nostocaceae based on analyses based on 16S rRNA gene sequence. While morphologically most similar to *Nodularia*, phylogenetic placement of *A. bohemensis* based on 16S rRNA was distant from that taxon. *Aulosira* actually falls at the base of the Nostocaceae clade, with possible sister taxa in *Trichormus, Mojavia* and *Nostoc*. Despite the variety of phylogenetic analyses performed, we were unable to obtain bootstrap support for its position in any tree. Secondary structure of both the 16S-23S internal transcribed spacer (ITS) and the 16S rRNA molecule was determined for several taxa in the basal Nostocales. Despite conservation of secondary structure in most helices, we found differences in structure informative for phylogeny."
(a) John Carroll University (b) Institute of Soil Biology (c) University of North Florida

P04019 Differential rate of divergence in mitochondrial and chloroplast genome sequences from geographically separate strains of *Heterosigma akashiwo*.
Jacobs, Michael A-presenter mikejac@u.washington.edu(a) Karol, Kenneth G (b) Zhou, Yang (a) Sims, Elizabeth H (a) Gillett, Will D (a) Cattolico, Rose Ann (a)

"Analysis of the complete chloroplast and mitochondrial genome sequences of the stramenopile *Heterosigma akashiwo* reveals significant genetic divergence between two geographically distinct strains (West Pacific: NIES 293, and West Atlantic: CCMP 453) of this toxic alga. Our data are in contrast to those obtained when common markers (rRNA and partial rbcL sequences), were used to elucidate population genetics. *H. akashiwo* chloroplast and mitochondrial genomes were sequenced by cloning into large insert vectors (fosmids) and finished to base-pair accuracy. Results show that the chloroplast genomes are closely conserved, with most variation found localized to intergenic regions. In contrast, the mitochondrial genomes, though similar in size (38.6 bp and 38.7 bp for NIES 293 and CCMP 452, respectively), contain many single nucleotide polymorphisms within genes as well as intergenically. The rate of evolution is inferred to be accelerated in mitochondria relative to that of chloroplasts for this taxon. Gene organization of the H. akashiwo mitochondrion is unique relative to other stramenopiles, although the encoded genes are common and include 34 known mitochondrial proteins, two rRNAs, (rnl and rns), and 25 tRNAs. Because fosmid clones are greater in length than that of a mitochondrial genomic unit, we have confirmed the multimeric physical structure of this organellar DNA. Phylogenetic analysis of 20 taxa using 10 genes placed *Heterosigma akashiwo* closest to the phaeophytes within the monophyletic clade of stramenopiles. The highly diverged regions of the mitochondrial genome are good candidates for use as makers to elucidate population genetics for the species."
(a) University of Washington (b) The New York Botanical Garden

P05004 "The recognition of *Caulerpa integerrima* (Zanardini) comb. et stat. nov. (Bryopsidales, Chlorophyta) from the Red Sea"
Wynne, Michael J.-presenter mwynne@umich.edu(a) Verbruggen, Heroen (b) Angel, Dror (c)
"Evidence based on both morphological and molecular data is presented to demonstrate that *Caulerpa freycinetii* C Agardh var. *integerrima* Zanardini, a name that has long been treated within the taxonomic synonymy of *C. serrulata* (Forsskal) J. Agardh, is to be resurrected and recognized at the species level as *C. integerrima* (Zanardini) comb. et stat. nov., for an alga thought to be endemic to the Red Sea. Three species in the genus with which it is superficially similar, *C. bartoniae, C. brachypus*, and *C. serrulata*, are separated from it using *tuf*A gene, complemented by morphological characters, including the entire margins and the 1.5-2.0 mm thickness of the assimilators. Another species also described from the Red Sea, *Herpochaeta* [*Caulerpa*] *requienii* Montagne, is also discussed and eliminated from consideration. Weber-van Bosse's assignment of this species as a forma of *C. racemosa* is accepted, namely, *C. racemosa* var. *lamourouxii* f. *requienii* (Montagne) Weber-van Bosse."
(a) Department of Ecology and Evolutionary Biology and Herbarium, University of Michigan, Ann Arbor, MI 48109, USA (b) Phycology Research Group and Center for Molecular Phylogenetics and Evolution, Ghent University, Krijgslaan 281, building S8, Gent, Belgium (c) Recanati Institute for Maritime Studies, University of Haifa, Mt Carmel, Haifa 31905, Israel

PB04021 The phylogeography of *Sargassum fusiforme* (Fucales, Heterokontophyta) in the northwestern Pacific
Cheang, Chi Chiu [(A)]; Chu, Ka Hou [(A)], Ang, Put O. [(A)]
Sargassum fusiforme is one of the most common brown macroalgal species in the Northwestern Pacific intertidal coast. This edible seaweed (mainly in the countries of East Asia) is of ecological and economical importance, attracting considerable efforts in studying its cultivation. This species also offers therapeutic function, e.g. antioxidant activity, which makes it a potentially profitable and promising target species for large scale mariculture in countries like China. It is amazing, however, that neither the genetic population structure nor the evolutionary history of this species has been investigated despite its ecological and economic significance. This study utilized the phylogeographic approach based on both the nuclear (ITS2) and cytoplamic (plastid Rubisco spacer and mitochondrial TrnW_I spacer) markers to assess the genetic diversity and the population structure of this species so that a better understanding of the genetic resources of the native populations could be obtained. Considerable genetic variations were demonstrated among the nine populations collected along the native ranges of this species, revealing two main lineages based on ITS2 and TrnW_I sequences. The geographical region associated with the genetic break between these two lineages was found to be situated in-between the eastern and southwestern Japan. The divergence of the lineages was suspected to be related to the formation of glacial refugia in this region during the Quaternary period.
(A): The Chinese University of Hong Kong

P04008 Phenotypic plasticity reconciles incongruous molecular and morphological taxonomies: *Macrocystis* is a monospecific genus
Demes, Kyle W-presenter kglenn@mlml.calstate.edu(a) Graham, Michael H (a) Suskiewicz, Thew W (a)
"The giant kelp genus *Macrocystis* C. Agardh (Laminariales, Phaeophyceae) is one of the worlds most ecologically and economically important seaweed taxa, yet its taxonomy remains uncertain. Although the genus currently contains four accepted species based on variable holdfast and blade morphology [*M. pyrifera* (Linnaeus) C. Agardh, *M. integrifolia* Bory de Saint-Vincent, *M. angustifolia* Bory de Saint-Vincent, and *M. laevis* C. Hay], numerous recent studies on *Macrocystis* interfertility, genetic relatedness, and morphological plasticity all suggest that the genus is monospecific. We reviewed this evidence and present an explanation for the extreme phenotypic plasticity that results in morphological variability within *Macrocystis*, driven by the effects of environmental factors on early development of macroscopic sporophytes. We propose that the genus be collapsed back to a single species, with nomenclatural priority given to *M. pyrifera*."
(a) Moss Landing Marine Laboratories

Start	End	Event	Location
11:00 AM	12:30 PM	**Algal Phylogenetics & Taxonomy-III** 11:00 - P05022: Michael Lynch - *Investigating deep phylogenetic relationships within the Rhodophyta by RNA secondary structure and nuclear gene sequence analysis* 11:15 - P05014: Sandra Lindstrom - *Contrasting phylogeographic patterns of two high intertidal dioecious species of Porphyra (Bangiales, Rhodophyta) in the northeast Pacific* 11:30 - P05055: Max Hommersand - *Molecular distance is correlated with biogeographic separation in marine red algae, whereas morphological variation is conditioned by environmental change, especially adaptations to different temperature isotherms* 11:45 - P05010: Craig Schneider - *Molecular investigations of foliose Rhodymeniophycidae (Rhodophyta) from Bermuda, western Atlantic*. 12:00 - P05003: Paul Geraldino - *Phylogenetic relationships within the genus Hypnea (Gigartinales, Rhodophyta) based on multigene data* 12:15 –P05042: Daniela Gabriel - *The red algal genus Titanophora (Schizymeniaceae, Nemastomatales) in the Gulf of Mexico*	Ni'ihau 312

P05022 Investigating deep phylogenetic relationships within the Rhodophyta by RNA secondary structure and nuclear gene sequence analysis

Lynch, Michael D.J.-presenter mdjlynch@sciborg.uwaterloo.ca(a) Cannone, Jamie J. (b) Gutell, Robin R. (b) Muller, Kirsten M. (a)

"Resolving supraordinal taxonomy within the Rhodophyta has proven problematic, and while the increasing availability of gene sequence data has resulted in the clarification of some of these relationships, much still remains unresolved. Furthermore, phylogenetic methods commonly applied to existing sequence data (e.g., RNA) often violate requirements for accurate phylogenetic reconstruction, reducing the efficacy of these methods. The objective of this study was to resolve non-monophyletic and poorly-supported taxonomy within the Rhodophyta through the application of structure-based empirical and parametric models of RNA evolution. Additionally, novel RNA secondary structure morphometrics were used to complement phylogenetic analyses and screen for elements of secondary structure defining taxonomic lineages (synapomorphic structural motifs). The incorporation of secondary structure information into phylogenetic analyses significantly increased resolution and phylogenetic support for higher-order branching across the Rhodophyta relative to previous studies. This increased resolution provided insights into ancient evolutionary events such as branching order and the taxonomic relationships among the unicellular Rhodophyta. Additionally, synapomorphic elements of nSSU rRNA secondary structure were observed. This work establishes the utility of analyzing secondary structure information of nuclear rRNA genes for resolving deep phylogenetic relationships. As a consequence of these results we propose taxonomic revisions to the Rhodophyta specifically reflecting higher-order phylogenetic relationships. Division, class, subclass and ordinal proposals are discussed. "

(a) Department of Biology, University of Waterloo (b) The Institute for Cellular and Molecular Biology, University of Texas at Austin

P05014 "Contrasting phylogeographic patterns of two high intertidal dioecious species of *Porphyra* (Bangiales, Rhodophyta) in the northeast Pacific"

Lindstrom, Sandra C-presenter sandracl@interchange.ubc.ca(a)

"*Porphyra pseudolanceolata* and *Porphyra schizophylla* occur on high intertidal rocky shores on exposed to semi-exposed coasts along the Pacific coast of North America. Both species first appear in winter, but *Porphyra pseudolanceolata* usually appears earlier and higher and also disappears sooner than *P. schizophylla*, which can persist through the summer months at some southern sites (both persist into summer near their western distribution limit in the Aleutian Islands). Sequencing of the *rbc*L gene of numerous isolates of both species throughout their geographic ranges indicates that both species contain cryptic species which have been confused under these names. These and other sequence data show that *Porphyra schizophylla* is restricted to California, where it ranges from Monterey County in the south to Mendocino County in the north. North of there, this species is replaced by *P. norrisii*, which extends north and west to at least Attu Island at the western end of the Aleutians, essentially the same distribution as *P. pseudolanceolata*. (This distribution is also exhibited by a number of other species of red algae.) Nested within the distribution of *P. pseudolanceolata* are at least two isolated populations of *P. hiberna*, originally described from the Monterey Peninsula. *Porphyra norrisii* shows significant phylogeographic structure, but *P. pseudolanceolata* does not."

(a) University of British Columbia

P05055 "Molecular distance is correlated with biogeographic separation in marine red algae, whereas morphological variation is conditioned by environmental change, especially adaptations to different temperature isotherms"

Hommersand, Max H-presenter hommersnd@bio.unc.edu(a)

"When geographical information is extracted from phylogenetic analyses of molecular data some biogeographic relationships appear to be correlated with vicariance events, whereas others are more readily explained by long distance dispersal. Analyses of rbcL sequences of red algae belonging to the subclass Rhodymeniophycidae are consistent with the hypothesis that time dependent separations of genera and species found in different biogeographic regions correlate with levels of rbcL base pair differences among related taxa, irrespective of the dispersal mechanism. By comparison, the type and degree of morphological change is related to species adaptations to differences in the environment that characterize different habitats. Differences in form or habit are sometimes seen over short periods of geological time with time measured as levels of base pair separation. On the other hand, differences in developmental morphology are associated with large base pair differences and are particularly striking when species have migrated from one temperature isotherm to another. Although changes in the rbcL gene should not influence morphogenesis directly, differences in the amino acid composition of the large subunit of RUBISCO are affected by temperature changes to a degree that corresponds to changes in developmental morphology. Examples that illustrate these observations will be taken from selected families of Rhodymeniophycidae including the Gigartinaceae, Sarcodiaceae, Halymeniaceae and Delesseriaceae."

(a) Univrsity of North Carolina

P05010 "Molecular investigations of foliose Rhodymeniophycidae (Rhodophyta) from Bermuda, western Atlantic."

Schneider, Craig W-presenter cschneid@trincoll.edu(a) Lane, Christopher E (b) Saunders, Gary W (c)
http://www.trincoll.edu/~cschneid/

"Using various combinations of the genetic markers COI, EF2, LSU and *rbc*L, we are assessing the taxonomic boundaries and phylogenetic affinities of foliose red algal specimens collected from the isolated warm temperate islands of Bermuda in the western Atlantic. Some are being assessed with specimens including collections from their type localities in Bermuda. Through our DNA analyses, we continue to uncover taxonomic problems, some in the form of cryptic species, but our collections have also included species completely new to science, which we characterize using both molecular and anatomical/reproductive features. On the other hand, some collections that look to be distinct species are, after sequence analysis, merely phenotypic variants displaying morphologies correlated with the environment from which they were collected. Examples of such morphological variability with genetic uniformity will be demonstrated using *Halymenia* and *Cryptonemia*. Our phylogenetic analyses indicate that two Bermudian species currently assigned to the genera *Kallymenia* and *Nemastoma* require assignment to other genera to correct their taxonomic position. We are also using gene sequences from local collections historically identified as species with European type localities (e.g., *Platoma cyclocolpum*) to compare with eastern Atlantic/Mediterranean specimen-derived sequences to assess whether or not they are truly conspecific."
(a) Department of Biology, Trinity College (b) Department of Biological Sciences, University of Rhode Island (c) Department of Biology, University of New Brunswick

P05003 "Phylogenetic relationships within the genus *Hypnea* (Gigartinales, Rhodophyta) based on multigene data"
Boo, Sung Min (a) Geraldino, Paul John L-presenter pj_huey@yahoo.com(a)
"Since the classification of the gigartinalean red algal genus *Hypnea* has been controversial, there is a need for a critical reassessment of species delimitation and phylogeny within the genus using extensively sampled collections, including those collected from the type localities. In this study, we determined *rbc*L sequences from 28 specimens, and 20 sequences from *cox*1, *psa*A and SSU genes representing 20 species of *Hypnea*. The phylogenetic trees of the four individual gene sequences were congruent. Two new species such as, *H. asiatica* sp. nov. and *H. caespitosa* sp. nov, were described here. *H. asiatica* occurs in Korea, Japan, and Taiwan and is distinguished by percurrent main axes, branches with abruptly curved adaxial branchlets, and the presence of lenticular thickening in the walls of medullary cells. *H. caespitosa* occurs in the warm waters of Malaysia, the Philippines, and Singapore and is characterized by a relatively slender main axis having a pulvinate growth habit, with entangled, anastomosing and subulate uppermost branches, and unilaterally borne tetrasporangial sori. All phylogenetic trees showed the distant relationship of *H. caespitosa* to *H. pannosa* from Baja California, as well as *H. asiatica* to *H. charoides* from Western Australia. The *rbc*L + *psa*A tree supported the monophyly of the genus with high bootstrap values and posterior probabilities and the recognition of three clades within the genus. The three clades correspond to the three sections, *Virgatae, Spinuligerae*, and *Pulvinatae*, originally proposed by J. Agardh in 1852, although exceptions are *H. japonica* in *Pulvinatae* and *H. spinella* in *Spinuligerae*. "
(a) Department of Biology, Chungnam National University

P05042 "The red algal genus *Titanophora* (Schizymeniaceae, Nemastomatales) in the Gulf of Mexico"
Gabriel, Daniela-presenter danielalgabriel@gmail.com(a,b) Fredericq, Suzanne (b)
"*Titanophora* (J. Agardh) Feldmann currently comprises nine species inhabiting tropical and subtropical waters worldwide. The genus is distinguished from other members of the Schizymeniaceae primarily by the calcification of the frond and the presence of an involucre surrounding the carposporophyte. The present study provides a characterization of four distinct *Titanophora* species that were dredged throughout the Gulf of Mexico. On the basis of comparative vegetative and reproductive morphology and chloroplast-encoded *rbc*L sequence analysis, a new record for the Gulf, *T. submarina* Bucher & J.N. Norris, and two species new to science in addition to *T. incrustans* (J. Agardh) Boergesen are recognized for the region."
(a) University of the Azores, Department of Biology (b) University of Louisiana at Lafayette, Department of Biology

Start	End	**Event**	Location
8:30 AM	10:15 PM	PSA Talks **Algal Ecology & Physiology** 8:30 – P06008: Raymond Lewis - *Reduced salinity increases oogenesis in various kelps (Order Laminariales)* 8:45 – P06004: Regina Radan - *Differential toxin response of Pseudo-nitzschia multiseries as a function of nitrogen source: batch and continuous cultures* 9:00 – P04005: Philip Bucolo - *Phototactic responses of swarmers of the Antarctic epiphyte Elachista antarctica* 9:15 – P04011: Kathryn Van Alstyne - *The release of dopamine by Ulvaria obscura and its allelopathic effects on algae and invertebrate larvae* 9:30 – P06006: Sarah Kiemle - *What do the cell walls of two primitive true taxa of the charophycean green algae, Chlorokybus atmophyticus and Klebsormidium flaccidum, tell us about the evolution of the land plant cell wall?* 9:45 - P06010: Graham Peers - *An ancient light harvesting protein is critical for the regulation of algal photosynthesis* 10:00 – P06002: Yingjun Wang - *LCIB: a novel gene family involved in the microalgal CO2-concentrating mechanism*	Lana'I 314

P06008 Reduced salinity increases oogenesis in various kelps (Order Laminariales)
Lewis, Raymond J.-presenter ray.lewis@wheaton.edu(a) Matthews, Benjamin J. (a) Chambers, Molly K. (a) Coburn, Melinda E. (a) Foxwell, Tyler J. (a)
"Lowering salinity has been reported to increase oogenesis in some species of kelp gametophytes. In this investigation, isolated female and male gametophytes of several species of kelps (Order Laminariales) were combined in ESNW seawater medium ranging from 12 to 33 psu salinity levels to determine the effects on oogenesis. Gametophytes of *Macrocystis pyrifera* produced the highest number of eggs at 23 psu salinity, with greatly decreasing oogenesis at lower and higher salinities. In *Costaria costata*, oogenesis was observed from 12 to 33 psu, with the highest numbers of eggs at 18-21 psu. Subsequent production of sporophytes showed the same pattern. Egg production of *Saccharina japonica* was highest at 24-27 psu, with about 85% of maximum egg production being observed at 18-21 psu. Oogenesis decreased at higher and lower salinities. Sporophyte production in this species showed the same pattern as egg production, indicating that egg production was the key determining factor for sporophyte production. Gametophytes of *Undaria pinnatifida* produced the greatest number of eggs at 12-15 psu, with high egg production continuing at higher salinities up to 27 psu, above which egg production dropped. This species had optimal sporophyte production at 21-27 psu, indicating something other than egg production was influencing sporophyte production at lower salinities. In all of these species, egg production was greater when the

salinity was reduced below the 30-33 psu salinities typically found in the habitats of these kelps."
(a) Department of Biology, Wheaton College

P06004 Differential toxin response of *Pseudo-nitzschia multiseries* as a function of nitrogen source: batch and continuous cultures
Radan, Regina L-presenter rlradan@hotmail.com(a,b) Cochlan, William P (a,b) Trainer, Vera L. (c)
"In natural marine systems, *Pseudo-nitzschia multiseries* will experience a variety of nutrient conditions including nutrient-replete, nutrient-limited and nutrient-starved environments depending on the geographic location and time of year. To understand how nitrogen sufficiency affects cellular growth and domoic acid (DA) production, we utilized batch and continuous culturing systems to emulate the range of in situ N availability expected during the development and decline of toxigenic diatom blooms. Duplicate, unialgal batch cultures of *P. multiseries* (culture strain NWFSC 245 collected from Sequim Bay, WA) were grown on NO_3^-, NH_4^+ or urea, and all three N substrates supported exponential growth equally well. Duplicate continuous culture experiments were set up using very similar concentrations of NO_3^-, NH_4^+ or urea as the sole N source for N-limited growth. Once cultures achieved steady state for 3-4 consecutive days, the chemostat cultures were harvested for analyses of toxin and dissolved nutrient concentrations. After harvesting, the remaining cells in the culture vessels were allowed to grow in batch mode without additional N amendment for approximately two cell generations and additional samples were collected for DA toxin analyses from the N-starved culture. At all three levels of N-sufficiency, although both inorganic and organic forms of N supported the growth of this pennate diatom, the urea-grown cells produced the highest amount of particulate DA per cell compared to those cells growing on either NO_3^- or NH_4^+. These results demonstrate that urea may play an important role in regulating the toxicity of developing coastal blooms, and needs to be considered when evaluating the potential effects of cultural eutrophication on the growth of harmful diatoms."
(a) Cellana LLC (b) Romberg Tiburon Center for Environmental Studies, San Francisco State University (c) NOAA Northwest Fisheries Science Center

P04005 Phototactic responses of swarmers of the Antarctic epiphyte *Elachista antarctica*
Bucolo, Philip-presenter apbucolo@uab.edu(a) Amsler, Charles D (a) McClintock, James B (a) Baker, Bill J (b)
"The endophyte/epiphyte *Elachista antarctica*, a filamentous brown alga found throughout the waters surrounding the Western Antarctic Peninsula appears to be restricted to one host macrophyte, *Palmaria decipiens*. *E. antarctica* swarmers released from unilocular and plurilocular sporangia are bombarded by many abiotic variables such as light. Light can significantly affect swimming behavior of released swarmers. Responses to light may influence dispersal and aid in detection of suitable settlement locations in marine microenvironments and may influence duration of motility. In this study we measured phototactic capabilities of *E. antarctica* swarmers after release. *E. antarctica* isolates collected near Anvers Island were cultured and their swarmers from plurilocular and/or unilocular reproductive structures were pipetted onto glass slides and imaged on a thermal stage using red light. A fiberoptic light source supplied white light from one side of the microscope stage providing treatments of 10, 80, and 200 µ;mol photons m^{-2} s^{-1} of irradiance. Control treatments were not treated with the white light. Phototactic movements of swarmers were determined using computer-assisted motion analysis software. Positive phototactic movements were significantly different than controls at medium and high light treatments. Swarmer taxis in low light treatments was not significantly different than their controls."
(a) University of Alabama Birmingham (b) University of South Florida

P04011 The release of dopamine by *Ulvaria obscura* and its allelopathic effects on algae and invertebrate larvae
Van Alstyne, Kathryn L-presenter kathy.vanalstyne@wwu.edu(a) Harvey, Elizabeth (a) Cataldo, Marianne (a) Salas, Amanda (a) Gifford, Sue-Ann (a) Nicely, Alexandra (a) Gehman, Alyssa (a) Chomiczewski, Lauren (a)
"The green alga *Ulvaria obscura* is common in blooms of ulvoid macroalgae in the northeast Pacific. *Ulvaria* is unique in that it produces copious quantities of the catecholamine dopamine, which is known to act as a feeding deterrent towards invertebrate herbivores. Anecdotal evidence suggests that dopamine can be released into the surrounding seawater where it may have allelopathic effects. To quantify the release of dopamine and its effects, we conducted both laboratory and field experiments. Dopamine release rates were measured in *Ulvaria* following re-immersion in seawater after 90 minutes of desiccation on a beach during a sunny day. Within 3 hours, tissue dopamine concentrations had declined by 90%. Dopamine concentrations in the surrounding seawater peaked after 1 hour, after which they declined as the compound was oxidized. Incubation in seawater containing dopamine at concentrations comparable to those we measured in the field experiment resulted in decreases in the germination of *Fucus distichus* ssp. *edentatus* zygotes, decreases in the growth of *Ulva lactuca*, and reductions in the survival of *Cancer magister* zoeae, but not the survival of *Cancer oregonensis* megalopae and juveniles."
(a) Western Washington University

P06006 "What do the cell walls of two primitive true taxa of the charophycean green algae, *Chlorokybus atmophyticus* and *Klebsormidium flaccidum*, tell us about the evolution of the land plant cell wall?"
Kiemle, Sarah N.-presenter snkiemle@mtu.edu(a) Domozych, David S. (b) Gretz, Michael R. (a)
"Charophycean green algae (CGA) represent a distinct group of morphologically diverse green algae, which are ancestral to land plants. Land plant cell wall (CW)polymers have been described from the CGA. *Chlorokybus atmophyticus* and *Klebsormidium flaccidum* are two taxa which reside at the phylogenetic base of the CGA and display two of the most simple morphological habits, the sarcinoid packet and unbranched filament. The wall polymers of these primitive CGA were examined to determine their role as indicators of plant CW evolution. The external region of the *C. atmophyticus* CW was labeled with the arabinogalactan protein (AGP)-directed monoclonal antibodies, JIM8 and JIM13. CWs were chemically fractioned and the hot water-(HW) and CDTA-soluble fractions contained large amounts of arabinose, galactose and uronic acid. Methylation analysis revealed 4-linked galactosyl, 2-linked arabinosyl, and 2,3,4-linked arabinosyl residues, which also suggested the presence of AGP. *K. flaccidum* HW- and CDTA-soluble fractions contained a predominance of arabinose, galactose, and glucose along with significant uronic acid content. The CW did not label with JIM13 or JIM8. The hemicellulose fractions of both species consist primarily of glucosyl residues. The 1M KOH soluble fraction of *C. atmophyticus* contained a predominance of 3-linked- with lesser amounts of 4-linked-glucosyl residues. *K. flaccidum* also contained 3-linked-glucosyl residues with very small amounts of 4-linked glucosyl residues. Mixed-linked glucans are believed to be important in cross-linking cellulose microfibrils in land plant CWs. Detailed chemical profiling of CW macromolecules in charophytes and lower green plants will be needed in order to accurately characterize evolutionary trends in CW structure and function."
(a) Department of Biological Sciences and Biotechnology Research Center, Michigan Technological University (b) Department of Biology, Skidmore College

P06010 An ancient light harvesting protein is critical for the regulation of algal photosynthesis
Peers, Graham-presenter gpeers@nature.berkeley.edu(a) Truong, Thuy B (a) Elrad, Dafna (b) Grossman, Arthur R (b) Niyogi, Krishna K (a)
"Light is a necessary substrate for photosynthesis, but its absorption by pigment molecules such as chlorophyll can cause severe oxidative damage

and result in cell death. The excess absorption of light energy by photosynthetic pigments has led to the evolution of protective mechanisms that operate on the time scale of seconds to minutes and involve feedback regulated de-excitation of chlorophyll molecules in photosystem II (qE). Despite the significant contribution of eukaryotic algae to global primary production, little is known about their qE mechanism, in stark contrast to flowering plants. The qE capacity of a model eukaryotic alga, *Chlamydomonas reinhardtii*, is dependent on environmental conditions and is inducible by growth in high light. We have characterized a non-photochemical quenching deficient mutant of *Chlamydomonas* (*npq4*) that lacks two of the three genes encoding LHCSR (formerly called LI818). Compared to wild-type cells, *npq4* has increased susceptibility to photoinhibition and reduced fitness when the light environment is rapidly switched from low to high fluxes. LHCSR is rapidly accumulated after a shift from low light to excess light conditions and its expression remains high even after weeks of growth in excess light conditions. The LHCSR protein is an ancient member of the light-harvesting complex superfamily, and homologs are found throughout photosynthetic eukaryote taxa, except red algae and vascular plants. LHCSR expression correlates with high light stress in the Prasinophyte algae, *Ostreococcus tauri*. We suggest that plants and algae employ different proteins to dissipate excess energy in a dynamic light environment."
(a) University of California, Berkeley (b) Carnegie Institution, Plant Biology

P06002 LCIB: a novel gene family involved in the microalgal CO2-concentrating mechanism
Wang, Yingjun-presenter wangyj@iastate.edu(a) Spalding, Martin (a)
"Microalgal photosynthesis accounts for a significant portion of the carbon capture and sequestration on earth and influences biomass production and the global environment. Many microalgae species possess a CO_2-Concentrating Mechanism (CCM) to cope with the limited CO_2 supply in their natural habitats. The recent observation that a lesion in the *LCIB* gene in Chlamydomonas directly affects internal C_i accumulation demonstrates the important role played by its gene product, LCIB, in the Chlamydomonas CCM (Wang and Spalding, 2006). BLAST searches revealed three additional homologues of *LCIB* in the Chlamydomonas genome, *LCIC*, *LCID* and *LCIE*. Multiple lines of evidence suggested that LCIB and LCIC form a complex: Western blots reveal that LCIC, even though actively transcribed, is down-regulated at the protein level in the *LciB* mutants, *ad1* and *pmp1*; Screening of LCIB-interacting proteins by yeast two-hybrid system revealed an apparent strong LCIB-LCIC interaction; and LCIC was co-purified with LCIB by *in vivo* affinity purification. Immunofluorescence examination indicated a plastid localization of LCIB/LCIC, which is consistent with the prediction of N-terminal plastid signal peptides in these proteins. Re-localization of LCIB in chloroplasts upon changing CO_2 concentration also was observed. We propose that LCIB is an integral component of a multi-subunit plastid complex, and that its function relies on its interaction with other proteins. Based on the currently published genome sequences, *LCIB*-like genes also exist in several other species of green microalgae, cyanobacteria and bacteria, suggesting an ancient origin of the LCIB gene family and its widespread occurrence in microorganisms. "
(a) Iowa State University, The Department of Genetics, Development and Cell Biology

Start	End	Event	Location
8:30 AM	10:30 AM	PSA Talks **Algal Cellular & Molecular Biology** 8:30 –P02005: John La Claire - *Gene expression profiling growth stages of Prymnesium parvum (Haptophyta) in culture and in nature* 8:45 – P07002: Alejandra Gonzalez - *Morphometric and molecular studies identify cryptic species in the Lessonia nigrescens complex along the chilean coast* 9:00 – P02006: Rose Ann Cattolico - *Chloroplast genomes, genes and gene expression in two strains of Heterosigma akashiwo- a bloom forming alga.* 9:15 – P07001: Megan Black - *Heterosigma akashiwo world-wide population diversity* 9:30 – P07003: Michelle Casanova - *Can we use vegetative morphology for species determination in Nitella (Characeae, Charophyceae)?* 9:45 – P02002: Nedeljka Rosic - *Gene expression analysis and housekeeping genes selection for real-time RT-PCR in symbiotic dinoflagellates during thermal and light stress* 10:00 – P02003: Yusuke Matsuda - *Modeling of structure of CO2 responsive promoter in the marine diatom Phaeodactylum tricornutum.* 10:15 – P04006: Su Yeon Kim - *Molecular approach for the current distribution pattern of Pterocladiella capillacea (Gelidiales, Rhodophyta)*	Moloka'I 315

P02005 Gene expression profiling growth stages of *Prymnesium parvum* (Haptophyta) in culture and in nature
La Claire, John W-presenter laclaire@uts.cc.utexas.edu(a)
"*Prymnesium parvum* blooms frequently produce the potent ichthyotoxins, prymnesin-1 and prymnesin-2. There are currently no simple *in vitro* assays for these toxins, because purified prymnesin standards are not yet available. An alternative approach is to correlate gene expression patterns and levels with the presence or absence of toxins and with the different stages of bloom development known to be toxin-producing. This information will allow us to unravel the timing of toxin production by a bloom and to develop molecular biomarkers for toxic vs. non-toxic bloom stages. To examine gene expression broadly, DNA microarrays were printed from a suite of 3,500 oligonucleotides, representing 3,415 genes that are expressed by *P. parvum*. Messenger RNA was purified from axenic cultures at four different phases of growth. After amplification and fluorescent labeling, RNA was hybridized to microarrays for pair-wise comparisons. Approximately 1,055 genes exhibited statistically-significant differential expression at the mRNA level ($p \leq 0.001$) over the four growth phases (early, mid-, late and post-log). Approximately 715 of these exhibited 2-fold or greater up- or down-regulation in one or more of the stages. The identities of ~275 of these genes are known through homologous sequence searches of gene databases. Among these are a few that encode proteins believed to be involved in prymnesin biosynthesis. Custom subsets of differentially-expressed genes were characterized further to determine their suitability as biomarkers for the individual growth stages and for distinguishing toxic vs. non-toxic blooms at the transcipt level. Biomarker genes will provide valuable molecular tools for analyzing the growth and toxin-synthesizing status of naturally-occurring *P. parvum* blooms."
(a) University of Texas at Austin

P07002 Morphometric and molecular studies identify cryptic species in the *Lessonia nigrescens* complex along the chilean coast
Alejandra, Gonzalez-presenter apgonzalez@uchile.cl(c) Jessica, Beltran (a,b) Juan, Correa (a,b) Bernabe, Santelices (a,b)
"The kelp *Lessonia nigrescens* Bory has a wide distribution along South-Eastern Pacific, from 12 to 55 LS. A marked genetic discontinuity within the species occurs between 30 and 32LS suggesting the hypothesis that two cryptic, allopatric species could be included in the presently accepted range

extension of the species. To test this hypothesis, we studied 26 morphological characters in 4 populations of *L. nigrescens*, two of them located to the north and two of them the south of 30-32LS. Simultaneously, we used three neutral markers (atp8/ITS1/Rubisco spacer) to evaluate genetic similarities. Our results evidenced two groups of populations distinguishable morphologically and genetically. Morphologically, both population groups are statistically identifiable by a combination of six anatomical characters. Genetically, the two population groups exhibited high level of divergence among them, equivalent to that found between them and other closely related sympatric species of Lessonia. In light of our results, we suggest that the population groups correspond to two cryptic species. The nomenclatural changes arising from this study will have to wait for additional research. For the time being, we propose the name *L. nigrescens* to be used for the populations located south of the 30-32LS and *Lessonia* sp. be used for the populations located to the north of that latitude. Support FONDAP 15010001-1 P.7 "
(a) Pontificia Universidad Catolica de Chile (b) Center for Advanced Studies in Ecology and Biodiversity (c) Universidad de Chile

P02006 "Chloroplast genomes, genes and gene expression in two strains of *Heterosigma akashiwo*- a bloom forming alga."
Cattolico, Rose Ann-presenter racat@u.washington.edu(a) Deodato, Chloe R (a) Rocap, Gabrielle (a) Jacobs, Michael A (a)
"*Heterosigma akashiwo* (Raphidophyceae) chloroplast genomes for both NIES 293 (West Pacific: 159,370kb) and CCMP 452 (West Atlantic: 160,149kb) strains have been sequenced. Architecturally both genomes contain a large (~20kb) repeat that defines the isomerization boundaries of the molecule. The multiple smaller tandem and inverted repeats found in these genomes appear to be located in a gene-specific manner when compared to placement in other raphidophyte chloroplast genomes, suggesting a functional constraint. Genes have been identified in *H. akashiwo* that have not been observed in any other chloroplast genome sequenced to date (e.g., *tyrC*: a tyrosine recombinase), some that may be unique to raphidophytes (006/062: a putative G protein-coupled receptor) and those rarely encoded in non-rhodophytic taxa (*tsg1/trg1*: a two-component signal transduction array). Both synonymous (30) and non-synonymous (36) single nucleotide polymorphisms are found in 35 of the 197 encoded chloroplast genes. Quantitative PCR analyses show that NIES 293 and CCMP 452 cultures grown under identical conditions exhibit distinct differences in gene expression when 20 genes representing photosynthetic, Calvin cycle, energy generation or metabolic function are examined. These data suggest that genetic identity may significantly influence the population fitness of this bloom-forming organism. "
(a) University of Washington

P07001 *Heterosigma akashiwo* world-wide population diversity
Black, Megan M.D.-presenter mmdblack@u.washington.edu(a) Deodado, Chloe (a) Jacobs, Michael (c) Hardin, William R. (a) Cattolico, Rose Ann (a,b)
"The raphidophyte alga, Heterosigma akashiwo is a single species with a cosmopolitan distribution. Five genes NAD2, NAD4, NAD5, NAD7, and COX1 in the mitochondrial genome reveal significant genetic variation with at least nine different genotypes. These genes have 115 SNPs out of 7648bp; most of the base pair changes code for synonymous amino acids. High variation in the mitochondria is in contrast to the lack of variation within the rDNA, but is consistent physiological differences observed between strains. Fourty-eight strains were sequenced, including subclones separated by thirty and ten years in culture that showed no change indicating that these genes are stable when not under selective pressure. The biogeography of the genotypes reveals that there has been extensive transport of cells around the world. "
(a) Biology Department, University of Washington (b) Oceanography Department (c) Genome Center

P07003 "Can we use vegetative morphology for species determination in *Nitella* (Characeae, Charophyceae)?"
Casanova, Michelle T-presenter amcnova@netconnect.com.au(a) Karol, Kenneth G (b)
"In any algal group the most useful traits for identification of species by non-experts are gross morphological features. Family Characeae (stoneworts, or charophytes) has a long taxonomic history, and a number of good morphological features that can allow identification to genus, section and subsection with few problems. In most areas of the world the majority of species are easily distinguished by local workers, however, it is usual for one or more (evolving) species groups to present taxonomic problems. In Australia one of these groups was called *Nitella pseudoflabellata* by R.D.Wood. In the genus *Nitella* recent taxonomic work has focused on the value of oospore morphology and nucleotide sequences for determining species and relationships among species. However, oospore morphology (best seen using scanning electron microscopy) and nucleotide sequences are features that are not accessible to most workers in plant identification or ecology. So, can we create a useable taxonomy based on the most discriminating features of *Nitella* species (i.e. oospore morphology and nucleotide sequences), then use aspects of gross morphology to create a key? In this study several species within the *Nitella pseudoflabellata* A. Braun complex are examined in relation to all three kinds of traits: oospore morophology, nucleotide sequences and vegetative morphology, in order to determine the most useful vegetative features for species discrimination. The results confirm that those vegetative characters used by early taxonomists (e.g. number of furcations, length and number of dactyls) are valuable for distinguishing among genetically distinct entities within this group. "
(a) Royal Botanic Gardens Melbourne (b) The New York Botanic Garden

P02002 Gene expression analysis and housekeeping genes selection for real-time RT-PCR in symbiotic dinoflagellates during thermal and light stress
Rosic, Nedeljka N-presenter n.rosic@uq.edu.au(a) Rodriguez-Lanetty, Mauricio (a) Pernice, Mathieu (a) Hoegh-Guldberg, Ove (a)
"Mapping the stress response of reef-building corals and their symbiotic dinoflagellates (genus *Symbiodinium*) is important in our attempt to understand the influence of global climate change on the world's most biodiverse marine ecosystem. We exposed cultured *Symbiodinium* (clade C) to a range of environmental stresses that included elevated temperatures (29^{o}C and 32^{o}C) under high (100 µ;mole quantum/m^{2}/s Photosynthetic Active Radiation) and low (15 µ;mole quantum/m^{2}/s) irradiances. Using microarray technology, we assessed the response of 3072 expressed sequence tags (ESTs) and were able to identify a series of gene candidates involved in the stress response. High temperature (32^{o}C) and increased light level effected expression of genes such as monoamine oxidase, heat shock proteins, sterol regulatory element binding transcription factor and Tob1. Variation in the light levels alone altered expression of genes encoding carbonic anhydrase, G protein beta subunit-like and photosynthetic genes peridinin chlorophyll-a binding protein. We also used real-time RT-PCR to test the stability of expression of a number of housekeeping genes (HKGs) in cultured *Symbiodinium* under thermal and light stress. Among the twelve analyzed HKGs, S-adenosyl methionine synthetase, S4 ribosomal protein, Cox1, Cyclophilin and calmodulin showed the most stable pattern of expression. Finally, we analysed the expression of Hsp90 by real-time RT-PCR analysis using the most stable HKGs as a reference. As expected, Hsp90 expression levels were increased by increased temperature and further boosted when thermal stress was combined with high irradiance. To conclude, the HKGs presented here will be a useful reference in future studies of gene expression in symbiotic dinoflagellates. "
(a) Centre for Marine Studies, University of Queensland, Australia

P02003 Modeling of structure of CO_2 responsive promoter in the marine diatom *Phaeodactylum tricornutum*.
Matsuda, Yusuke-presenter yusuke@kwansei.ac.jp(a) Inoue, Takuya (a) Yamashiki, Ryosuke (a) Yoshida, Satoshi (a)

"Marine diatoms (Bacillariophyceae) play a major role in photosynthetic fixation of inorganic carbon on the earth. Their CO_2 acquisition is supported by active uptakes of CO_2 and bicarbonate, and subsequent flux controls of influent Ci. This system is termed as CCM. It is also proposed in some diatom species that a C_4-like pathway may also contribute to CO_2 supply to Rubisco. Flux of intracellular Ci is controlled by carbonic anhydrases (CAs). A marine diatom *P. tricornutum* possesses two chloroplastic beta CAs and the transcription of one of these CA genes (*ptca1*) is repressed under high CO_2 condition. The promoter region of *ptca1* (P*ptca1*) was known to be composed of at least three cAMP response elements, which are highly homologous to CRE, P300, and Skn1 motifs. However, it was revealed in the present study that new three tandem motif, CCRE1-3 play a key role in CO_2 response of the P*ptca1*. Since these CO_2-responsive cis-elements were the typical cAMP responsive sequence, cAMP related transcription factors potentially target the CCREs were searched in the genome database of *P. tricornutum*. Eight genes which belong to the CREB/ATF family were retrieved from the database and were cloned from *P. tricornutum* cDNA library. These 8 cDNAs were expressed as His-tag protein in *E. coli* and successfully solubilized 3 products were purified by Ni-sepharose chromatography. Gel-shift assay carried out with these products and tandem repeat sequence of the labeled CCRE demonstrated that 3 CREB/ATF proteins in *P. tricornutum* specifically bound to CCRE sequence. The required structure of promoter to respond to the ambient CO_2 in a marine diatom based upon interactions among cAMP, CCREs and CREB/ATF family proteins will be discussed."
(a) Department of Bioscience, Kwansei-Gakuin University

P04006 "Molecular approach for the current distribution pattern of *Pterocladiella capillacea* (Gelidiales, Rhodophyta)"
Kim, Su Yeon-presenter poop95@naver.com(a) Boo, Sung Min (a)
"*Pterocladiella capillacea* has rhizoidal filaments produced from the cortical cells and later abundant in the medullar layers, which are sources for marine pulp developed by a commercial company in Korea. The species is a suitable taxon for studying phylogeography of marine organisms since it commonly occurs in temperate to tropical waters in the world. In order to understand its current distribution pattern, we collected more than 200 samples in Korea, Japan, New Zealand, Ireland, France, Canary Island, and USA. Two molecular markers were selected; plastid *rbc*L for recognizing the species and *cox*1for analyzing haplotypes. A total of 10 haplotypes from 67 specimens including published data of samples from Australia, Japan, and Venezuela were found. An identical haplotype occurred in Mediterranean sea (three sites), Australia (one site), and New Zealand (two sites). In *cox*1 gene, nineteen haplotypes were found in 75 specimens of the species. The haplotype analysis produced biogeographical structure. The same haplotype occurred in New Zealand and Canary Island. These results indicate both scenarios of distribution; recent dispersal of some haplotypes by human-mediated activities and historical migration of the species. On-going analysis of the specimens will give us a better understanding of the phylogeography of the species. "
(a) Department of Biology, Chungnam National University

Start	End	**Event**	Location
11:00 AM	12:45 PM	**Applied Phycology-II** 11:00 – P01009: Zackary Johnson - *Isolation and characterization of marine phytoplankton as a next generation biofuel* 11:15 – P01008: Lisa Pickell - *Mid-scale screening of marine phytoplankton for large scale production of biofuel* 11:30 – P02001: Rachel Miller - *Oil biosynthesis in chlamydomonas reinhardtii* 11:45 – P01004: Eugene Zhang - *Characterisation of australian microalgae for biodiesel production* 12:00 – P02007: Todd Lane - *Digital transcriptomic analysis of silicate starvation induced triacylglycerol* 12:15 – P06011: Skye Thomas-Hall - *Analysis of lipid accumulation in microalgae* 12:30 – P04016: Charles Yarish - *Multi-Component Evaluation to Minimize the Spread of Aquatic Invasive*	Moloka'I 315

P01009 Isolation and characterization of marine phytoplankton as a next generation biofuel
Johnson, Zackary I-presenter zij@hawaii.edu(a) Bidigare, Robert R (a) Brown, Susan L (a) Bruyant, Flavienne (b) Cochlan, William (c,d) Cullen, John J (b) Huntley, Mark E (a,d) Redalje, Donald G (e) de Scheemaker, Gabriel (f)
http://www2.hawaii.edu/~zij
"Global energy demands continue to rise, but global fossil fuel production is struggling to keep up. Further, fossil fuels contribute to the increase in global atmospheric carbon dioxide and are a major driver of global climate change. To address these concerns, Cellana BV is pursuing the use of marine phytoplankton as a next generation, low carbon-profile biofuel. Marine phytoplankton have three major advantages over other biofuels including (1) they do not use freshwater (2) they do not require arable land and (3) phytoplankton can grow much faster than traditional plant-based biofuels. Working with Cellana, our group has isolated phytoplankton strains from diverse locations and some have significant lipid content. Using an efficient experimental strategy, we have analyzed promising candidates from this collection and discovered a wide range of growth rates and lipid compositions in conditions simulating environmental scenarios associated with commercial applications. Here we describe our screening process and show that some phytoplankton have characteristics that make them excellent candidates for commercial-scale production suggesting that marine phytoplankton are the leading contenders for large scale production of next generation biofuels."
(a) University of Hawaii (b) Dalhousie University (c) Romberg Tiburon Center for Environmental Studies, San Francisco State University (d) Cellana LLC (e) The University of Southern Mississippi (f) Cellana BV

P01008 Mid-scale screening of marine phytoplankton for large scale production of biofuel
Pickell, Lisa D-presenter lisa.pickell@cellana.info(a) Pollard, Michael (a) Herndon, Julian (a) Cochlan, William P (a) Huntley, Mark E (a)
"A multi-stage, mid-scale culturing system was constructed to evaluate and contrast the growth characteristics of various marine phytoplankton species under simulated large-scale cultivation conditions for future use in biofuel production. Screening at the mid-scale level permits optimization and characterization of growth rates, macronutrient dynamics, and inoculation conditions for use in large-scale culturing systems, but with a substantial reduction in time, operating costs and resources. Here we describe the design and operation of a microalgal screening system, where experiments were conducted simultaneously in 24, pH-controlled, aerated 50-L polycarbonate culture vessels under ambient light and temperature conditions. In the simulated photobioreactors (PBRs), each algal species was initially grown as triplicate batch cultures for characterization of growth rates and nutrient utilization (4-5d), followed by inoculation into corresponding open pond simulators; these cultures were subsequently harvested after 2-3 days of N starvation. Prior to pond inoculation, the PBRs were conducted as semi-continuous cultures, and diluted daily with enriched seawater media to maintain constant biomass and exponential growth over extended periods. Monitoring (2-3 d^{-1}) of optical density, *in vivo* cellular fluorescence capacity (F_v/F_m), chlorophyll *a*, particulate carbon and nitrogen, and dissolved macronutrients permitted accurate assessment of

physiological health and degree of N sufficiency in both pond and PBR culture simulators. This novel mid-scale screening approach demonstrates high precision estimation of growth, nutrient drawdown and dilution rates, and provides an effective platform for selection of ideal phytoplankton candidates for large-scale production of biofuels."
(a) Cellana LLC

P02001 Oil biosynthesis in chlamydomonas reinhardtii
Miller, Rachel-presenter mill1663@msu.edu(a,b) Moellering, Eric (a,c) Li, Xiaobo (a,c) Vieler, Astrid (a,d) Benning, Christoph (a,d)
"With the rising cost of fuel, many are looking to biofuels as an alternative. One possible feedstock for biofuels is oil produced by microalgae. Microalgae produce significant amounts of triacylglycerol (TAG), often in response to environmental stress, which can be harvested and converted into biodiesel. In order to make microalgal biodiesel economically viable, more needs to be known about how TAG is synthesized, and how the process is regulated. Towards this end, we have initiated several approaches to study TAG synthesis in the model green alga *Chlamydomonas reinhardtii*. We used 454 sequencing to analyze global transcript levels in *C. reinhardtii* under normal and TAG-inducing conditions. This has led to the identification of several putative transcription factors that may regulate the synthesis of TAG. The role of these putative transcription factors is being confirmed by further analysis of transcript levels, and by modified expression in *C. reinhardtii*. In a more focused approach, we are looking for diacylglycerol acyltransferases (DGATs), which catalyze the final step in TAG synthesis. Several putative DGATs have been identified based on sequence similarity to plant DGATs, and they are being characterized by expression in yeast. These approaches, combined with the results of other research in the Benning lab, will provide the basis for the engineering of TAG biosynthesis in microalgae. This research is supported by funding from the Air Force Office of Scientific Research. "
(a) Michigan State University (b) Cellular and Molecular Biology Program (c) Dept. of Energy Plant Research Laboratory (d) Department of Biochemistry

P01004 Characterisation of australian microalgae for biodiesel production
Zhang, Eugene-presenter e.zhang@uq.edu.au(a,b) Thomas-Hall, Skye (a) Timmins, Matthew (a) Oey, Melanie (a) Kruse, Olaf (c) Hankamer, Ben (b) Schenk, Peer (a)
http://www.solarbiofuels.org
"Microalgae provide a promising potential to address the current global energy demands and climate changes associated with increasing CO_2 levels. The ability for microalgae to photosynthesise and convert CO_2 into organic compounds such as triacylglycerides as a potential source for biodiesel production make them a prime candidate for further study and development. Under certain conditions, various types algae are able to produce and accumulate high amounts of intracellular lipids suitable for biodiesel development. This talk will present the results from various promising new green algal specimens isolated from Australian waterways. The efficiency of photosynthesis has been significantly improved by modification of antennae pigments. An assessment of the potential of these strains as biodiesel producers is presented."
(a) University of Queensland, School of Biological Sciences (b) University of Queensland, Institute of Molecular Bioscience (c) University of Bielefeld

P02007 Digital transcriptomic analysis of silicate starvation induced triacylglycerol formation in the marine diatom *Thalassiosira pseudonana*.
Lane, Todd W-presenter twlane@sandia.gov(a) Pawate, Ashtamurthy (a) Linquist, Erika D (b) Kirton, Edward S (b) Lane, Pamela D (a) Simmons, Blake A (a)
"The completion of the sequencing of two marine diatom genomes, *Thalassiosira pseudonana* 3H and *Phaeodactylum tricornutum* CCAP1055/1 by the Joint Genome Institute (JGI), the continuing development of systems for genetic manipulation, and the overall advances in Molecular Biology and Biotechnology will allow a more systematic approach to understanding and manipulating the ability of these organisms and other microalgae to form triaclyglycerols (TAGs). In a previous study we characterized the ability of these strains to produce TAGs in response to nitrate and silicate starvation. To extend this work we have, in collaboration with the JGI, conducted a digital transcriptomic analysis by ultra high throughput sequencing (UHTS), of gene expression in *T. pseudonana* in during the onset of TAG accumulation in response to silicate starvation. We have conducted a 65 hour timecourse experiment, measuring increase in cell number and estimated relative TAG abundance in a silicate limited culture. We have created RNA libraries from four timepoints during the timecourse for transcriptomic analysis; one taken during logarithmic growth phase and three at intervals during the transition from logarithmic phase to stationary phase and the onset of triacylgycerol accumulation. We carried out a two Illumina UHTS runs per library for a total 49.8 million reads. 95% of these reads were alignable with the genome. Approximately 9600 expressed sequence tag (EST) clusters that aligned to existing gene models were identified. An additional 8000 EST clusters were identified that did not align to existing models. We have utilized this data estimate changes in transcript abundance, in response to silicate starvation, of the genes encoding the metabolic pathways resulting in TAG formation. "
(a) Sandia National Laboratories (b) Joint Genome Institute

P06011 Analysis of lipid accumulation in microalgae
Thomas-Hall, Skye R-presenter skye.thomas-hall@cellana.info(a) Paul, Blair M (a) Stone, Thomas V (a) Brown, Susan L (b,a) Johnson, Zackary I (b,a) Bidigare, Robert R (b,a) Cochlan, William P (a) Huntley, Mark E (a)
"Microalgae have long been recognized for their potential to produce hydrocarbons suitable for use as biofuels. The great attraction of algae is that their potential biofuel production capacity far exceeds that from land-based crops. However, at present there is a major discrepancy between predicted hydrocarbon yields based on laboratory experiments and the measured hydrocarbon yields of large-scale algal production systems. Algae possess a variety of lipid types ranging from the less commercially useful membrane-bound polar lipids to large droplets of unbound triacylglycerols (TAGs) which are of prime interest as a biofuel source. Identifying those algal species capable of producing large stores of readily extractable neutral lipid droplets is the first step in the development of algal biofuels as an economically viable fuel source. Identification of algal strains with high lipid content can be achieved quickly by utilizing the polarity-sensitive Nile Red stain and visualization by microscopy or semi-quantification by spectophotometry. Nile Red fluorescence can also be utilized for tracking lipid production as a function of time, with the caveat that this stain only approximates lipid content, results need to be verified with standard gravimetric methods. Cellana LLC's prime objective is to realize and commercialize microalgal systems for the production of biofuels, animal feedstock and carbon sequestration. To achieve this goal we are screening numerous algal species, optimizing the algal production system and analyzing growth and lipid productivity rates in near real-time. Presented will be an overview of our production system with a specific focus on the analysis of lipid accumulation in algal species grown in 50,000 L batch ponds. "
(a) Cellana LLC (b) University of Hawaii

P04016 "Multi-Component Evaluation to Minimize the Spread of Aquatic Invasive Seaweeds and Harmful Algal Bloom Microalgae via the Live Bait Vector, *Ascophyllum nodosum*, into Long Island Sound "

Yarish, Charles-presenter charles.yarish@uconn.edu(a) Haska, Christina (a) Lin, Senjie (a) Kraemer, George P (b)
http://www.stamford.uconn.edu/profile_YarishCharles.htm

"The ecological and economic health of Long Island Sound (LIS) is currently threatened by introductions of invasive species. To mitigate the risk of introducing non-native species, potential vectors must be evaluated. This project has investigated *Ascophyllum nodosum* packaging that accompanies bait worms as a vector of macroalgae and harmful microalgae to LIS. Bait was purchased from retail establishments at locations ranging from northeastern Long Island Sound along the Connecticut shoreline to the southwestern part of the Sound in Nassau County, New York. Using a combination of visual and microscopic inspection, and molecular biological techniques to detect the presence of macro- and micro-algal cells, the study questioned whether (i) non-native organisms were being imported via bait worms, and if so whether; (ii) non-native organisms vary according to purchase location, or; (iii) time of year. Overall, 14 species of macroalgae and two species of harmful microalgae (*Alexandrium fundyense*, and *Pseudo-nitzschia multiseries*) were discovered among the *A. nodosum*. The Gulf of Maine now harbors a diverse suite of non-native organisms. These may be exported to other areas of the U.S. via national bait wholesalers and cause ecological harm to the receiving ecosystem. In addition to potential ecological impacts associated with the import of non-native organisms, economic harm is also possible. For example, commercial shellfish beds may be closed to harvesting when harmful microalgae bloom in coastal waters. With ca. 470 retail bait shops in NY and CT, the chances of introduction of harmful non-natives is not trivial. For example, during our 18 month study of four locations, we discovered the harmful non-native microalga *Pseudo-nitzschia multiseries* in 58% of our samples. "

(a) University of Connecticut (b) Purchase College, State University of New York

Start	End	**Event**	Location
2:30 PM	4:10 PM	**Minisymposium 1: Education Outreach - Evolution & Innovation in Plant Biology Outreach for Elementary, Community College, Undergraduate, & Professional Science Educators** - Chair: Jane Ellis 2:30 - M0101: Jeremy Pritchard - *Simple but Dangerous ideas; strategies to help in teaching evolution* 2:55 - M0102: Christina Reynaga-Pena - *Development and assessment of didactic packages including DVDs on plant biology experiments for rural schools in Mexico* 3:20 - M0103: Kabi Neupane - *Advances in Biosciences Education for Community Colleges: The journey from summer workshop to year-round independent research project* 3:45 - M0104: Erin Dolan - *Undergraduate-level Inquiry: Benefits and challenges of engaging in classroom-based research*	Lana'I 314

M0101 Simple but Dangerous ideas; strategies to help in teaching Evolution

Pritchard, Jeremy-presenter J.pritchard@bham.ac.uk(a,b)
http://www.biosciences.bham.ac.uk/links/teachers

"Darwins theory of Evolution by Natural Selection is the most important tool used by the modern Biologist, but despite its simplicity, it is often misunderstood. This talk presents a historical perspective to the development of Darwins ideas as a useful and objective tool for pedagogical purposes. Charles Darwin was not the first to think about Evolution but he was the first to get it right. Ideas about Evolution can be traced back the ancient Greeks and beyond, but the development of ideas about changes in plant and animals has often been seen as dangerous, conflicting with the social and religious dogma of the times. Charles Darwin was implicitly and explicitly aware of the impact of his ideas. In refining his theories he drew on influences from his education and family background in the West Midlands and beyond. Training and engagement of all students at high school and in the initial stages of their University education is of central importance, whether they are molecular biologists or ecologists. A range of resources are being designed to directly address specific evolutionary issues including counter-views The talks illustrates the strategy of taking an historical perspective to diffuse subjective critiques, uses human evolution to provide immediate context and includes demonstration of a computer resources that demonstrates evolution of complexity by random variation. It is hoped that this talk will provide an opportunity for comparing UK, US and other countrys experiences and help exchange idea of good practice. A range of associated resources are freely available on the web (http://www.biosciences.bham.ac.uk/links/teachers/teachers.htm). "

(a) Univerity of Birmingham (b) Society of Experimental Biology

M0102 Development and assessment of didactic packages including DVDs on plant biology experiments for rural schools in Mexico

Reynaga-Pena, Cristina G.-presenter creynaga@ira.cinvestav.mx(a) Valderrama-Chairez, Maria L. (b) Tiessen, Axel (a)
http://www.sientelaciencia.com

"An important number of public elementary schools in Mexico are schools in rural areas, where didactic resources are very limited. This work intends to facilitate the first scientific experience of children in those schools to diverse topics such as plant and cell biology among other. Currently, there is a great need for the development of didactic materials for teaching science to vulnerable populations in Mexico such as children in rural areas. In the context of our present education and outreach activities we have implemented a 3-day workshop for children of rural areas, where we include a section on Plant Biology. Most experiments were chosen by the precondition of needing only easily available materials. We have included experiments on plant cells, photosynthesis, metabolism, tropisms, DNA extraction, plant pigments and pH determination. From our experience, the presentation of experiments by a competent scientist usually gives the student a different perspective, encouraging them to continue doing experiments in the long term. So, to multiply and expand the experience, we are videotaping and editing a series of DVDs that will be distributed among all possible schools to help the rural school teacher. Currently, we are about to deliver our first DVD in 1500 schools of the State of Guanajuato, Mexico. The impact on the children's attitude to science as well as the utility for the teacher as a didactic resource will be evaluated."

(a) Centro de Investigacion y de Estudios Avanzados del IPN Unidad Irapuato (b) CUCBA, Universidad de Guadalajara

M0103 Advances in Biosciences Education for Community Colleges: The journey from summer workshop to year-round independent research project

Neupane, Kabi R-presenter kabi@hawaii.edu(a) Lum, Jamie M (a,b) Messa-Oh, Christine (a,b) Perez, Pierriden A (b) Christopher, David A (b)

"The Advances in Biosciences Education (ABE) workshop was formed to maximize broader impacts under a NSF-sponsored research grant entitled 'Functional Genomics of the Protein Disulfide isomerase Family in Arabidopsis Plants.' Five faculty and 24 students from four community colleges lacking research programs received hands-on training with experimental materials and techniques employed in the ongoing research grant. The

three-week long workshop held each summer for four years provided basic training in recombinant DNA, molecular biology, genomics, bioinformatics and state-of-the art fluorescence and electron microscopy by using *Arabidopsis* as the experimental material. By pairing students with their teachers, the workshop format fostered collaborative problem solving, helping students prepare for graduate-level research or the job market, while giving teachers an opportunity to hone their professional skills and develop new educational resources. As an outcome of the workshop, two undergraduate students and a community college faculty mentor pursued an independent project during the academic year on an economically important tropical ornamental plant (*Anthurium adreanum*) in Hawaii. They utilized their knowledge gained from the workshop to characterize and identify >600 genes from senescing tissues of Anthurium. Several senescence-related genes were present in transcriptome, which are currently being assembled for publication and for use for undergraduate education and research."
(a) Leeward Community College (b) University of Hawaii

M0104 Undergraduate-level Inquiry: Benefits and challenges of engaging in classroom-based research
Dolan, Erin L-presenter edolan@vt.edu(a) Alkaher, Iris (a) Hlousek-Radojcic, Alenka (b)
http://www.prep.biotech.vt.edu
"Partnership for Research and Education in Plants for Undergraduates (PREP-U) engages students in research in the contexts of their courses. They address the unanswered question of how disabling genes in *Arabidopsis thaliana* influences the plants' interactions with two herbivores, two-spotted spider mites (*Tetranychus urticae*) and root-knot nematodes (*Meloidogyne incognita*). Students design and conduct their own investigations to determine differences in herbivory of wild-type versus mutant plants and then report their findings to scientists interested in the genes being studied. Here we focus on students' perceptions of the benefits and challenges of participating in classroom-based research and its relevance for teaching and learning. PREP-U was implemented in one plant physiology and four introductory biology courses in fall 2008. Twenty-five sets of pre/post interviews were conducted with participating students. Content analysis was done to identify emergent categories and major themes. Students reported a wide range of benefits and challenges and perceived that some aspects were both benefits and challenges. For example, students indicated the importance of independent work, but also asked for more guidance and support. Students valued the opportunity to understand how science is done, but noted their struggles to develop rationales and explain results. Our results point to the complexity of inquiry teaching in undergraduate classrooms. Instructors must achieve a balance between preparing students to conduct investigations and allowing them freedom to make progress independently. We believe that the extent of instructors' involvement, especially how they gauge students' needs to inform their instructional decisions, influences students' perceptions of research."
(a) Virginia Tech (b) Richard Bland College

Start	End	**Event**	Location
2:30 PM	4:10 PM	**Minisymposium 2: Gibberellins & Abscisic Acid** - Chair: Tai-ing Sun 2:30 - M0201: Kohji Murase & Rodolfo Zentella - *Structure-function analysis of GA receptor and DELLA protein in Arabidopsis* 2:55 - M0202: Camille M. Steber - *Relieving DELLA Repression of Stem Elongation and Flowering, Evidence for a Proteolysis Independent Mechanism for GA signaling* 3:20 - M0203: Wan-Chi Lin - *ABA Receptors? Not Sure. Signaling Molecules? Yes*. 3:45 - M0204: David H. Huizinga - *Isoprenylcysteine methylation and demethylation regulate abscisic acid signaling in Arabidopsis*	Kaua'I 311

M0201 Structure-function analysis of GA receptor and DELLA protein in Arabidopsis
Murase, Kohji (a,b) Zentella, Rodolfo-presenter zentella@duke.edu(a) Hirano, Yoshinori (b) Hu, Jianhong (a) Olszewski, Neil (c) Jeong, Sun Yong (a) Hakoshima, Toshio (b) Sun, Tai-ping (a)
http://fds.duke.edu/db/aas/Biology/staff/zentella
"DELLA proteins are central negative regulators of the gibberellin (GA) signaling pathway. DELLAs act as transcriptional regulators, which likely modulate downstream gene expression through interactions with other transcription factors. Inactivation of DELLAs is essential for the promotion of plant growth and development. Recent advances have shown that the GA receptor, GID1, binds DELLAs in the presence of GA. This triggers DELLA poly-ubiquitination by the ubiquitin E3 ligase SCF^{SLY1} complex and degradation via the 26S proteasome. We have determined the crystal structure, at a 1.8 Angstrom resolution, of a complex composed of GID1, GA and the amino-terminus signal sensing domain of a DELLA protein. We describe how the binding of the GA molecule to GID1 causes a conformational change in the receptor that creates a binding surface for the highly conserved DELLA, VHYNP and LExLE motifs of DELLA proteins. In addition, we are investigating how DELLA proteins function and are regulated by post-translational modifications. There is evidence suggesting that DELLA activity is modulated by phosphorylation and *O*-GlcNAcylation. Using a variety of biochemical and genetic approaches we aim to understand the role of specific post-translational modifications within DELLAs. We will present evidence that DELLAs are phosphorylated and *O*-GlcNAcylated in planta. In addition, we are analyzing transgenic plants expressing mutant DELLAs in order to understand the function of different motifs within the protein."
(a) Dept. of Biology, Duke University (b) Graduate School of Information Science, Nara Institute of Science and Technology (c) Dept. of Plant Biology, University of Minnesota

M0202 "Relieving DELLA Repression of Stem Elongation and Flowering, Evidence for a Proteolysis Independent Mechanism for GA signaling"
Steber, Camille M.-presenter csteber@wsu.edu(a,b) Ariizumi, Tohru (b)
http://www.wsu.edu/~csteber/
"GA stimulates germination, stem elongation, and flowering by lifting DELLA protein repression of these responses via both proteolysis dependent and independent pathways. There are five members of the DELLA protein family in Arabidopsis with partially overlapping functions. GA biosynthesis lifts DELLA repression by triggering DELLA proteolysis via the ubiquitin-proteasome pathway. Perception of GA by the GA receptors GIBBERELLIN INSENSITIVE DWARF1 (GID1a, b, and c) enables GID1/GA to recognize and bind the DELLA protein. It appears that the SLY1 protein binds and ubiquitinates DELLA only when it is in the GID1/GA/DELLA complex. Polyubiquitinaiton by the SCF-SLY1 E3 ubiquitin ligase then targets DELLA for proteolysis. If DELLA proteolysis were the only mechanism for DELLA inactivation, then the level of DELLA protein should correlate with the degree of dwarfism and other GA phenotypes. In contrast, *sly1* mutants accumulate more DELLA protein but display less severe dwarf and germination phenotypes than the GA biosynthesis mutant *ga1-3* or the *gid1abc* triple mutant. Interestingly, *GID1* overexpression rescued the *sly1* dwarf and infertility phenotypes without decreasing the accumulation of the DELLA protein REPRESSOR OF GA1-3 (RGA). *GID1* rescue of *sly1* mutants appeared to be dependent on the level of GID1 protein, GA, and the presence of a functional DELLA motif. Since DELLA shows increasing protein interaction

with GID1 with increasing GA levels in vivo, it appears that GA-bound GID1 can block DELLA repressor activity by direct protein interaction with the DELLA domain. Thus, a SLY1 independent mechanism for GA signaling may function without DELLA degradation."
(a) USDA-ARS, Wheat Genetic Unit (b) Washington State University, Dept of Crop and Soil Science

M0203 ABA Receptors? Not Sure. Signaling Molecules? Yes.
Lin, Wan-Chi-presenter linwc@mail2000.com.tw(a) Huang, Kuan-ying (a) Lu, Yung Yu (a) Ho, Tuan-hua David (a,b)
"Several putative ABA receptors have been reported in recent years, however the validity of some of these reports has been seriously questioned (e.g. McCourt and Creelman [2008], Curr Opin Plant Biol, 11:474; Risk et al [2008] Nature, 456: &916&163 5&8722;6). We have investigated the role of these proteins in the cereal aleurone tissue where well-defined ABA responses can be analyzed with precision. Three established signaling pathways exist in this tissue: ABA induction of LEA genes, and GA induction & ABA suppression of α-amylase. ABA induction of LEA genes is enhanced by over-expression of either GCR2 or ABAP1, but significantly suppressed by GCR2 RNAi or ABAP1 RNAi. However, the other two signaling pathways, i.e. GA induction & ABA suppression of α-amylase, are not affected at all by either GCR2 and ABAP1 over-expression or their RNAi. Furthermore, the suppression effect of GCR2 RNAi can be overcome by over-expression of ABAP1, suggesting that ABAP1 works downstream from GCR2. Following a similar approach, we have determined that both GCR2 and ABAP1 work downstream from a plasma membrane-localized receptor kinase, RPK1, which has been shown to be an important signaling molecule for ABA action. The GCR2-GFP fusion protein is localized in the cytoplasm with a punctate pattern near plasma membrane. The ABAP1-GFP protein is initially localized in the cytoplasm with a punctate pattern more pronounced than that of GCR2-GFP. Upon prolonged incubation, the ABAP1-GFP is preferentially localized in the nucleus. Based on these observations, we propose that these regulatory molecules work in the sequence of RPK1 (plasma membrane) GCR2 (cytoplasm) ABAP1 (cytoplasm to nucleus) ABI5 (nucleus) LEA expression. "
(a) Institute of Plant and Microbial Biology, Academia Sinica, Taipei 115, Taiwan (b) Dept of Biology, Washington University, St. Louis, MO 63130

M0204 Isoprenylcysteine methylation and demethylation regulate abscisic acid signaling in *Arabidopsis*
Huizinga, David H-presenter dhuizing@iupui.edu(b) Omosegbon, Olutope (b) Omery, Bilal (b) Crowell, Dring N (a)
"Isoprenylated proteins bear an isoprenylcysteine methyl ester at the carboxyl terminus. Although isoprenylated proteins have been implicated in meristem development and negative regulation of abscisic acid (ABA) signaling, the functional role of the terminal methyl group has not been described. Here, we show that transgenic *Arabidopsis thaliana* plants overproducing isoprenylcysteine methyltransferase (ICMT) exhibit ABA insensitivity in stomatal closure and seed germination assays, establishing ICMT as a negative regulator of ABA signaling. In contrast, transgenic plants overproducing isoprenylcysteine methylesterase (ICME) exhibit ABA hypersensitivity in stomatal closure and seed germination assays. Thus, ICME is a positive regulator of ABA signaling. To test the hypothesis that ABA signaling is under feedback control at the level of isoprenylcysteine methylation, we examined the effect of ABA on *ICMT* and *ICME* gene expression. Interestingly, ABA induced *ICME* gene expression, establishing a positive feedback loop whereby ABA promotes ABA responsiveness of plant cells via induction of *ICME* expression, which presumably results in the demethylation and inactivation of isoprenylated negative regulators of ABA signaling. These results suggest strategies for metabolic engineering of crop species for drought tolerance by targeted alterations in isoprenylcysteine methylation."
(a) Idaho State University (b) Indiana University-Purdue University Indianapolis

Start	End	**Event**	Location
2:30 PM	4:10 PM	**Minisymposium 3: Genome Integrity** - Chair: Anne Britt 2:30 - M0301: Igor Kovalchuk - *Progeny of stressed plants exhibit dramatic changes in genome stability, methylation pattern, stress tolerance and metabolites profile* 2:55 - M0302: Anne B. Britt - *The NAC domain transcription factor Suppressor of Gamma Response 1 (Sog1) governs programmed response to DNA damage* 3:20 - M0303: Sascha Biedermann - *The DDB1a interacting proteins CSA and DDB2 are critical factors for UV-B tolerance in Arabidopsis thaliana* 3:45 - M0304: Vipula K. Shukla - *Precise genome modification in the crop species Zea mays using zinc-finger nucleases*	Moloka'I 315

M0301 "Progeny of stressed plants exhibit dramatic changes in genome stability, methylation pattern, stress tolerance and metabolites profile"
Kovalchuk, Igor-presenter igor.kovalchuk@uleth.ca(a) Boyko, Alex (a) Kathiria, Palak (a) Yao, Youli (a) Blevins, Todd (b,b)
"The fact that plants are able to quickly adapt to stress may suggest the involvement of epigenetic mechanisms of inheritance. We hypothesized that epigenetic alterations are a general mechanism of plant adaptation to stress, and are the initial mechanism of permanent genomic changes leading to genome evolution. We used transgenic *Nicotiana tabacum* and *Arabidopsis thaliana* plants carrying luciferase-based substrate for the analysis of homologous recombination frequency (HRF) and methylation patterns. We exposed plants to abiotic (temperature, water, salt, UV) and biotic (viral and bacterial pathogens) stresses and analyzed changes in the progeny. We found the progeny of stressed plants to show changes in genome stability, reflected by higher level of HRF, as well as changes in global genome hypermethylation and loci-specific hypomethylation, as shown using MeDIP, cytosin extention and COBRA assays. Changes in HRF persisted when plants were propagated with stress and were less dramatic when plants were propagated without stress. Changes at the genome and epigenome were paralled by multiple changes in plant physiology, including higher tolerance to stress, changes in metabolic profile, in the level of phenolic compounds as well as the expression of DNA repair and stress tolerance genes. Since these experiments suggested possible role of epigenetic machinery in transgenerational changes, we performed the same experiments in *dcl2*, *dcl3* and *dcl4* mutants, and indeed found the mutants to be partially impaired in establishment of transgenerational changes, including the increase in HRF and stress tolerance. This work suggests that epigenetic mechanisms indeed may play essential role in plant adaptation."
(a) University of Lethbridge (b) Biology Department, Washington University

M0302 The NAC domain transcription factor Suppressor of Gamma Response 1 (Sog1) governs programmed response to DNA damage
Britt, Anne B.-presenter abbritt@ucdavis.edu(a) Yoshiyama, Kaoru (a) Furukawa, Tomoyuki (a) Conklin, Phillip (a) Curtis, Marc (b) Hays, John (b)
"All living things possess mechanisms to detect the presence of DNA damage and transduce that signal to induce a variety of responses. These responses include the activation of repair, the arrest of the cell cycle, and the induction of programmed cell death. In Arabidopsis the response to chromosome-breaking agents includes the robust upregulation of hundreds of genes, including many genes that are clearly involved in DNA repair. This is a specific response to double strand breaks: the spectrum of genes induced by gamma radiation does not include the genes known to be

induced by a variety of other abiotic stressors, and the transcriptional response is entirely dependent on the PI3K-like protein kinase ATM, a protein known to be activated by double strand breaks. Such a response is unprecedented- mammals and yeast do not exhibit this robust and specific induction of repair-related genes in response to double strand breaks. As a result of a search for mutants defective in gamma-induced cell cycle arrest, a line carrying a 'gamma resistant' mutation, termed sog1, was identified. This mutation was mapped and cloned, and SOG1 was revealed to be a member of the large family of NAC domain transcription factors. Further analysis of sog1 revealed that this gene, like ATM, is required for the transcriptional response to gamma radiation. Here we will present evidence that SOG1 is also required for the programmed, tissue-specific cell death response to DNA damage observed in Arabidopsis. Thus SOG1, although unrelated to the mammalian transcription factor and tumor suppressor TP53, evolved independently in multicellular plants to play the same essential role in governing DNA damage response."
(a) University Of California (b) Oregon State University

M0303 The DDB1a interacting proteins CSA and DDB2 are critical factors for UV-B tolerance in *Arabidopsis thaliana*
Biedermann, Sascha-presenter sbiedermann@wsu.edu(a,b) Mooney, Sutton (b) Hellmann, Hanjo (b)
"Genotoxic stress imposed by UV-irradiation or chemical treatment is a permanent threat potentially affecting the genomic integrity of all live forms, but especially that of sessile organisms like plants. Consequently, several DNA repair mechanisms have evolved, one of which is the nucleotide excision repair (NER). NER comprises two separate pathways, the global genomic repair (GGR) and the pathway of transcription-coupled repair (TCR). Critical for recognition of UV-induced DNA damages to initiate GGR and TCR dependent repair processes are the proteins DDB2 (Damaged DNA Binding 2), and Cockayne Syndrome A (CSA), respectively. Both DDB2 and CSA assemble with an ubiquitin E3 ligase that contains the cullin CUL4 and the substrate adaptor DDB1. This assembly leads to the ubiquitination and subsequent degradation of DDB2, CSA and additional proteins via the 26S proteasome. Although GGR and TCR have been intensively described in mammalian cells, only poor knowledge is present for plants. Here, we report that loss of the CSA and DDB2 orthologs from *Arabidopsis thaliana* leads to an increased sensitivity of affected plants to UV stress. We provide evidence that these plants exhibit an impaired NER-dependent repair of UV induced thymidine dimers. Additionally we demonstrate that CSA assembles into a CUL4-DDB1a complex and that it is degraded by the 26S proteasome in a CUL4 and UV-B dependent manner. Furthermore we describe tissue specific expression patterns of *Arabidopsis* CSA and DDB2 using promoter:GUS constructs and RT-PCR analysis, and investigate subcellular localization of the two proteins. In summary, this work describes for the first time functions of *Arabidopsis* CSA and underscores the significance of CUL4-based E3 ligases, DDB2, and CSA for DNA repair in higher plants. "
(a) Angewandte Genetik, Freie Universitaet Berlin (b) School of Biological Sciences, Washington State University

M0304 Precise genome modification in the crop species *Zea mays* using zinc-finger nucleases
Shukla, Vipula K-presenter vkshukla@dow.com(a) Doyon, Yannick (b) Choi, Vivian (b) Rock, Jeremy (b) Ying-Ying , Wu (b) Katibah, George (b) Gao, Zhifang (a) McCaskill, David G (a) Simpson, Matthew A (a) Blakeslee, Beth (a) Greenwalt, Scott A (a) Butler, Holly J (a) Miller, Jeffrey C (b) Hinkley, Sarah J (b) Lei, Zhang (b) Rebar, Edward J (b) Gregory, Philip D (b) Urnov, Fyodor D (b) DeKelver, Russell C (b) Moehle, Erica A (b) Worden, Sarah E (a) Mitchell, Jon C (a) Arnold, Nicole L (a) Gopalan, Sunita (b) Xiandong, Meng (b)
"Agricultural biotechnology is limited by the inefficiency and unpredictability of conventional random mutagenesis and transgenesis. Here we report a broadly applicable, versatile alternative: the use of designed zinc-finger nucleases (ZFNs) that induce double-stranded breaks (DSB) at their target locus. We have used ZFNs to modify endogenous loci in plants of the crop species *Zea mays*, and show that ZFNs designed to cleave a maize gene disrupt their target via DSB repair by error-prone non-homologous end-joining. We further demonstrate that simultaneous expression of ZFNs and delivery of a simple heterologous donor molecule leads to high frequency targeted addition of an herbicide-tolerance gene at the intended locus. Modified maize plants transmit these genetic changes to the next generation. Insertional disruption of one target locus, *ZmIPK1*, results in both herbicide tolerance and alterations of the inositol phosphate profile in developing seeds. ZFNs can be utilized in any plant species amenable to DNA delivery; therefore our results establish a new paradigm in plant genetic manipulation for basic science and agricultural applications."
(a) Dow AgroSciences, LLC (b) Sangamo BioSciences

Start	End	**Event**	Location
2:30 PM	4:10 PM	**Minisymposium 4: Secondary Metabolism** - Chair: Kazuki Saito 2:30 - M0401: Kazuki Saito - *Transcriptome coexpression analysis and comprehensive metabolite profiling led to decoding gene-metabolite correlations in Arabidopsis flavonoid metabolism* 2:55 - M0402: Hyun Joo Koo - *Evolution and biosynthesis of medicinally important terpenoids curlone and the turmerones in turmeric and ginger* 3:20 - M0403: Charles E. Stewart - *Convergent biosynthetic evolution in type III polyketide synthases* 3:45 - M0404: Hong Han - *The Biosynthesis of Triterpenoid Glutinol and Friedelin in Kalanchoe daigremontiana*	Maui 316A

M0401 Transcriptome coexpression analysis and comprehensive metabolite profiling led to decoding gene-metabolite correlations in *Arabidopsis* flavonoid metabolism
Yonekura-Sakakibara, Keiko-presenter keikoys@psc.riken.jp(a) Tohge, Takayuki (a) Matsuda, Fumio (a) Nakabayashi, Ryo (b) Takayama, Hiromitsu (b) Niida, Rie (a) Watanabe-Takahashi, Akiko (a) Inoue, Eri (a) Saito, Kazuki (a,b)
" To complete the metabolic map for an entire class of compounds, it is essential to identify gene-metabolite correlations of a metabolic pathway. We used liquid chromatography-mass spectrometry (LC-MS) to identify the flavonoids produced by *Arabidopsis thaliana* wild-type and flavonoid biosynthetic mutant lines. The structures of novel and known flavonoids were deduced by LC-MS profiling of these mutants. Candidate genes presumably involved in the flavonoid pathway were delimited by transcriptome coexpression network analysis using public databases, leading to the detailed analysis of two flavonoid pathway genes, *UGT78D3* and *RHM1*. The levels of flavonol 3-*O*-pentosides were reduced in *ugt78d3* knockdown mutants. Recombinant UGT78D3 protein could convert quercetin to quercetin 3-*O*-arabinoside. The strict substrate specificity of UGT78D3 for flavonol aglycones and UDP-arabinose strongly suggest that UGT78D3 is the first flavonol arabinosyltransferase characterized. The structures of unknown *Arabidopsis* flavonols were confirmed by direct comparison of flavonol 3-*O*-arabinoside-7-*O*-rhamnosides enzymatically synthesized by UGT78D3. In *rhm1* knockout mutants, flavonol 3-*O*-rhamnoside levels were lower but levels of flavonol 3-*O*-glucosides were higher. These results suggest that the rate of flavonol glycosylation is affected by the supply of UDP-rhamnose produced by RHM1. The precise identification of flavonoids in conjunction with transcriptomics thus led to the identification of a novel gene function and a more complete understanding of a plant metabolic network. We will also discuss a gene encoding a novel anthocyanin glycosyltransferase. reference: Yonekura-Sakakibara and Tohge *et al.*, Plant Cell,

2008, 20: 2160-2176. "
(a) RIKEN Plant Science Center (b) Graduate School of Pharmaceutical Science, Chiba University

M0402 Evolution and biosynthesis of medicinally important terpenoids curlone and the turmerones in turmeric and ginger
Koo, Hyun Jo-presenter hjk@email.arizona.edu(a) Gang, David R (a)
"Turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*) are known for their anti-inflammatory and anti-cancer activities, which are attributed to the presence of gingerols, curcumin and related diarylheptanoids. Curlone and the turmerones are sesquiterpenoids from turmeric that have been shown to possess antioxidant and antimutagenic properties. α-Zingiberene and β-sesquiphellandrene, which also possess important biological activities, have been found in both turmeric and ginger. In a database of over 50,000 ESTs from these species, we identified 20 putative monoterpene synthases and 10 putative sesquiterpene synthases, as well as P450 monooxygenases that are good candidates for the oxidation step leading to curlone and the turmerones. Corresponding cDNAs were isolated from the respective species and recombinant proteins were expressed in E. coli or yeast. In vitro enzyme assays identified the following terpene synthases: camphene synthase, α-phellandrene synthase, β-phellandrene synthase, 1,8-cineole synthase, *p*-mentha-1,4(8)-diene (terpinolene) synthase, α-zingiberene/sesquiphellandrene synthase, β-selinene synthase, β-elemene synthase, γ-amorphene synthase, and α-humulene synthase. Comparative metabolite/gene expression profiling suggested that α-zingiberene/sesquiphellandrene synthase initiates the curlone/turmerone synthesis pathway in turmeric. Results from comparative modeling of the different terpene synthases and implications regarding the evolution of terpenoid metabolism in these species will also be discussed."
(a) University Of Arizona

M0403 Convergent biosynthetic evolution in type III polyketide synthases
Stewart, Charles E-presenter cstewart@salk.edu(a) Austin, Mike B (a) Bowman, Marianne (a) Noel, Joseph P. (a)
http://www.salk.edu/faculty/noel.html
"Type III polyketide synthases are structurally simple, yet biochemically complex enzymes involved in the biosynthesis of a dazzling array of metabolites important in medicine and agriculture. Two classic type III polyketide synthases are the chalcone synthases (CHS) and stilbene synthases (STS). CHS is ubiquitous in plants and is essential for the biosynthesis of tannins, anthocyanins, and other flavonoids. STS is taxonomically restricted and responsible for the biosynthesis of stilbenes such as the well-known red wine compound, resveratrol. The key difference between CHS and STS is their cyclization pattern; STS catalysis involves an aldol cyclization (C2 C7); whereas CHS choreographs a claisen cyclization (C6 C1). Previous work in our lab discovered an aldol switch responsible for shifting the cyclization specificity in CHS and STS. However, sequence comparisons of STSs from various species suggested that the aldol switch was not conserved. X-ray crystallographic analysis of STSs from grape and peanut indicate that while there is not a consensus sequence, a consensus structure is present. Mutagenesis and biochemical assays indicate that the amino acid residues responsible for shifting cyclization specificity are species-specific. These results support an evolutionary model whereby STSs independently diverged from CHS only to converge upon the same structural solutions for choreographing their aldol cyclizations. This work highlights the evolutionary relationship between enzyme architecture and the emergence of novel biochemical activities. Understanding how nature fine-tunes enzyme activity lays the foundation for expanding the biosynthetic potential of enzymes for the production of environmentally friendly pesticides, flavors, and fragrances."
(a) Salk Institute for Biological Studies

M0404 The Biosynthesis of Triterpenoid Glutinol and Friedelin in *Kalanchoe daigremontiana*
Han, Hong-presenter honghh@interchange.ubc.ca(a) Wang, Zhonghua (a) Jetter, Reinhard (a,b)
"Triterpenoids ($C_{30}H_{50}O$), a major group of plant secondary metabolites, are synthesized by enzymatic cyclization of a common precursor (2,3-oxidosqualene). The variations in 1,2-methyl and 1,2-hydride shifts as well as deprotonation result in the remarkable structural diversity of triterpenoids. The goal of this project was to identify the amino acid residues that determine product specificity in those triterpenoid synthases catalyzing the maximum number of 1,2-shifts, leading to the formation of glutinol and friedelin. Two novel triterpenoid synthase genes were isolated from *Kalanchoe daigremontiana* and heterologous expression in yeast, followed by GC-FID and GC-MS chemical analysis, showed that they code for a glutinol synthase and a friedelin synthase. Sequence comparisons between both enzymes, together with three-dimensional structure modeling, were used to predict amino acids involved in determining product specificity. To test these predictions, chimeragenesis and site-directed mutagenesis experiments were carried out. The product profiles of the altered enzymes were significantly changed, most notably in the percentages of glutinol and friedelin. These results provide some important new insights into the mechanisms of triterpenoid cyclization, and also have implications for the biological functions of plant triterpenoids. Glutinol and friedelin accumulate to high concentrations in the cuticular wax of *K. daigremontiana*, where they likely play an important role in protecting the plant against biotic and/or abiotic stress. As a result, understanding the genetics and biochemistry of triterpenoids will also provide us further insight into their physiological and ecological roles."
(a) University of British Columbia, Department of Botany (b) University of British Columbia, Department of Chemistry

Start	End	**Event**	Location
2:30 PM	4:10 PM	**Minisymposium 5: Reproductive Biology** - Chair: Mark Johnson 2:30 - M0501: Yongxian Lu - *Cation/proton transporters are key players in pollen tube guidance* 2:55 - M0502: Tetsuya Higashiyama - *Identification of Pollen Tube Attractants derived from the Synergid Cell* 3:20 - M0503: Mily Ron - *Mis-regulation of a nat-siRNA Pair in Sperm Cells Results in Single Fertilizations.* 3:45 - M0504: Mark A. Johnson - *HAP2(GCS1) is a sperm-expressed component of a deeply conserved fertilization mechanism*	Maui 316C

M0501 Cation/proton transporters are key players in pollen tube guidance
Lu, Yongxian-presenter yxlu@umd.edu(a) Chanroj, Salil (a) Sze, Heven (a)
http://www.cbmg.umd.edu/faculty/sze/sze.html
"Understanding the molecular & cellular bases of fertilization in plants is critical for enhanced reproduction and crop seed production. Successful fertilization depends on accurate delivery of sperms to the ovule by the pollen tube. Guiding signals from the female cells are being identified, though how the pollen senses and responds to the signals are largely not known. We are testing the role of two related genes CHX-A and CHX-B from a family of predicted cation/proton exchangers that are expressed preferentially in pollen (Sze et al. 2004 Plant Physiol 136: 2532). Single T-DNA insertion mutants (*aa* or *bb*) showed in vitro pollen germination and tube growth similar to that of wild-type. However, male gene transmission was

specifically blocked when both genes are defective. When wild-type pistil was pollinated with a limited number of pollen grains from *Aabb* plants, seed set was reduced to half of that from *AAbb* plants, suggesting *ab* pollen is infertile. In vivo pollen tube growth was monitored using mutants expressing the GUS reporter. *aB* pollen tube grew inside the transmitting tissues of the female reproductive organ, turned towards the ovule and discharged its content into the embryo sac. However, the double mutant pollen tube grew mainly inside the transmitting tissue and failed to target the ovule. To our knowledge, this is the first transporter mutant with a defect in pollen tube guidance. The results suggest that loss of these transporters disrupts the perception and/or transduction of female guidance signals to shift the axis of polar tip growth. GFP-tagged CHX-B was localized to endomembranes in the pollen tube. Studies are underway to determine the cellular and molecular bases of this phenotype. (Supported by DOE BES grant to HS)"
(a) University Of Maryland

M0502 Identification of Pollen Tube Attractants derived from the Synergid Cell
Higashiyama, Tetsuya-presenter higashi@bio.nagoya-u.ac.jp(a,b) Okuda, Satohiro (a) Tsutsui, Hiroki (a) Shiina, Keiko (a) Takeuchi, Hidenori (a) Kawano, Nao (a) Sprunck, Stefanie (c) Dresselhaus, Thomas (c) Kanaoka, Masahiro (a) Sasaki, Narie (a)
"For more than 140 years, pollen tube guidance in flowering plants has been thought to be mediated by chemo-attractants derived from target ovules. However, there has been no convincing evidence of any particular molecule being the true attractant that actually controls the navigation of pollen tubes towards ovules. Here we report first identification of pollen tube attractants derived from the synergid cell on the side of the egg cell (Okuda et al., Nature, in press). Our group developed the in vitro Torenia system, whereby pollen tubes growing through a cut style were attracted to a protruding embryo sac (Higashiyama et al., Plant Cell, 1998). By using this system and laser cell ablation technique, the synergid cell was shown to emit some diffusible attractant(s) (Higashiyama et al., Science, 2001). The attractant molecule was species preferential even in closely relating species, implying that the molecule had rapidly evolved (Higashiyama et al., Plant Physiol., 2006). Thus, we investigated genes expressed in the synergid cell of Torenia, by collecting isolated synergid cells. We finally identified attractant proteins derived from the synergid cell. We will present in this talk detailed properties of attractants molecules."
(a) Graduate School of Science, Nagoya University (b) Presto, JST (c) Cell Biology/Plant Physiology, University of Regensburg

M0503 Mis-regulation of a nat-siRNA Pair in Sperm Cells Results in Single Fertilizations.
Ron, Mily-presenter milyron@berkeley.edu(a) Alandete-Saez, Monica (a) McCormick, Sheila (a)
"We noticed that plants with T-DNA insertions in a gene we call T10 had reduced seed set (50-80% in homozygotes). Reciprocal crosses with WT indicated the male gametophyte was affected, although pollen of homozygous t10 plants appeared normal and could germinate, suggesting a defect in fertilization. T10 is in reverse orientation to the adjacent gene that we call M. The 3 UTR of T10 protrudes ~80 bp into the last exon of M, creating a transcript overlap. We hypothesized that T10 and M generate a pair of natural antisense small interfering RNAs (nat-siRNA) and that the transcript of T10 down-regulates M transcripts in sperm cells. Q-PCR with flowers confirmed that the T10 transcript was almost absent from the t10 mutant, while M transcripts were increased. Promoter-reporter lines confirmed that T10 is expressed specifically in sperm cells while M is expressed in both the vegetative and sperm cells. Co-expressing T10 and a GFP-M fusion in leaves resulted in down-regulation of GFP and a M-specific small RNA form was detected, supporting our hypothesis. Moreover, plants in which M was overexpressed in sperm had phenotypes similar to t10 mutants. DIC imaging of the undeveloped seeds 2-3 days after pollination showed that in the t10 mutant single fertilizations are prevalent (~40% of either embryo or endosperm) compared to their incidence in other known mutants. These results suggest that proper regulation of M in sperm cells is important for fertilization and supports the idea that sperm randomly fertilize the egg or central cell. "
(a) Plant Gene Expression Center and Dept. of Plant and Microbial Biology, USDA/ARS-UC-Berkeley,

M0504 HAP2(GCS1) is a sperm-expressed component of a deeply conserved fertilization mechanism
Johnson, Mark A-presenter Mark_Johnson_1@Brown.edu(a) Wong, Julian L (a) Frank, Aubrey C (a) Chang, Alexander (a) Leydon, Alexander R (a)
http://brown.edu/Departments/Molecular_Biology/pgl/
"Pollen tubes carry two sperm to the female gametophyte where one fuses with the egg to form the embryo and the other fuses with the central cell to form the endosperm. This process of double fertilization is at the core of seed crop production and is as mysterious as it is fascinating. We know very little about the mechanisms required for interactions between sperm and the female gametes that lead to fusion and initiation of development. We discovered *hap2-1* in a screen for mutations in Arabidopsis that disrupt the function of the male gametophyte. *hap2-1* pollen tubes are defective in pollen tube guidance, but deliver sperm to ovules at reduced frequencies. However, *hap2-1* sperm are completely incapable of fertilizing either the egg or the central cell. Recently, it has been show that the HAP2 ortholog is essential for gamete fusion in the green alga, *Chlamydomonas reinhardtii*, and in the protozoan parasite, *Plasmodium falciparum*. These results, combined with the conservation of HAP2 in plants and other organisms, lead us to propose that HAP2 is an anciently conserved component of a widely used fertilization mechanism. Our primary hypothesis is that HAP2 is directly involved in mediating fusion of the sperm and female gamete plasma membranes. We are taking a number of approaches to understand the biochemical function of HAP2, which has a transmembrane domain but no other domains of known function. Genetic dissection of HAP2 suggests that the large region of HAP2 N-terminal to the transmembrane domain, predicted to be outside of the sperm cell, is essential for function. Analysis of the C-terminal portion of the protein indicates that the charge of amino acid side chains is critical for function. We will discuss models for HAP2 function based on these results."
(a) Brown University

Start	End	**Event**	Location
2:30 PM	4:10 PM	**Minisymposium 6: Choloroplast Signalling & Gene Expression** - Chair: Maureen Hanson 2:30 - M0601: Masahiro Sugiura - *Novel termination-dependent translation in chloroplasts* 2:55 - M0602: Wade Heller - *A comparative genomics approach identifies RARE1: a pentatricopeptide repeat protein mediating chloroplast accD transcript editing* 3:20 - M0603: Takehito Inaba - *Coordination of plastid protein import and nuclear gene expression by plastid-to-nucleus retrograde signaling pathway* 3:45 - M0604: Barry Pogson - *Chloroplast-Nuclear signaling: A Tale of Phosphatases, Gene silencing and Histone Modifications*	O'ahu 313A

M0601 Novel termination-dependent translation in chloroplasts
Sugiura, Masahiro-presenter sugiura@nsc.nagoya-cu.ac.jp(a,b) Maki, Yukawa (a)
"The chloroplast genome of higher plants contains ca. 80 protein-coding genes. Translational control is the major step of chloroplast gene expression, and it is important for the stoichiometric production of individual subunits in photosynthetic complexes. To study mechanisms of translation unique to chloroplasts, we have developed an *in vitro* system from tobacco chloroplasts. This system is highly active enough to measure the relative rate of translation. The *ndhC* and *ndhK* genes are partially overlapped and cotranscribed in many land plants. The downstream *ndhK* mRNA possesses 4 possible AUG initiation codons in many dicot plants. Using our *in vitro* system, we defined that the major initiation site of tobacco *ndhK* mRNAs is the third AUG that is located 4 nt upstream from the *ndhC* stop codon. Mutation of the *ndhC* stop codon arrested translation of the *ndhK* cistron. Frameshift of the *ndhC*-coding strand inhibited also *ndhK* translation. The results indicated that *ndhK* translation depends on termination of the preceding cistron, namely translational coupling. Surprisingly, removal of the *ndhC* 5'-UTR and its AUG still supported substantial translation of the *ndhK* cistron. This translation was abolished again by removing the *ndhC* stop codon. Although translation of the downstream cistron of an overlapping mRNA is generally very low, we found that the *ndhC/K* mRNA produces NdhK and NdhC in similar amounts. As the stoichiometry of NdhK and NdhC is suggested to be 1:1, the *ndhC/K* mRNA is translated not only by a translational coupling event but also by a novel termination-dependent pathway. For the latter pathway, free ribosomes are likely to be loaded on the *ndhC* coding region, migrate to the *ndhC* stop codon and start to translate the *ndhK* cistron."
(a) Graduate School of Natural Sciences, Nagoya City University (b) Sugiyama Human Research Center, Sugiyama Jogakuen University

M0602 A comparative genomics approach identifies RARE1: a pentatricopeptide repeat protein mediating chloroplast *accD* transcript editing
Heller, Wade P.-presenter wph7@cornell.edu(a) Robbins, John C. (a) Hanson, Maureen R. (a)
"Proper chloroplast function depends on regulated interactions between nuclear and plastid genes and their products. Nuclear-encoded proteins affect nearly every aspect of chloroplast gene expression--from transcription to proteolysis. The molecular apparatus responsible for C-to-U RNA editing in chloroplasts is encoded by nuclear genes. Recognition of the correct C target of editing is directed by site-specific trans-factors that interact with sequences 5' of edited nucleotides. A few such trans-factors have been identified in Arabidopsis mutants lacking RNA editing of particular Cs, and all are members of the pentatricopeptide-repeat (PPR) protein family. The Arabidopsis PPR family comprises over 450 proteins, some members of which are known to be mediate aspects of organellar expression in addition to RNA editing, including polycistron cleavage, splicing and translation. PPR proteins contain degenerate 35aa repeats, some having additional C-terminal motifs, including the 'E' (extended) domain, and the 'DYW' domain, which is believed to have catalytic activity. At present there are 4 known editing factors, including RARE1, that have DYW domains in addition to the PPR and E domains. *RARE1* was identified by a comparative genomics analysis of Arabidopsis and rice PPR genes in combination with virus-induced gene silencing. Assays of editing in *rare1* insertional mutants confirmed the *accD* transcript editing defect of *RARE1* silenced plants. Wild-type plants edit *accD* transcripts to restore an evolutionarily conserved leucine residue in the encoded protein, β-carboxyltransferase. Despite a complete lack of *accD* editing, homozygous *rare1* mutants are unexpectedly robust and produce abundant progeny."
(a) Department of Molecular Biology & Genetics, Cornell University

M0603 Coordination of plastid protein import and nuclear gene expression by plastid-to-nucleus retrograde signaling pathway
Kakizaki, Tomohiro (a) Matsumura, Hideo (b) Nakayama, Katsuhiro (a) Che, Fang-Sik (c) Terauchi, Ryohei (b) Inaba, Takehito-presenter tinaba@iwate-u.ac.jp(a)
"Plastids, such as chloroplasts, are a diverse group of organelles that perform essential metabolic and signaling functions within all plant cells. It is generally believed that plastids originated from a unicellular photosynthetic bacterium that was taken up by a eukaryotic host cell. During evolution, most of the genes encoded by the bacterial ancestor have been transferred to the host nuclear genome. Therefore, the expression of nuclear-encoded plastid proteins and import of those proteins into plastids are indispensable for plastid biogenesis. One possible cellular mechanism that coordinates these two essential processes is retrograde signaling from plastids to the nucleus. However, the molecular details of how this signaling occurs remain elusive. Using the *ppi2* mutant of Arabidopsis, which lacks the atToc159 protein import receptor, we demonstrate that the expression of photosynthesis-related nuclear genes is tightly coordinated with their import into plastids. Genetic studies indicate that the coordination of plastid protein import and nuclear gene expression is independent of the accumulation of Mg-protoIX and the activity of ABI4. Instead, it may involve GUN1 and the transcription factor AtGLK. The expression level of *AtGLK1* is tightly correlated with the expression of photosynthesis-related nuclear genes in mutants defective in plastid protein import. Furthermore, the activity of GUN1 appears to down-regulate the expression of *AtGLK1* when plastids are dysfunctional. Taken together, we suggest that AtGLK1 may act as a positive regulator that coordinates plastid protein import and nuclear gene expression in response to the functional state of plastids."
(a) Cryobiofrontier Research Center, Iwate University (b) Iwate Biotechnology Research Center (c) Faculty of Bio-Science, Nagahama Institute of Bio-Science and Technology

M0604 "Chloroplast-Nuclear signaling: A Tale of Phosphatases, Gene silencing and Histone Modifications"
Pogson, Barry J-presenter barry.pogson@anu.edu.au(a) Estavillo, Gonzalo (a) Cazzonelli, Chris (a) Cuttriss, Abby (a) Wilson, Pip (a) Pornsiriwong, Wannarat (a)
http://www.anu.edu.au/bambi/people/academic/pogson.php
"Chloroplast-nuclear signaling involves retrograde signals from the chloroplast to the nucleus and the opposite process of nuclear modifications altering chloroplast function is referred to an anterograde signaling. We have demonstrated that high light-mediated retrograde signaling requires the nucleotidase/phosphatase, SAL1 (1). Knockouts in Arabidopsis of SAL1, *alx8* and *fry1-1*, have elevated transcripts of *APX2*, *ZAT10* and *DREB2A* (2). In addition, the plants have lower levels of H_2O_2, enhanced tolerance to drought and altered leaf morphology. SAL1 either breakdowns IP3 and/or 3'(2')-phosphoadenosine 5'- phosphate (PAP). Two studies have implicated PAP activity as critical for the leaf phenotype due to PAP inhibition of exoribonucleases, which in turn inhibit gene silencing (3, 4). The implications of gene silencing for the chloroplast-nuclear signaling functions of SAL1 will be discussed. With respect to anterograde signalling, the chloroplast and carotenoid regulation mutant, *ccr1*, demonstrated that the histone methyltransferase, SDG8, is required for expression of the *CAROTENOID ISOMERASE* gene. The ccr1 knockout of SDG8 has altered histone methylation, reduced *CRTISO* mRNA, reduced lutein and increased shoot branching (5). The altered carotenoid profile may partially affect shoot branching, potentially by perturbed biosynthesis of the carotenoid substrates of strigolactones. Thus, lutein, a carotenoid critical for photosynthesis and photoprotection, appears to be regulated by a chromatin modifying enzyme. 1. Wilson*, Estavillo* et al (2009) Plant J. online 2. Rossel et al (2006) Plant Cell Environ. 29: 269-281 3. Gy et al (2007) Plant Cell 19: 3451-3461 4. Kim and von Arnim (2009) Plant J. online 5. Cazzonelli*, Cuttriss* et al. (2009) Plant Cell online "
(a) ARC Centre of Excellence in Plant Energy Biology, Australian National University

Start	End	Event	Location
4:45 PM	6:25 PM	**Minisymposium 7: Minority Affairs - Ka Hunaola Lā`au (Plant Cell Biology)** - Chair: John Harada 4:45 - M0701: Kawika Winters - *Seeing the Waonāhele from Amongst the Trees: A Culturally-Based, Whole-System View of Plant Biology* 5:10 - M0702: Beronda Montgomery-Kaguri - *Right Place, Right Time: Spatiotemporal Phytochrome Regulation of Plant Growth and Development* 5:35 - M0703: Elison Blancaflor - *Filling the gap between cytoskeletal remodeling and membrane trafficking in the regulation of tip growth in plants* 6:00 - M0704: Magdalena Bezanilla - *Controlling Actin Dynamics is Required for Tip Growth*	Lana'I 314

M0701 "Seeing the *Waonhele* from Amongst the Trees: A Culturally-Based, Whole-System View of Plant Biology"
Winter, Kawika-presenter kwinter@ntbg.org(a,b)
"Plant biology is scalable from the cellular to the whole system. Viewing a subject from a different scale than one is accustomed to can provide useful perspective. In this presentation I will take a step back and look at the whole system perspective of plant biology through a cultural lens. An example we will be presented that specifically examines the social-ecological system of Hawai`i to get a sense of the rich interactions between the native Hawaiian culture and plants. Emphasis will be placed upon Hawaiian resource management systems and their associated body of philosophy, as well as the role that plant biology has in perpetuating these for future generations."
(a) National Tropical Botanical Garden (b) University of Hawai`i at Manoa

M0702 "Right Place, Right Time: Spatiotemporal Phytochrome Regulation of Plant Growth and Development"
Montgomery, Beronda L-presenter montg133@msu.edu(a)
"Photoperception and the developmental changes that occur in response to light are among the most important adaptations of photosynthetic organisms. Prior physiological studies resulted in the identification of spatially distinct photoreceptor pools that control discrete aspects of light-dependent growth and development in plants through light-dependent, intercellular coordination. Despite the progress that has been made in understanding the mechanisms of phytochrome signaling, molecular evidence about spatial-specific phytochrome signaling is limited. Thus, we initiated transgenic plant studies to broaden our molecular understanding of spatial-specific phytochrome responses. We used the gene encoding the mammalian enzyme biliverdin IX- reductase (*BVR*) whose constitutive expression has been shown to alter light-dependent growth and development by metabolic inactivation of the phytochrome chromophore precursors. Our studies are based on the premise that individual aspects of phytochrome-mediated growth and development, which are controlled by pools of phytochromes in distinct cells and tissues, can be uncoupled by targeted expression of *BVR* in Arabidopsis. The rationale for this research is that the targeted use of this molecular tool for the inactivation of specific pools of phytochromes and consequential characterization of the cellular mechanisms controlling distinct aspects of light-mediated growth and development is providing novel insight into tissue-specific, intercellular and inter-organ regulatory roles of phytochromes in plants. We induced spatial-specific *BVR* expression using distinct Arabidopsis promoters, including constitutive, leaf- and shoot-apex-specific promoters, to investigate the phenotypic consequences of cell- and tissue-specific phytochrome deficiencies. Plants that exhibit mesophyll-specific phytochrome chromophore depletion display severe defects in a number of phyA-mediated far-red high irradiance responses (FR-HIR), as well as in phyA-mediated blue light responses. We conclude that mesophyll-localized phyA regulates inter-organ signals that control cellular elongation and tissue-specific pigment accumulation. We also discuss additional spatial-specific phytochrome responses that we have uncovered using this approach. Through the identification of specific cellular mechanisms and candidate genes involved in the regulation of discrete aspects of light-mediated growth and development in plants, our findings are adding significantly to our knowledge of the complex signaling cascades and cellular biology controlled by phytochromes *in vivo*."
(a) Michigan State University

M0703 Filling the gap between cytoskeletal remodeling and membrane trafficking in the regulation of tip growth in plants
Blancaflor, Elison B-presenter eblancaflor@noble.org(a) Yoo, Cheol-Min (a) Sparks, J. Alan (a) Quan, Li (a)
http://www.noble.org/PlantBio/Blancaflor/index.html
"To achieve its final form and orientation, a plant must coordinate the growth of its individual cells. Crucial to this function is the establishment of polarity where cell wall precursors and membrane material are targeted to specific regions of the cell. This differential sorting of cellular components is largely responsible for many of the diverse cell and organ shapes that comprise the plant body. To study factors that define cell shape in plants, we use Arabidopsis root hairs as a model system. Root hairs elongate by a process known as tip growth where expansion is restricted to a small region of the cell leading to the formation long tubular cells. To facilitate the process of tip growth, a number of interconnected signaling pathways come into play. For instance, vesicles carrying cell wall precursors and membrane material are directed to the tip of the cell via the cytoskeleton. Through a forward genetic screen we identified an ADP ribosylation factor (ARF)-GTPase activating protein (GAP) as a new molecular player that could function as a signaling scaffold between several components of the root hair tip growth machinery. When the gene encoding this ARF-GAP protein (*AGD1*) is mutated, root hairs display a wavy growth pattern instead of the straight growth pattern, typical of wild-type plants. The cytoskeleton, cytoplasmic calcium tip oscillations, tip targeting of other small GTPases, vacuolar membrane dynamics and the distribution of fluorescent phosphoinositide sensors were altered in root hairs of *agd1*. Our data indicate that AGD1 could serve as an important link through which several components of the root hair tip growth machinery interact. "
(a) The Samuel Roberts Noble Foundation

M0704 Controlling Actin Dynamics is Required for Tip Growth
Vidali, Luis (a) vanGisbergen, Peter (a,c) Guerin, Christophe (b) Franco, Paula (a) Li, Ming (a) Burkart, Graham (a) Augustine, Robert C. (a) Blanchoin, Laurent (b) Bezanilla, Magdalena-presenter bezanilla@bio.umass.edu(a)
"Tip growth is a critical form of polarized cell growth in all plants. In the moss *Physcomitrella patens*, the colonizing protonemal tissue emerges from the spore and propagates by tip growth. Thus tip growth is essential for propagation of the species. We are using this very facile molecular genetic system to dissect the molecular basis of tip growth. With a combination of RNAi, complementation, allele replacement, live-cell imaging, and biochemical characterization of purified components, we have discovered that proteins essential for the regulation of actin turnover are required for tip growth. We have observed a highly dynamic cortical array of actin filaments emanating from the very apex of the cell. This array is most likely generated by an exceedingly rapid actin elongation factor, the class II formins. Formins are well-known actin nucleating/elongating factors present in all eukaryotes. Class II formins in moss are localized to the apex of the cell via their N-terminal phosphatase tensin (PTEN)-like domain. Only class II, not class I, formins actin elongation activity is required for polarized growth. We have also shown that class II formins work with the small actin

monomer binding protein, profilin to generate the apical actin network. To recycle the actin filament network, we have shown that actin depolymerizing factor (ADF) is required. We propose that the apical actin network may serve as tracks for secretion of new cell wall at the apex of the cell. In support of this, we have shown that myosin-XI, an actin based molecular motor is also required for tip growth. We will present the most current model for actin-mediated polarized growth in plant cells."
(a) University of Massachusetts Amherst (b) Institut de Recherches en Technologie et Sciences pour le Vivant (c) Wageningen University and Research Centre

Start	End	**Event**	Location
4:45 PM	6:25 PM	**Minisymposium 8: Hormone Biology** - Chair: Yuji Kamiya 4:45 - M0801: Yuji Kamiya - *Indole-3-acetaldoxime dependent auxin biosynthesis in Arabidopsis* 5:10 - M0802: John G. Tallman -*Like heat, L-NG-monomethyl arginine (L-NMMA), an inhibitor of arginine-dependent nitric oxide (NO) production, blocks auxin signaling for gene expression and interferes with hormone-dependent cell expansion and division in cultured Nicotiana glauca guard cell protoplasts (GCP).* 5:35 - M0803: Abidur Rahman - *Transcytosis of PIN2 in arabidopsis is regulated by protein phosphatase 2A and PID kinase* 6:00 - M0804: Noriyuki Nishimura - *Identification of new ABI1-mediated ABA signaling components in Arabidopsis.*	Kaua'I 311

M0801 Indole-3-acetaldoxime dependent auxin biosynthesis in *Arabidopsis*
Sugawara, Satoko (a) Hishiyama, Shojiro (c) Jikumaru, Yusuke (a) Hanada, Atsushi (a) Nishimura, Takeshi (b) Koshiba, Tomokazu (b) Zaho, Yunde (d) Kamiya, Yuji-presenter ykamiya@postman.riken.jp(a) Kasahara, Hiroyuki (a)
"Auxins are hormones that regulate many aspects of plant growth and development. Indole-3-acetaldoxime (IAOx) has been proposed to be a key intermediate in the synthesis of indole acetic acid (IAA) and several other indolic compounds. Genetic studies of IAA biosynthesis in Arabidopsis have suggested that 2 distinct pathways involving the *CYP79B* or *YUCCA (YUC)* genes may contribute to IAOx synthesis and that several pathways are also involved in the conversion of IAOx to IAA. We present the biochemical dissection of IAOx biosynthesis and metabolism in plants by analyzing IAA biosynthesis intermediates. We demonstrated that the majority of IAOx is produced by *CYP79B* genes in *Arabidopsis* because IAOx production was abolished in *CYP79B*-deficient mutants. IAOx was not detected from rice, maize, and tobacco, which do not have apparent *CYP79B* orthologues. IAOx levels were not altered in the *yuc1 yuc2 yuc4 yuc6* quadruple mutants, suggesting that the *YUC* gene family probably does not contribute to IAOx synthesis. We determined the pathway for conversion of IAOx to IAA by identifying 2 intermediates, indole-3-acetamide (IAM) and indole-3-acetonitrile (IAN), in *Arabidopsis*. When $^{13}C_6$-labeled IAOx was fed to *CYP79B*-deficient mutants, $^{13}C_6$-atoms were efficiently incorporated to IAM, IAN, and IAA. This biochemical evidence indicates that IAOx-dependent IAA biosynthesis, which involves IAM and IAN as intermediates, is not a common but a species-specific pathway in plants; thus IAA biosynthesis may differ among plant species."
(a) RIKEN Plant Science Center (b) Tokyo Metropolitan University (c) Forestry and Forest Product Research Institute (d) University of California at San Diego

M0802 "Like heat, L-N^G-monomethyl arginine (L-NMMA), an inhibitor of arginine-dependent nitric oxide (NO) production, blocks auxin signaling for gene expression and interferes with hormone-dependent cell expansion and division in cultured *Nicotiana glauca* guard cell protoplasts (GCP)."
Tallman, John G.-presenter gtallman@willamette.edu(a) Bufford, Jennifer L. (b) Anderson, David J. (a) Beard, Robert (a)
"At 32^{o}C, an auxin, 1-naphthaleneacetic acid (NAA) and a cytokinin, 6-benzylaminopurine (BAP) cause cultured GCP to expand 20-30 fold, deposit cell walls, re-enter the cell cycle and divide. Both NAA and BAP are required for GCP to survive in high percentages (50-80%) at 32^{o}C. After 9-12 h at 38^{o}C GCP survive in the same high percentages as at 32^{o}C but neither NAA nor BAP is required. GCP expand only 5-6 fold and neither deposit cell walls nor make the G1-to-S cell cycle transition. Transient gene expression analyses with thermostable *mGFP*-based reporters indicate that heat suppresses activation of the auxin-responsive BA promoter in cultured GCP, but not *mGFP* expression driven by the CaMV 35S constitutive promoter. Here we report that at the normally permissive temperature of 32^{o}C L-NMMA mimics the effects of sustained heat on GCP. Like heat, L-NMMA limits cell expansion; prevents cell wall deposition; eliminates the survival requirement for NAA and BAP; prevents the G1-to-S transition; and suppresses BA promoter activation (but not 35S CaMV promoter activity). These data suggest that at 32^{o}C arginine-dependent NO production is required for auxin signaling for gene expression and hormone-dependent cell expansion and cell division in cultured GCP."
(a) Department of Biology, Willamette University (b) Department of Botany, University of Hawaii

M0803 Transcytosis of PIN2 in arabidopsis is regulated by protein phosphatase 2A and PID kinase
Rahman, Abidur-presenter abidur@iwate-u.ac.jp(a) Takahashi, Maho (a) Shibasaki, Kyohei (a) Shuang, Wu (b) Tsurumi, Seiji (c) Baskin, Tobias I (b)
"PIN proteins, whose polarized deployment is essential for polar auxin transport, have recently been shown (in arabidopsis) to be targeted in part by transcytosis. The polarity shift of basally localized PIN proteins was shown to be induced by the fungal toxin, brefeldin A (BFA), through a GNOM-ARF GEF pathway. Here, we report that BFA affects PIN2 localization via protein phosphatase 2A (PP2A) and pinoid (PID) kinase. Long-term incubation in low concentration (10µ;M) BFA reduced root elongation and gravitropism and caused an irreversible shift in the polarity of basally localized PIN2 but not of PIN1. The altered PIN2 appears functional, insofar as the BFA treatment increased the rate of shoot-ward polar auxin transport. These responses (inhibition of elongation and gravitropism, altered PIN2 polarity) were induced by lower BFA concentrations (1 to 3µ;M) in protein phosphatase 2A (PP2A) mutants. In the mutant line complemented with PP2A-GFP, 10 µ;M BFA interfered with targeting of the reporter in cortical cells, as indicated by slower recovery from photobleaching, and intracellular localization. In contrast, BFA treatment did not alter the sub cellular localization of PID but enhanced its expression. Previously, this kinase has been shown, when over-expressed, to shift the polarity of PIN2 in the root cortex. Our results suggest that basal localization of PIN2 in the root cortex depends on a phosphorylation balance and further that BFA enhances the kinase activity, reversing the polarity of PIN2 and thereby altering auxin flux in the root. "
(a) Iwate University (b) Kobe University (c) University of Massachusetts, Amherst

M0804 Identification of new ABI1-mediated ABA signaling components in Arabidopsis.
Nishimura, Noriyuki-presenter nonishi@ucsd.edu(a) Sarkeshik, Ali (b) Nito, Kazumasa (c) Park, Sang-Youl (d) Wang, Angela (a) Lee, Stephen (a) Cutler, Sean (d) Chory, Joanne (c) Yates, John R (b) Schroeder, Julian I (a)

http://www-biology.ucsd.edu/labs/schroeder/
"Abscisic acid (ABA) regulates physiologically important stress and developmental responses. ABI1 encodes a protein phosphatase 2C that functions early in the ABA signaling cascade. To address the mechanism of ABI1-mediated ABA signaling, we generated tagged ABI1 Arabidopsis expression lines in an *abi1* knockout mutant and performed affinity column purification of ABI1-associated proteins. Transgenic tagged ABI1 plants show strong ABA insensitive phenotypes in seed germination, root elongation and stomatal responses. After silver staining, visible bands overlapped with controls, and specific bands associated with purified tagged ABI1 samples were consistently observed. Mass-spectrometrical analyses allowed identification of proteins associated with ABI1. These included some known ABA signaling components. These results suggested that this strategy has the potential of identifying new ABA signaling components. We found that a sub-group of a previously uncharacterized gene family interacted with ABI1 in an ABA dependent manner. The functional relationship between ABI1 and this protein family is being characterized."
(a) University of California, San Diego (b) The Scripps Research Institute (c) SALK Institute (d) University of California, Riverside

Start	End	**Event**	Location
4:45 PM	6:25 PM	**Minisymposium 9: Cell Cycle Regulation** - Chair: Dirk Inze 4:45 - M0901: Christine Foyer - *Redox homeostasis and regulation in the cell cycle* 5:10 - M0902: Dirk Inzé - *The molecular basis of organ growth* 5:35 - M0903: Hyun-Sook Pai - *Dual Functions of Nicotiana benthamiana Rae1 in Interphase and Mitosis* 6:00 - M0904: Yuh-Ru Julie Lee - *The WD40 repeat protein NEDD1 plays a role in microtubule organization during mitotic cell division in Arabidopsis thaliana*	Moloka'I 315

M0901 Redox homeostasis and regulation in the cell cycle
Foyer, Christine H-presenter christine.foyer@ncl.ac.uk(a) Pellny, Till K (b) Locato, Vittoria (b,c) Diaz Vivancos, Pedro (a) Markovic, Jelena (d) Pallardo, Federico V (d) De Gara, Laura (c)
"Cellular redox homeostasis plays an important role in the regulation of the plant cell cycle. However, little information is available on the precise functions of ascorbate, glutathione and pyridine nucleotides in this process. We therefore examined the the changes in these redox pools during the exponential growth of cultured Arabidopsis cells in relation to various cell cycle markers. In contrast to ascorbate and glutathione, which were present largely in the reduced forms, the pyridine nucleotide pools were highly oxidised over the period of exponential growth and only became more reduced once growth had ceased. The glutathione pool increased in parallel with poly (ADP-ribose) polymerase (PARP) activities and with the abundance of PARP1 and PARP2 mRNAs, at a time of high cell cycle activity, as indicated by transcriptome information. Marked changes in the intracellular partitioning of GSH between the cytoplasm and nucleus were also observed. Intracellular redox state was modulated during the growth cycle but redox homeostasis was maintained by interplay of the major redox pyridine nucleotides, glutathione and ascorbate pools. The correlation between PARP expression and activity and GSH accumulation and the finding that GSH can be recruited to the nucleus suggest a relationship between redox regulation and nuclear enzyme activity."
(a) University of Newcastle Upon Tyne (b) Rothamsted Research (c) CIR Universita Campus Bio-Medico, Rome (d) Depto. de Fisiologia, University of Valencia

M0902 The molecular basis of organ growth
Inze, Dirk G-presenter dirk.inze@psb.ugent.be(a) Gonzalez, Nathalie (a)
http://www.psb.ugent.be
"Many genes have been described in *Arabidopsis thaliana* that, when mutated or ectopically expressed, form larger structures, such as leaves or roots. These 'intrinsic yield genes' (IYGs) are involved in various processes whose interrelationship in mostly unknown (Gonzalez et al., 2009). Furthermore, all experiments carried out worldwide to measure the effects of IYGs on growth were performed under different conditions and using different *Arabidopsis* ecotypes, making comparisons virtually impossible. To this end, we have recently initiated a large-scale project 'Yield Booster' to compare the effects of IYGs in the same genetic background (Columbia 0) and to analyze the cellular and molecular bases underpinning the increased growth and biomass production. Kinematic analysis revealed that enhanced cell proliferation (and not cell expansion) is the main driving force of increased leaf growth, underpinning the central role of cell cycle. Various 'omics' technologies are being used to decipher the molecular networks orchestrating the observed growth effects. Genetic analysis demonstrated that several growth enhancing pathways are operational. The long-term goal is to develop computational models describing the molecular basis of plant organ growth and to use these models to improve crop productivity. Gonzalez, N., Beemster, G. T.S. and Inze, D. (2009). David and Goliath: What can the tiny weed Arabidopsis teach us to improve biomass production in crops? Curr. Opin. Plant Biol. In press "
(a) VIB Department of Plant Systems Biology, UGent

M0903 Dual Functions of *Nicotiana benthamiana* Rae1 in Interphase and Mitosis
Pai, Hyun-Sook-presenter hspai@yonsei.ac.kr(a)
"The nuclear pore complex protein Rae1 performs multiple functions in animal systems, acting in interphase as an mRNA export factor and during mitosis as mitotic checkpoint and spindle assembly regulators. In this study, we characterized multiple functions of Rae1 in plants. Virus-induced gene silencing of *Nicotiana benthamiana* Rae1, *NbRae1*, which encodes a protein with four WD40 repeats, resulted in growth arrest and abnormal leaf development. NbRae1 was mainly associated with the nuclear envelope during interphase, and NbRae1 deficiency caused accumulation of poly(A) RNA in the nuclei of leaf cells, suggesting defective mRNA export. In the shoot apex, depletion of NbRae1 led to reduced mitotic activities, accompanied by reduced CDK activity and decreased expression of cyclin B1, CDKB1-1, and histones H3 and H4. The secondary growth of stem vasculature was also inhibited, indicating reduced cambial activities. Differentiated leaf cells of *NbRae1*-silenced plants exhibited elevated ploidy levels. Immunolabeling in BY-2 cells showed that NbRae1 protein localized to mitotic microtubules and the cell plate-forming zone during mitosis, and recombinant NbRae1 directly bound to microtubules in vitro. Inhibition of NbRae1 expression in BY-2 cells using a β-estradiol-inducible RNAi system resulted in severe defects in spindle organization and chromosome alignment and segregation, which correlated with delays in cell cycle progression. Together, these results suggest that NbRae1 plays a dual role in mRNA export in interphase and in spindle assembly in mitosis."
(a) Yonsei University

M0904 The WD40 repeat protein NEDD1 plays a role in microtubule organization during mitotic cell division in *Arabidopsis thaliana*
Lee, Yuh-Ru Julie-presenter yjlee@ucdavis.edu(a) Zeng, Cui Jing Tracy (a) Liu, Bo (a)
"Microtubule (MT) organization depends on the evolutionarily conserved γ-tubulin complex. In plant cells, it is unclear how the activity of γ-tubulin-

dependent MT organization is regulated spatiotemporally during the cell cycle. An *A. thaliana* WD40 repeat protein, AtNEDD1, is homologous to the animal NEDD1/GCP-WD proteins which interact with the γ-tubulin complex. Using immunofluorescence microscopy, we have determined that AtNEDD1 decorated spindle MTs preferentially toward the spindle poles at metaphase in root meristematic cells. The protein appeared at phragmoplast MTs toward their distal minus ends at telophase. The *AtNEDD1* gene was essential, and the T-DNA insertional *nedd1* allele was only found in a heterozygous mutant state. Genetic analyses revealed that the *nedd1* allele severely affected fertility. Anti-tubulin staining showed that approximately half of the dividing microspores from the heterozygous mutant plant exhibited aberrant MT organization. In the dividing mutant microspores, spindles were no longer restricted to the cell periphery and underwent abnormal elongation at metaphase. The phragmoplast array was also affected as demonstrated by MT aggregation between reforming nuclei and the absence of a bipolar configuration. Consequently, defective microspores failed to form a continuous cell plate, and the generative cell was not produced. Our results suggest that AtNEDD1 plays a critical role in MT organization during mitosis, and its function is likely linked to that of the γ-tubulin complex."
(a) University of California, Davis

Start	End	**Event**	Location
4:45 PM	6:25 PM	**Minisymposium 10: Emerging Model Systems** - Chair: Janet Slovin 4:45 - M1001: Todd Michael - *Duckweeds as model aquatic plants* 5:10 - M1002: Janet Slovin - *Diploid strawberry (Fragaria vesca) a reference species for the Rosaceae family* 5:35 - M1003: John Vogel - *Brachypodium distachyon: a new model for the grasses* 6:00 - M1004: Todd Mockler - *Brachypodium distachyon Transcriptomics*	Maui 316A

M1001 Duckweeds as model aquatic plants
Michael, Todd P-presenter tmichael@waksman.rutgers.edu(a) Trumbull, Julia E (a) Wang, Wenqin (a) Zdepski, Ana (a) Lutz, Kerry (a) Kerstetter, Randall (a)
"*Lemna* has long been a model organism for biochemical studies and much of the pioneering work in plant photoperiodism was carried out with this organism. *Lemna*, commonly known as duckweed, is a member of an aquatic monocot family Lemnoideae, which also includes four additional genera *Spirodela*, *Landoltia*, *Wolffia* and *Wolffiella*. The individual plants range in size from 1.5 cm long (*Spirodela polyrhiza*) to less than one millimeter (*Wolffia globosa*). The base chromosome number of this Family is 10, and the genome sizes have a 10-fold range (150 to 1500 MB) representing diploids to octaploids. Many species of duckweeds are currently developed for industrial uses. For instance, the Environmental Protection Agency uses *Lemna minor* and *Lemna gibba* for ecotoxicological bioassays and for water quality testing, and other species are being developed as well. The plants readily grow on agricultural and municipal wastewater, an abundant, infinitely renewable, low-cost substrate that is not typically used for agricultural applications in developed countries. The plants are perennials with worldwide distribution; growing anywhere there is fresh water and sunlight. These tiny plants require little mechanical support or vascular tissue; the smallest members of the family completely lack differentiated xylem and phloem. As a result of expending little energy on supportive structures, the composition of vegetatively propagating fronds resembles maturing leaves with high levels of protein and carbohydrate and negligible lignin. *Spirodela*, *Lemna*, and *Wolffia* species form specialized over-wintering fronds, called turions that accumulate high levels of starch (40 to 70%). Their high starch content increases density causing them to sink to the bottom of the water column where they are more likely to survive freezing conditions. The change in density that accompanies starch accumulation would provide an ideal system for continual harvest of high starch fronds. Since duckweeds are small, morphologically reduced (although with root and leaf-like structure), fast-growing, easily cultivated under aseptic conditions, transformable, crossable, and particularly suited to biochemical studies (direct contact with media), it is an ideal system for biological research. Currently, the 150 Mb *Spirodela polyrhiza* genome is slated for sequencing by the DOE-JGI Community Sequencing Program (CSP)."
(a) Waksman Institute of Microbiology and Rutgers, The State University of New Jersey

M1002 Diploid strawberry (*Fragaria vesca*) a reference species for the Rosaceae family
Slovin, Janet-presenter Janet.Slovin@ARS.USDA.GOV(a) Shulaev, Vladimir (d) Folta, Kevin (b) Sargent, Daniel (c) Folkerts, Otto (d)
"Fresh and processed products of the Rosaceae plant family (almonds, apples, apricots, blackberries, peaches, pears, plums, cherries, strawberries, raspberries, roses) in the U.S. are valued at over $7 billion per year. Expansion of the genomics, genetics, and germplasm knowledge base of flower, fruit, and nut development, ripening, senescence, and microbial contamination is essential for maximizing and maintaining the quality of these crops. The diploid woodland strawberry, *Fragaria vesca*, has been developed as a system for rapid discovery in strawberry genetics and genomics for the Rosaceae family. *F. vesca*, has a genome size of ~200 Mb, and is a subgenome constituent of the polyploid cultivated strawberry. Advantages of *F. vesca* include: self-fertility, fecundity, small plant size, ~3.5 months generation time, diverse germplasm base, and very small genome with ~ 20x sequence coverage achieved using 454 technology. A diploid genetic map is populated with markers, documented inbred lines are available, and a highly efficient transformation system facilitates insertion mutagenesis and direct assessment of gene function with overexpression or RNAi. Substantial deep sequencing of transcripts from many tissues, developmental stages, and treatments has revealed a substantial number of sequences that do not share identity with transcripts from other species. Their function is being explored using RNAi and over-expression. A well-characterized *F. vesca* system enables us to develop useful assays to evaluate genes for their function in plant stress responses, fruit quality, disease resistance and a host of other horticulturally relevant traits important to the Rosaceae family as a whole."
(a) US Dept. of Ag./Agric. Research Serv. (b) University of Florida (c) East Malling Research (d) VBI, Virginia Tech

M1003 *Brachypodium distachyon*: a new model for the grasses
Vogel, John-presenter john.vogel@ars.usda.gov(a) Bragg, Jennifer (a) Anderson, Olin (a) Garvin, David (g) Bevan, Michael (h) Mayer, Klaus (b) Wu, Jiajie (c,a) Rokhsar, Daniel (d) Schmutz, Jeremy (e) Mockler, Todd (f) Huo, Naxin (c,c) Gu, Yong (a) Lazo, Gerard (a)
http://brachypodium.pw.usda.gov/
"*Brachypodium distachyon* (Brachypodium) is rapidly emerging as a model system to study questions unique to the grasses. This emergence is coincident with an increased need for basic research in grass biology to develop perennial grasses as a source of renewable fuel. The list of genomic resources available to Brachypodium researchers is increasing exponentially. We recently completed the sequencing and analysis of the entire Brachypodium genome using a whole genome shotgun sequencing strategy based on Sanger sequencing. The vast majority (99.6%) of the 272 Mb of genomic sequence was assembled into 10 scaffolds ranging from 8 to 38 Mb. These scaffolds were then verified and arranged into five chromosome scale assemblies using a high-density SNP-based genetic linkage map. Automated annotation of this compact genome revealed ~25,500 genes strongly supported by transcriptome sequencing. With the Brachypodium genome sequence in hand we can examine the relationship between

genomes from the three major groups of grasses; the Panicoids (Sorghum), the Bambusoids (rice) and the Pooids (Brachypodium). Other resources we have developed include: an extremely efficient Agrobacterium-mediated transformation protocol (average efficiency 44%), BAC libraries, BAC end and EST sequences, a physical map, and mutagenesis protocols. In addition, the generation of a sequence-indexed insertional mutant population is underway with >3,000 mutants generated to date. When taken together, these resources enable researchers to utilize Brachypodium for a wide array of experimental approaches, including those that require complete genome sequence. An overview of the Brachypodium genome project, Brachypodium resources and the identification of mutants relevant to biomass crops will be presented."
(a) USDA-ARS Western Regional Research Center (b) Munich Information Center for Protein Sequences (c) University of California, Davis (d) DOE Joint Genome Institute (e) Hudson Alpha Institute of Biotechnology (f) Oregon State University (g) USDA-ARS Plant Science Research Unit (h) John Innes Centre

M1004 *Brachypodium distachyon* Transcriptomics
Priest, Henry D. (a) Fox, Samuel E. (a) Scott, Givan A. (a) Filichkin, Sergei A. (a) Michael, Todd P. (b) Mockler, Todd C.-presenter tmockler@cgrb.oregonstate.edu(a)
http://mocklerlab.cgrb.oregonstate.edu/
"*Brachypodium distachyon* is a model for temperate grasses and bioenergy crops. To facilitate genomic studies in Brachypodium we used Illumina (Solexa) sequencing to sample a collection of cDNA libraries representing a diverse array of tissues, treatments and developmental stages. The resulting EST data were aligned to the Brachypodium genome and used to assemble transcriptional units, including alternative splice variants. The high depth of sequencing and broad unbiased coverage available from the Illumina platform increases the chance of identifying low abundance transcripts. Our analysis provides a comprehensive view of the Brachypodium transcriptome and facilitates annotation efforts. We used our empirical annotation of the transcriptome to aid design of a versatile oligonucleotide microarray platform that includes exon scanning and genome tiling features. We are using these arrays to generate a Brachypodium expression atlas comparing tissues over development, diurnal and circadian time-courses, and stress conditions. This atlas will provide a hypothesis-generating foundation for elucidating the transcriptional networks underlying traits of major importance economically important crops including wheat, barley and potential bioenergy grass crops. "
(a) Department of Botany and Plant Pathology and Center for Genome Research and Biocomputing, Oregon State University, Corvallis, Oregon, 97331 (b) Waksman Institute of Microbiology, Rutgers, Piscataway, NJ, 08854

Start	End	**Event**	Location
4:45 PM	6:25 PM	**Minisymposium 11: Abiotic Stress** - Chair: Joerg Kudla 4:45 - M1101: Joerg Kudla - *Regulation and function of calcium sensor proteins and their interacting kinases in abiotic stress responses* 5:10 - M1102: June M. Kwak - *Two MAP kinases preferentially expressed in guard cells positively regulate ROS-mediated ABA signaling* 5:35 - M1103: Hargurdeep S. Saini - *Enhancement of salinity tolerance by engineering a chloride-volatilizing enzyme into plants* 6:00 - M1104: Shutian Li - *Nuclear activity of ROXY1, a glutaredoxin interacting with TGA factors, promotes petal development in Arabidopsis*	Maui 316C

M1101 Regulation and function of calcium sensor proteins and their interacting kinases in abiotic stress responses
Held, Katrin (a) Eckert, Christian (a) Waadt, Rainer (a) Batistic, Oliver (a) Kudla, Joerg-presenter jkudla@uni-muenster.de(a)
"Calcium serves as a critical messenger in many adaptation processes and in abiotic stress responses. Calcium-binding proteins are involved in sensing and relaying these signals to downstream signaling and adaptation responses. Calcineurin B-like proteins (CBLs) represent a group of calcium sensor proteins that are closely related to Calcineurin B and Neuronal Calcium Sensors (NCS). CBLs interact with a group of serine-threonine kinases designated as CBL-interacting protein kinases (CIPKs). In Arabidopsis, 10 CBL-type calcium sensor proteins form an interaction network with 26 CIPKs. Preferential complex formation of individual CBLs with defined subsets of CIPKs appears to be one of the mechanisms generating the temporal and spatial specificity of calcium signals in plant cells. Reverse genetics and cell biological approaches have begun to unravel the functional principles of this signaling network. I will present results of our identification of novel *cbl* loss-of-function mutants that function in salt stress tolerance and of our investigation of the sub-cellular localization of all CBLs from Arabidopsis. Moreover, I report that dual lipid modification by myristoylation and palmitoylation of CBL1 is crucial for proper function in salt stress signaling and determines the membrane targeting of CBL/CIPK complexes. In addition, our reverse genetics analyses indicate that alternative complex formation of CIPK-type kinases with different CBLs, enables simultaneous regulation of the extrusion of Na^+ ions in root tissues and the sequestration of Na^+ into the vacuole in green tissues. Moreover, CBL proteins, which were initially identified as salt stress signaling components, mediate the regulation of multiple ion transport processes in the plant cell. "
(a) Universitaet Muenster, Institut fuer Botanik

M1102 Two MAP kinases preferentially expressed in guard cells positively regulate ROS-mediated ABA signaling
Kwak, June M.-presenter jkwak@umd.edu(a) Song, Charlotte (a) Shin, Dong-Jin (a) Takeda, Koji (a) Gu, Dan (a) Cho, Daeshik (a) Lee, Sangmee (a) Giordo, Roberta (a,b) Ellis, Brian (c) Leonhardt, Nathalie (d)
"Abscisic acid (ABA) plays an essential role in protection of plants from environmental stresses such as drought, salt, and cold. Reactive oxygen species (ROS) have been suggested to function in guard cell ABA signaling. To further genetically dissect guard cell ABA-ROS signaling, we identified two MAPK genes, GCMPK3 and GCMPK4, that are preferentially and highly expressed in guard cells. To provide direct genetic evidence, RNAi-based gene silencing plant lines were generated in which both genes are simultaneously silenced. In parallel, Arabidopsis single and double mutants carrying deleterious point mutations in these genes were identified. ABA-induced stomatal closure was strongly impaired in two independent RNAi lines in which both GCMPK3 and GCMPK4 transcripts were significantly silenced. Consistent with this result, gcmpk3-1/4-1 double mutants showed an enhanced transpirational water loss and ABA- and H2O2-insensitive response in stomatal movement assays, whereas mutants carrying a mutation in one of these genes did not show any altered phenotype, indicating functional redundancy in these genes. A GCMPK4-YFP fusion construct rescued the gcmpk3-1/4-1 double mutant phenotype in ABA-induced stomatal movements, demonstrating that the mutations in these genes caused the phenotype. GCMPK4 protein is localized in the cytosol and the nucleus, and ABA enhances the protein kinase activity of GCMPK4. Together, these results provide genetic evidence that GCMPK3 and GCMPK4 function downstream of ROS to positively regulate guard cell ABA signaling."
(a) University Of Maryland (b) University of Sassari (c) University of British Columbia (d) CNRS-CEA-Universite Aix-Marseille II

M1103 Enhancement of salinity tolerance by engineering a chloride-volatilizing enzyme into plants
Saini, Hargurdeep S.-presenter hsaini@uwaterloo.ca(a) Kaur, Simendeep (b) Babayeva, Sima (b) Koonjul, Priyum (c)

"Several organisms possess enzymes that can catalyze one-step methylation of Cl^- ions to chloromethane gas using *S*-adenosyl-L-methionine as methyl donor. Presence of this enzyme in organisms that live in saline habitats has been interpreted as a mechanism for Cl^- detoxification via its volatilization (Science 249: 160-162), but this possibility has never been experimentally tested. While searching for chloride-methylating enzymes in plants, we identified a thiol methyltransferase (TMT) in cabbage that, aside from its natural role in the methylation of thiol compounds produced upon glucosinolate hydrolysis, was also able to methylate Cl^- ions with greater efficiency than any other similar enzyme reported (J Biol Chem 270: 9250-9257; Plant Cell Environ. 23: 165-174). We cloned the gene encoding this TMT (Plant Mol. Biol. 50: 511-521), and engineered it under the control of CaMV 35S promoter into tobacco, which otherwise lacks the ability to methylate Cl^-. Transgenic tobacco plants acquired the ability to efficiently transform Cl^- to chloromethane over extended periods, parallel with a dramatic enhancement in their salinity tolerance. Whereas both wild type and transgenic plants grew normally in 50 NaCl, transgenic plants grew significantly better at higher concentrations of the salt. The latter were able to complete their life cycle and produce viable seed at 200 mM NaCl, which was lethal to the wild-type plants. The results convincingly demonstrate that volatilization of Cl^- is a detoxification event, which can contribute to the plant's ability to withstand salinity stress. This ability can, therefore, be used to engineer crop species with enhanced salt tolerance. The impact of Cl^- volatilizing transgenic plants on the atmospheric budget of chloromethane will, however, require careful estimation before such plants could be introduced in the field. "
(a) Faculty of Environment, University of Waterloo, Waterloo, Canada (b) IRBV, Universite de Montreal, Montreal, Canada (c) Valeo Management L.P., Montreal, Canada

M1104 "Nuclear activity of ROXY1, a glutaredoxin interacting with TGA factors, promotes petal development in Arabidopsis "
Li, Shutian-presenter shutian.li@biologie.uni-osnabrueck.de(a,b) Lauri, Andrea (a) Ziemann, Mark (a,c) Busch , Andrea (a,b) Bhave, Mrinal (c) Zachgo, sabine (a,b)
"Glutaredoxins (GRXs) are ubiquitous glutathione-dependent oxidoreductases that catalyze reversible reduction of disulfide bonds and regulate protein activities in a variety of cellular processes. In plants, three subclasses of GRXs are defined according to their respective active sites. GRXs with the CPYC and CGFS active sites are common to pro- and eukaryotes, while GRXs with the CC-type motif have so far only been identified in land plants. *ROXY1*, encoding a CC-type GRX, is known to regulate petal primorida initiation and further petal morphogenesis in Arabidopsis. Intracellular localization studies revealed a nucleocytoplasmic expression of ROXY1. However, exclusively redirecting ROXY1 either to the cytoplasm or the nucleus proved that nuclear localization of ROXY1 is indispensable and thus crucial for its activity in flower development. Yeast two-hybrid screens identified TGA factors as ROXY1-interacting proteins and their nuclear interactions in planta were further confirmed using BiFC assays. Overlapping expression patterns of *ROXY1* and TGA genes during flower development support their biological relevance in petal development. Deletion analysis demonstrates the importance of the C-terminus for its functionality and for mediating ROXY1/TGA protein interactions. Phenotypic analysis of the *roxy1 pan* double mutant and an engineered chimeric repressor mutant from *PERIANTHIA* indicates a dual role of ROXY1 in petal development. Collectively, our data show that nuclear activity of ROXY1 controls petal primordial formation likely by modifying PAN posttranslationally. Additionally, ROXY1 affects later petal morphogenesis probably by modulating other TGA factors that act redundantly during differentiation of second whorl organs. "
(a) Max-Planck Institute for Plant Breeding Research (b) Department of Botany, University of Osnabrueck (c) Environment and Biotechnology Centre, Faculty of Life and Social Sciences, Swinburne University of Technology

Start	End	**Event**	Location
4:45 PM	6:25 PM	**Minisymposium 12: Light Signalling** - Chair: Robert Larkin 4:45 - M1201: Robert M. Larkin - *Integration of light and plastid signals* 5:10 - M1202: Md. Sayeedul Islam - *Photoreceptor systems for light-dependent intracellular positioning of mitochondria in Arabidopsis thaliana* 5:35 - M1203: Meng Chen - *HEMERA, an essential regulator linking phytochrome nuclear bodies and light signaling in Arabidopsis* 6:00 - M1204: Hongtao Liu - *Blue light-specific regulation of CIB1 protein expression in Arabidopsis*	O'ahu 313A

M1201 Integration of light and plastid signals
Ruckle, Michael E. (a,b) Larkin, Robert M.-presenter larkinr@msu.edu(a,b)
http://www.prl.msu.edu/Facultypages/larkin.html
"Light and plastid signals promote chloroplast biogenesis and are among the most potent inducers and repressors of photosynthesis-related gene expression, respectively. These signals can be likened to a gas and brake system that promotes efficient chloroplast biogenesis and function. To learn more about the regulation of photosynthesis-related gene expression by plastid signals we performed a new *genomes uncoupled* (*gun*) mutant screen in *Arabidopsis thaliana*. The expression of genes that encode proteins active in photosynthesis is uncoupled from chloroplast function in *gun* mutants, and the expression of the nuclear and the plastid genomes is uncoupled in particular *gun* mutants. To identify mutants with defects in both light and plastid signals, we screened this new collection of *gun* mutants for photomorphogenic phenotypes and found that *cryptochrome 1* (*cry1*) mutants are *gun* mutants. Our subsequent genetic analysis of plastid and light signaling mutants indicates that a plastid signal can rewire a light signaling network, converting it from a positive to a negative regulator of photosynthesis-related genes such as those that encode the light-harvesting chlorophyll *a*/*b*-binding proteins and the small subunit of Rubisco. We found that these interactions contribute to chloroplast biogenesis, especially in high-intensity light conditions. We also found that plastid signals can broadly affect photomorphogenesis and that plastid and light signaling can promote or antagonize each other, depending on the response studied, thus providing evidence that plastid signals can help integrate chloroplast biogenesis with photomorphogenesis. To test these ideas further, we are screening for factors that contribute to this integration of light and plastid signals. The current status of the work will be presented."
(a) Michigan State University-Department of Energy Plant Research Laboratory, Michigan State University (b) Department of Biochemistry and Molecular Biology, Michigan State University

M1202 Photoreceptor systems for light-dependent intracellular positioning of mitochondria in Arabidopsis thaliana
Islam, Md. Sayeedul-presenter islam@bio.sci.osaka-u.ac.jp(a) Niwa, Yasuo (b) Takagi, Shingo (a)
"While mitochondria movement in plant cells is actin- and/or microtubule-dependent, light-dependent intracellular positioning and movement of mitochondria remain to be elucidated. We asked whether mitochondria in leaf palisade cells of Arabidopsis thaliana stably expressing mitochondria-targeted GFP occupy different intracellular positions under different light conditions. The pattern of light-dependent redistribution of mitochondria was essentially identical to that of chloroplasts, namely, mitochondria occupy the periclinal regions under weak blue light (wBL; 470 nm, 4 µ;mol m-2s-1) and the anticlinal regions under strong blue light (sBL; 100 µ;mol m-2s-1), respectively. Strong red light (660 nm, 100 µ;mol m-2s-1) had a small effect to induce accumulation of mitochondria on the inner periclinal regions. We semi-quantitatively analyzed the mode of movement of

individual mitochondria along the outer periclinal walls. Within 30 min of BL illumination, mitochondria movement was acclerated in a fluence-rate dependent manner, whereas it was gradually decelerated resulting in co-localization with chloroplasts. In the presence of a photosynthetic inhibitor DCMU, the normal accumulation and avoidance responses of chloroplasts were impaired. In addition, mitochondria no longer took any specific positions and were distributed all over the cytoplasm regardless of fluence rate of BL. These results strongly suggest the involvement of different photoreceptors in the regulation of mitochondria movement, namely, phototropins for the early acceleration and photosynthesis for the late deceleration. "
(a) Osaka University (b) Shizuoka University

M1203 "HEMERA, an essential regulator linking phytochrome nuclear bodies and light signaling in *Arabidopsis*"
Chen, Meng-presenter chen.meng@duke.edu(a,b) Galvao, Rafaelo M (a) Li, Meina (a) Burger, Brian (b) Bugea, Jane (b) Chory, Joanne (b,c)
http://www.plasticgenome.org/
"Phytochromes are red and far-red photoreceptors regulating every facet of plant development and growth. Two early phytochrome signaling events have been described: (A) Light directly regulates the relocation of phytochrome A (phyA) and phytochrome B (phyB) from the cytoplasm to the nucleus, where they interact and colocalize with a group of bHLH transcription factors (PIFs) on discrete subnuclear foci called phytochrome nuclear bodies and regulate transcription; (B) Light triggers rapid degradation of phyA and some of the PIFs. The function of phytochrome nuclear bodies in relationship to phytochrome signaling events is unknown. We carried out a unique genetic screen looking for phyB:GFP mislocalization mutants. This screen identified a novel photomorphogenetic mutant, *hemera* (*hmr*). Strikingly, besides defects in phyB:GFP nuclear body formation, the *hmr* mutant is impaired in all phytochrome responses examined, including chloroplast biogenesis and phyA degradation, suggesting that HMR is an essential regulator linking phytochrome nuclear body formation and light signaling. The tall and albino phenotypes of *hmr* make it the founding member of a new class of photomorphogenetic mutant. In addition, the *hmr* mutant is the first phytochrome signaling mutant defective in phyA proteolysis, which suggests a biochemical role of HMR and phytochrome nuclear bodies in protein degradation. Further characterization of HMR will likely to provide great insight into the mechanistic link between phytochrome nuclear bodies and early phytochrome signaling events."
(a) Department of Biology, Duke University, Durham, NC 27708, USA (b) Plant Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 29037, USA (c) Howard Hughes Medical Institute, The Salk Institute for Biological Studies, La Jolla, CA 92037, USA

M1204 Blue light-specific regulation of CIB1 protein expression in Arabidopsis
Liu, Hongtao-presenter liuhongtao76@gmail.com(a) Lin, Chentao (a)
"Cryptochromes (CRY) are photolyase-like blue-light receptors that mediate light responses in plants and animals. How plant cryptochromes act in response to blue light is not well understood. We have recently reported the identification and characterization of the Arabidopsis CIB1 (cryptochrome-interacting basic-helix-loop-helix). CIB1 interacts with CRY2 (cryptochrome 2) in a blue light-specific manner, and it acts with additional CIB1-related proteins to promote CRY2-dependent activation of FT gene expression and floral initiation and. CIB1 binds to the FT protmoter in vivo. We proposed that the blue light-dependent interaction of cryptochrome(s) with CIB1 and CIB1-related proteins represents an early photoreceptor signaling mechanism in plants. Consistent with our hypothesis that CIB1 is specifically involved in signaling of blue light receptor CRY2, we discovered that CIB1 protein expression is regulated specifically by blue light. CIB1 protein is degraded in the absence of blue light. CIB1 is degraded, via a ubiquitin/proteasome pathway, in the dark, red, and FR light. CIB1 degradation is suppressed in blue light, resulting in accumulation of CIB1 in blue light. Possible molecules associated with CIB1 expression will be discussed"
(a) MCDB,University of California, Los Angeles

MINISYMPOSIA/ TALKS – TUESDAY, JULY 21

Start	End	**Event**	Location
4:40 PM	6:10 PM	PSA Talks **Algal Phylogenetics & Taxonomy-IV** 4:40 –P05011: Ed Theriot - *A preliminary multigene phylogeny of the diatoms* 4:55 –P05025: Elizabeth Ruck - *Comparing chloroplast, nuclear, and mitochondrial phylogenies in the Surirellales (Bacillariophyta)* 5:10 – P05024: Matt Ashworth - *Holes and poles: a molecular approach to the phylogeny of the ocellate and pseudocellate diatoms* 5:25 –P05028: Teofil Nakov - *Preliminary molecular phylogeny of the Cymbellales (Bacillaryophyceae)* 5:40 – P05012: Cheong Xin Chan - *Rampant gene transfer in dinoflagellates and its implications to the tree of life* 5:55 - P05007: Sung Mi Cho - *Phylogenetic relationships of Heterokontophyta based on six genes data*	Ni'ihau 312

P05011 A preliminary multigene phylogeny of the diatoms
Theriot, Edward C.-presenter etheriot@mail.utexas.edu(a) Ruck, Elizabeth (a) Ashworth, Matthew (a) Nakov, Teofil (a) Jansen, Robert K. (a)
"Until recently, formal inferences on the diatom phylogeny have been based entirely on the nuclear encoded small subunit of the ribosomal gene (nSSU). Where multiple genes have been employed, the focus has been on taxa within diatoms. Here we analyze the potential of chloroplast genes (*rbc*L and *psb*C)to contribute to our understanding of the diatom phylogeny. These genes have been used successfully at lower taxonomic levels in diatoms. Preliminary results indicate that signal to noise ratios may be low, but that the chloroplast genes have great potential to aid in the inference of the overall diatom phylogeny. We will also formally test traditional and recently proposed phylogenetic hypotheses for the diatoms."
(a) University of Texas at Austin

P05025 "Comparing chloroplast, nuclear, and mitochondrial phylogenies in the Surirellales (Bacillariophyta)"
Ruck, Elizabeth C.-presenter eruck@mail.utexas.edu(a) Theriot, Edward C. (a)
"The order Surirellales currently consists of 9 genera in 3 families: Entomoneidaceae, Auriculaceae, and Surirellaceae. Surirellales species exhibit diverse valve morphologies and are considered the most advanced of the pennate diatoms due to the possession of canal raphe systems and elaborate keels. Although Surirellales monophyly has been supported in previous analyses of both morphological and molecular datasets, inadequate taxon sampling has always been a concern and the phylogenetic relationships at the family level and below have not been examined. As part of a large-scale phylogenetic study of the Surirellales, morphological characters and DNA sequence data from the nuclear, chloroplast, and mitochondrial genomes have been gathered from all 3 families and 8 of the 9 genera. We present preliminary estimates of Surrirellales phylogeny from these datasets, analyzed individually and in combination. Monophyly of the families and major genera, and phylogenetic congruence between morphological and DNA sequence data, are evaluated and discussed."
(a) University of Texas at Austin

P05024 Holes and poles: a molecular approach to the phylogeny of the ocellate and pseudocellate diatoms
Ashworth, Matt-presenter mashworth@mail.utexas.edu(a) Theriot, Ed C (b)
"Inferring the evolutionary relationships of the ocellate and pseudocellate diatoms is a problem that can no longer be ignored as the distinction between the araphid pennate and multipolar centric diatoms has become blurred by the increased use of molecular markers in phylogenetic surveys. These diatoms have previously been classified based on the presence of elevated pore fields known as ocelli (if they are enclosed by a solid rim) or pseudocelli (if they are not enclosed by a distinct rim), pore structure on the valves, presence of external or internal ribs on the valves, and even the number of angles, or poles, on each valve. However, these characters overlap so much between taxa that there is still much uncertainty regard to the evolutionary relationships of these wide-ranging, primarily marine diatoms. Presented as an example in this talk, strains of individual taxa such as *Biddulphiopsis titiana* are capable of expressing bipolar and tripolar valve morphology. We have begun to reconstruct the phylogeny of these diatoms using nuclear (SSU rRNA) and plastid (*rbc*L and *psb*C) markers and find that the presence of ocelli might not be as useful a diagnostic character as previously thought."
(a) Section of Integrative Biology, University of Texas, Austin (b) Texas Memorial Museum, University of Texas, Austin

P05028 Preliminary molecular phylogeny of the *Cymbellales* (*Bacillaryophyceae*)
Nakov, Teofil-presenter teofil.nakov@mail.utexas.edu(a) Theriot, Edward C. (a)
"As currently circumscribed, the order *Cymbellales* Mann (*Bacillaryophyceae*) contains taxa that exhibit both isogamy (e.g. *Rhoicosphenia* Grunow) and physiological anisogamy (members of the *Anomoeoneidacae* Mann, *Cymbellaceae* Greville and *Gomphonemataceae* Kutzing). The different modes of sexual reproduction in conjuction with the wide array of variation in frustule morphology and symmetry raise doubts over the monophyly of the order. This is further confounded with the possible plesiomophic state of chloroplast morphology, previously thought to be a synapomorphy for the *Cymbellales*. We test the monophyly of the order *Cymbellales* (*Bacillariophyceae*) and relationships between genera currently included of the order using molecular data from the nuclear and chloroplast genomes. Preliminary analyses indicate i) problems in identifying the sister group to the order, ii) basal position of *Rhoicosphenia* and *Anomoeoneis* within the order and iii) unclear inter-generic relationships between *Cymbella*, *Gomphonema* and *Placoneis* due to moderate bootstrap support of higher nodes. The monophyly of the genera *Encyonema* and *Placoneis* is strongly supported whereas *Gomphonema* and *Cymbella* appear to be paraphyletic lineages containing species of *Gomphoneis* and *Cymbopleura* . The need for a more complete taxon sampling and inclusion of more molecular markers in inferring relationships of the *Cymbellales* at the family or genus level is emphasized. "
(a) University of Texas at Austin

P05012 Rampant gene transfer in dinoflagellates and its implications to the tree of life
Chan, Cheong Xin-presenter chancx@gmail.com(a,c) Moustafa, Ahmed (b,c) Bhattacharya, Debashish (a,c)
http://cyanophora.biology.uiowa.edu/home/

"Dinoflagellates, known for their phagotrophic nature, can ingest food particles or extract cell contents from prey through myzocytosis. The capture of an exogenous cell is used to explain plastid origin through endosymbiosis, whereby the ingested prey (endosymbiont) is permanently maintained as an organelle, resulting in endosymbiotic gene transfer (EGT) from the prey to the host genome. Whereas previous studies have shown that dinoflagellates underwent multiple endosymbioses, the extent of gene uptake in the dinoflagellate nucleus *via* EGT or other means of lateral gene transfer (LGT) is yet to be examined in a rigorous manner. In this study, we adopt a systematic, phylogenomic approach to investigate the origins of dinoflagellate nuclear genes using 12,329 contigs of expressed sequence tags (ESTs) from the causative agent of 'red tides', *Alexandrium tamarense*. We found at least 6,227 (50.5%) of these genes to be exclusive to *A. tamarense*, whereas the remaining 6,102 genes (49.5%) arose from a diverse collection of non-lineal eukaryotic and prokaryotic sources, encompassing all major phyla in the tree of life (ToL). We identified 3,995 genes (32.4%) that have arisen *via* non-lineal gene transfer. The (putatively) transferred genes encode a variety of functions, including some that are plastid- and/or mitochondrion-targeted. Our results demonstrate remarkably diverse gene origins in dinoflagellate owing to rampant gene transfer between dinoflagellates and other lineages of unicellular organisms. This exciting finding however suggests that resolving the dinoflagellate branch of the ToL may pose a great challenge due to their highly chimeric genomes."
(a) The Roy J. Carver Center for Comparative Genomics and Department of Biology, University of Iowa (b) Interdisciplinary Program in Genetics, University of Iowa (c) Present address: Department of Ecology, Evolution and Natural Resources and Institute of Marine and Coastal Science, Rutgers University

P05007 Phylogenetic relationships of Heterokontophyta based on six genes data
Cho, Sung Mi-presenter smcho@cnu.ac.kr(a) Boo, Ga Hun (a) Cho, Ga Youn (b) Yoon, Hwan Su (c) Andersen, Robert (c) Boo, Sung Min (a)
"Much of evolutionary uncertainty of photosynthetic stramenopiles (Heterokontophyta) arises from limited and patchy molecular data, many of which are from either single gene sequences or a complete absence of sequence data from key species. We analyzed six genes such as *psa*A, *psb*A, *psb*C, and *rbc*L in plastid and LSU and SSU in nucleus from 51 heterokontophytes, covering representatives of all 16 classes in the division. Although separate gene analysis revealed less resolution on phylogeny of heterokontophytes, the combined tree of six genes data improved resolution that the heterokontophytes are monophyletic, consisting of basal and crown clades. Dictyochophyceae was basal, and then Pinguiophyceae followed. These two classes share the scattered genophore and proximal direction and number of transitional helix as plesiomorphy. Crown groups consisted of four subclades, each of which was strongly supported; Eustigmatophyceae was a sister to the clade of Synurophyceae and Chrysophyceae. Bacillariophyceae was closely related to Bolidophyceae. The clade including Raphidophyceae, Xanthophyceae, Phaeothamniophyceae, Schizocladiophyceae and Phaeophyceae was strongly supported. However, Pelagophyceae was independent from other groups. Morphological transformation of all clades is discussed. "
(a) Chungnam National University, Korea (b) National Institute of Biological Resources, Korea (c) CCMP, Bigelow Laboratory for Ocean Sciences, USA

Start	End	Event	Location
4:40 PM	5:55 PM	PSA Talks **Algal Ecology & Population Biology II** 4:40 –P04010: Michael Stekoll - *Competition between and co-existence of algal crusts and subtidal kelps* 4:55 –P04014: Charles Amsler - *Filamentous algal endophytes in macrophytic Antarctic algae: prevalence in hosts and palatability to mesoherbivores* 5:10 –P04022: Bruce Parker - *Shrinkage and disappearance of Mountain Lake, Virginia, USA* 5:25 –P05001: Poonam Sharma - *Studies on the taxonomy and biodiversity of microalgae occurring in fresh water habitats of Shivalik Himalayas of Jammu and Kashmir, India: Applications in aquaculture, pollution and bioremediation.* 5:40 –P04001: Nathan Smucker - *Acid mine drainage and remediation impacts on lotic biofilm structure and extracellular enzyme activities during succession*	Maui 316B

P04010 Competition between and co-existence of algal crusts and subtidal kelps
Daniel, Okamoto K. (b) Stekoll, Michael S.-presenter ffmss@uaf.edu(a,b) Eckert, Ginny L. (b)
"Understanding coexistence among dominant plant guilds often requires identifying the nature of interactions among coexisting competitors. Using a combination of field surveys, plus laboratory and field experiments we investigated the role of dominant subtidal encrusting algae in inhibiting recruitment of kelp species. In laboratory experiments kelp spores were observed to recruit on red encrusting coralline algae. Bur very little kelp recruitment occurred on either red or brown algal crusts, although kelp spores appeared to settle and germinate on the brown crusts. Removal of crusts in field plots drastically increased recruitment success of kelps. In unscraped plots kelp recruits did not appear on crusts, but appeared only on non-crust surfaces in non-trivial numbers, presumably due to high reproductive output. This inhibitory interaction by the algal crusts may explain the exclusion of kelps in some crust-dominated habitats, especially if combined with reductions in zoospore supply or survivorship of recruits. Yet despite inhibition of kelp recruitment by crustose algae, the high reproductive capacity of kelps likely compensates for the competitive space-occupancy advantage of algal crusts, possibly explaining why this interaction may not always translate to competitive exclusion of kelps in nature. "
(a) University of Alaska Southeast (b) University of Alaska Fairbanks

P04014 Filamentous algal endophytes in macrophytic Antarctic algae: prevalence in hosts and palatability to mesoherbivores
Amsler, Charles D-presenter amsler@uab.edu(a) Amsler, Margaret O (a) McClintock, James B (a) Baker, Bill J (b)
"Five individuals each from thirteen common species of large macroalgae ('macrophytes') from the western Antarctic Peninsula were surveyed for the presence of filamentous algal endophytes. Of the thirteen species surveyed, endophytes were rare or absent in five. The remaining species all supported endophytes in most or usually all individuals with maximum endophyte densities per species ranging from 3% to 75% of the thallus area. Thallus fragments with endophytes were placed into culture and 99 unialgal, filamentous brown algal strains were isolated. The ITS1 gene was sequenced in each strain to sort these into distinct genotypes. Ten distinct filamentous brown algal genotypes were present. Green endophytes did not grow well in culture and only two such species were isolated. No-choice feeding rate bioassays were performed with thallus fragments of all 13 macrophyte species and with cultures of nine filamentous endophyte species. Feeding rates on the endophytes were 2-3 orders of magnitude higher than rates on 12 of the macrophyte species and 2- to 6-fold higher than on the only truly palatable macrophyte. These data support the hypothesis that antarctic macrophytes are commonly endophytized and that the endophytes benefit from the association by being protected, at least in part,

from amphipod herbivory."
(a) University of Alabama at Birmingham (b) University of South Florida

P04022 "Shrinkage and disappearance of Mountain Lake, Virginia, USA"
Parker, Bruce C.-presenter genera@vt.edu(a) Rosenzweig, Michael S. (a)
"Mountain Lake, Virginia is an oligotrophic lake with an incompletely sealed natural dam comprised of Silurian Clinch sandstone boulders. During periods of prolonged low precipitation, subterranean losses of water through the dam can exceed inputs, resulting in lake shrinkage. Previous investigations have suggested that this lake may have drained at least six times within the last 4,100 years. A 30% decline in annual precipitation since 1972 has accompanied more frequent shrinkage and refilling episodes in recent years, and since 2004 the lake reached an entirely empty state by September 30, 2008. The preceding months of June-August, 2008 were the most devastating, involving changes in chemical composition, phytoplankton algae, and total loss of all aquatic macrophytes and resident fauna. "
(a) Virginia Tech, Biological Sciences

P05001 "Studies on the taxonomy and biodiversity of microalgae occurring in fresh water habitats of Shivalik Himalayas of Jammu and Kashmir, India: Applications in aquaculture, pollution and bioremediation."
Sharma, Poonam -presenter poonambot2005_ju@yahoo.co.in(a) Anand, Vijay Kumar (a) Jatindra, Priya (a)
"Shivalik Himalayas of Jammu and Kashmir represents the lower Western Himalayas that constitute the hills of moderate elevation, dipped gently towards the south with an altitudinal range of 300-1200 m above m.s.l. This region of J&K State is blessed with a vast expanse of Inland waters ie, rivers, streams, canals, lakes, groundwater impoundments, estuaries etc., which harbors extra-ordinarily rich and unique microalgal diversity.There are severe threats to these freshwater resources like construction of dams and barrages, siltation, anthropogenic pressure, soil erosion, water pollution from industries, agricultural (pesticides) runoff and municipal waste contamination, have all contributed to water quality and in turn have devastating effects not only on the algal diversity but also on the physico-chemical characteristics of the habitat water. In total, 206 species of microalgae belonging to four major groups i.e., Chlorophyta (36.5%), Cyanophyta (30.9%), Bacillariophyta (25.3%), Euglenophyta (7.3%) have been identified and enumerated from 30 fresh water bodies. Interestingly, species like ' *Chlorococcum humicola, Cosmarium granatum, Ankistrodesmus spiralis, Oedogonium curvum, Nostoc calcicola, Bulbochaete nana, Coelastrum microporum, Spirogyra polymorpha, Spirulina major, Chlorella vulgaris, Ulothrix acqualis, Pediastrum tetras,* ' etc., are important as they constituted basic food components of various herbivores fishes like ' *Chitala chitala, Clupisoma gerua, Ailia coila, Aorichthys aor, Wallago attu, Rhinomugil corsula, Channa marulius, Channa striatus, Ompok pabda, Ombok pabo* ' etc., inhabiting these habitats. This report deals principally with the role of microalgae in aquaculture and bioremediation "
(a) University of Jammu

P04001 Acid mine drainage and remediation impacts on lotic biofilm structure and extracellular enzyme activities during succession
Smucker, Nathan J-presenter ns218005@ohio.edu(a) Vis, Morgan L (a)
"Acid mine drainage (AMD) with high concentrations of metals and acidic pH is a devastating legacy of coal mining that impairs the biology and habitat of thousands of stream kilometers throughout the USA and the world. To improve assessments and restoration efforts, this research focused on how AMD and a CaO doser treatment impact the structure and function of lotic biofilms. We documented algal diversity, biomass, bacterial abundance, and extracellular enzyme activity (EEA) during succession on tiles over 5 weeks in 8 streams (3 AMD, 3 downstream of a doser, 1 control recovered from AMD, and 1 control with no AMD). Biomass accrual was greatest in the non-AMD control, least in sites downstream of the doser, and intermediate in non-treated AMD sites. Algal diversity and evenness was lowest in AMD streams, and distinct communities formed in the various stream types. Phosphatase was greatest in AMD sites, likely a response to P limitation due to non-organically bound P. Leucine-amino peptidase and β-glucosidase (GLU) were greatest in control streams. GLU was related to biomass, probably because algal exudates are the dominant source of biofilm polysaccharides. β-xylosidase was consistent among streams, indicating similar roles of allochthonous carbon. A principal components analysis of week 5 EEAs showed AMD streams clustered together, doser sites similar to moderate AMD impacts, and control streams isolated in the ordination. Chlorophyll a to P ratios and EEAs showed inefficient nutrient uptake (impaired function) in AMD and CaO treated streams causing implications for nutrient dynamics downstream. CaO treatment reduces the stream length needed for biofilms to recover, but complete recovery is unlikely within the 4.8 km reach sampled due to metal precipitates."
(a) Department of Environmental and Plant Biology, Ohio University

Start	End	**Event**	Location
4:40 PM	6:20 PM	**Minisymposium 13: Brassinosteroids** - Chair: Steven Clouse 4:40 - M1301: Yanhai Yin -*Network and Mechanism of Brassinosteroid Regulated Gene Expression and Responses in Arabidopsis thaliana* 5:05 - M1302: Tae-Wuk Kim - *Brassinosteroid signal transduction from cell surface receptor kinases to nuclear transcription factors* 5:30 - M1303: Xuelu Wang - *The primary signaling outputs of brassinosteroids are regulated by abscisic acid signaling* 5:55 - M1304: Steven D. Clouse - *Receptor kinases involved in brassinosteroid signal transduction phosphorylate protein translation initiation factors*	Lana'I 314

M1301 Network and Mechanism of Brassinosteroid Regulated Gene Expression and Responses in Arabidopsis thaliana
Li, Lei (a) Xiaofei, Yu (a) Chory, Joanne (e) Yin, Yanhai-presenter yin@iastate.edu(a) Zola, Jaroslaw (b) Guo, Michelle (a) Ye, Huaxun (a) Asami, Tadao (d) Aluru, Maneesha (b) Liu, Peng (c) Aluru, Srinivas (b) Rodermel, Steve (a)
"Plant steroid hormone brassinosteroids (BRs) play important roles throughout growth and development. BRs signal through membrane receptor BRI1 to regulate the activities of BES1/BZR1 family transcription factors. Genetic, genomic and computational approaches are used to understand how BES1 regulates gene expression and BR responses. First, we have used Chromatin Immunoprecipitation and genomic tilling arrays (ChIP-chip) and identified about 1600 BES1 direct target genes. At the chromatin level, BES1 binding sites were largely free of the trimethyl histone 3 lysine 27 (H3K27me3), but partially overlap with H3K9me3. The result suggests involvement of histone modifications and chromatin structure in BES1-regulated transcription, which is consistent with our recent finding that BES1 recruits two histone demethylases to activate gene expression (PNAS 2008 105:7618). BES1 target genes are involved in multiple aspects of BR responses, including cell elongation, self-regulation of BR signaling and

crosstalk with other signaling pathways. Transcription factors were highly enriched in BES1 target genes and the expression of which was strongly correlated with each other as shown in a gene regulatory network (GRN), revealed by ARACNe (Algorithm for the Reconstruction of Accurate Cellular Networks). The result demonstrates that BES1 functions through a transcriptional network to mediate BR responses. Moreover, we found that BES1 activates the expression of MYB30 transcription factor gene and subsequently cooperates with MYB30 protein to further activate downstream targets, thereby providing a novel mechanism to amplify BR signal (Plant J 2009 58: 275). Finally, we found that three related receptor-like kinases, HERCULES1 (HERK1), THESEUS1(THE1) and FERONIA (FER), are transcriptionally induced by BRs. While FER was recently found to mediate male-female interaction (FER) and THE1 was proposed to sense cell wall integrity, our genetic studies demonstrated that they are required for optimal cell elongation during vegetative growth as herk1 the1 double and fer RNAi mutants displayed a clearly reduced growth phenotype. Gene expression studies demonstrate that these RLKs define a previously unknown pathway that acts in concert with, but largely independent of the BR pathway to promote plant growth. Our results, therefore, provide significant insights into the network and mechanism of BR-regulation of plant growth and development. Supported by grants from NSF, DOE and USDA."
(a) Department of Genetics, Development and Cell Biology, Iowa State University (b) Department of Electrical and Computer Engineering, Iowa State University (c) Department of Statistics, Iowa State University (d) Department of Applied Biological Chemistry, The University of Tokyo, Japan (e) Howard Hughes Medical Institute, The Salk Institute for Biological Studies

M1302 Brassinosteroid signal transduction from cell surface receptor kinases to nuclear transcription factors
Kim, Tae-Wuk-presenter twgibio@stanford.edu(a) Guan, Shenheng (b) Sun, Yu (a) Deng, Zhiping (a) Tang, Wenqiang (a) Shang, Jian-Xiu (c) Sun, Ying (c) Burlingame, Alma L. (b) Wang, Zhi-Yong (a)
"Brassinosteroid (BR) regulates gene expression and development through a receptor kinase-mediated signaling pathway in plants. Although many components of BR signaling pathway have been identified and studied, a major gap still exists in the BR signaling pathway. In particular, how upstream signaling regulates the GSK3-like kinase (BIN2) remains unclear. Here we close the last gap of the BR signaling pathway by demonstrating the molecular function of BSU1 upstream of BIN2 and downstream of BSK1. We show that BSU1 inactivates BIN2 by dephosphorylating a phospho-tyrosine residue that is required for its kinase activity. Both in vitro BSU1 treatment and in vivo BR treatment cause tyrosine dephosphorylation of wild type BIN2 but not of mutant bin2-1, which causes BR-insensitive phenotypes. Quadruple loss-of-function mutant of BSU1 family displays an extreme dwarf phenotype similar to BR-deficient mutants, indicating that BSU1-mediated BIN2 dephosphorylation is essential for BR-dependent plant growth. In addition, we show that BSK1 directly interacts with BSU1 in a BRI1-phosphorylation-dependent manner. This study reveals a mechanism of GSK3 regulation in plants and demonstrates a fully connected BR signaling pathway from the BRI1 receptor kinase to BIN2 and its substrates BZR transcription factors."
(a) Department of Plant Biology, Carnegie Institution for Science, Stanford, CA 94305 (b) Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143 (c) Institute of Molecular Cell Biology, Hebei Normal University, Shijiazhuang, Hebei, 050016, China

M1303 The primary signaling outputs of brassinosteroids are regulated by abscisic acid signaling
Zhang, Shanshan (a) Cai, Zhenying (a) Cheng, Yinwei (a) Wang, Xuelu-presenter xueluw@gmail.com(a)
"Unlike animals, plants are sessile and need to constantly regulate their developmental and physiological processes to respond to various internal and external stimuli. Many phytohormones play essential roles in coordinately regulating these processes. Brassinosteroids (BRs) mainly play roles in promoting plant growth and development, while abscisic acid (ABA) is a hormone induced by many stress signals. Although it is known that BRs and ABA co-regulate hundreds of genes expression, whether their interaction is through modification or interaction of their primary signaling cascades or through independent signaling pathways remains a big mystery. In this study, we used biochemical and molecular markers of BR signaling and found that exogenous ABA rapidly inhibits BR signaling outputs as indicated by the phosphorylation status of BES1 and BR-responsive gene expression. Experiments using a bri1 null-allele, bri1-116, and analysis of subcellular localization of BKI1-YFP further reveal that the BR receptor complex is not required for this effect of ABA on BR signaling outputs. However, when the BR downstream component BIN2 is inhibited by LiCl, ABA fails to inhibit BR signaling outputs. Furthermore, using a set of ABA insensitive mutants, we found that the effect of ABA on the BR primary signaling pathway is dependent on the ABA early signaling components, ABI1 and ABI2. We propose that the signaling cascades of ABA and BRs primarily crosstalk after BR perception, but before the downstream transcriptional factor BES1, which also explains why a large proportion of BR-responsive genes are also regulated by ABA. Our studies provide significant insight into the molecular mechanisms by which BRs interact with ABA."
(a) State Key Laboratory of Genetic Engineering, and Institute of Plant Biology, School of Life Sciences, Fudan University

M1304 Receptor kinases involved in brassinosteroid signal transduction phosphorylate protein translation initiation factors
Wang, Xiaofeng (a) Kota, Uma (c) Blackburn, Kevin (c) Browning, Karen (b) Goshe, Michael B (c) Clouse, Steven D-presenter steve_clouse@ncsu.edu(a)
http://www4.ncsu.edu/~sclouse/
"Brassinosteroids (BRs) are essential plant hormones that regulate multiple aspects of plant growth and development and require two receptor kinases, BRASSINOSTEROID INSENSITIVE 1 (BRI1) and BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1), for hormone perception and signal transduction. We previously isolated a putative cytoplasmic substrate of BRI1 with homology to the mammalian TGF-beta receptor interacting protein (TRIP-1). TRIP-1 (also known as eIF3i) is a dual function protein that regulates TGF-beta signaling in mammals and also plays a critical role in the eIF3 protein translation initiation complex in animals, yeast and plants. Arabidopsis BRI1 interacts with TRIP-1 *in planta* and phosphorylates TRIP-1 on multiple Ser/Thr residues *in vitro*. Initiation is the rate-limiting step in eukaryotic protein translation and is often regulated by phosphorylation of specific initiation factor subunits in response to various signals. A proteomic screen for novel BRI1 and BAK1 interactors identified four additional eIF subunits as putative kinase domain substrates for BRI1 and/or BAK1. Moreover, *in vitro* kinase assays confirmed that these eIF subunits were phosphorylated by both BRI1 and BAK1. We postulate that BR-dependent phosphorylation of TRIP-1 and other eIF subunits by BRI1 and/or BAK1 may affect initiation factor activity and thus impact protein translation, providing a novel mechanism for BR regulation of plant growth. We are studying the intersection of BR signal transduction and protein translation initiation by using a variety of mass spectrometry approaches to identify *in vivo* phosphorylation sites of eIF subunits, followed by analysis of their functional significance with respect to BR signaling and the assembly and activity of the translation initiation complex."
(a) Dept. of Horticultural Science, North Carolina State University (b) Dept. of Chemistry and Biochemistry, University of Texas (c) Dept. of Molecular and Structural Biochemistry, North Carolina State University

Start	End	**Event**	Location
4:40 PM	6:20 PM	**Minisymposium 14: Cytoskeletal Dynamics** - Chair: Valerian Dolja & Magdalena Bezanilla 4:40 - M1401: William R. Eisinger - *Reduction in guard cell microtubule stability*	Kaua'I 311

		correlates with stomatal closure in Arabidopsis 5:05 - M1402: Chris Staiger - *Stochastic dynamics of actin filaments in the cortical array of Arabidopsis epidermal cells* 5:30 - M1403: Luis Vidali - *Class II formins and myosin XIs are required for tip growth* 5:55 - M1404: Valerian V. Dolja - *Myosin functions in organelle trafficking, F-actin organization, and plant development*	

M1401 Reduction in guard cell microtubule stability correlates with stomatal closure in Arabidopsis
Eisinger, William R.-presenter weisinger@scu.edu(a) Briggs, Winslow (b)
"Microtubules (MTs) establish the pattern of the transversely ordered cellulose microfibrils that are responsible for the functional shape of guard cells. However, the role of cortical MTs in mature, functional guard cells is less clear. At night when stomates are normally closed, most guard cells show few organized MTs. By contrast, imaging of GFP-labeled tubulin with confocal microscopy revealed that during the day, guard cells with open stomates have large numbers of radially oriented cortical MTs. However, using GFP labeled-tubulin expressed in Arabidopsis we found that microtubule (MT) numbers decrease about 50% as guard cells close their stomates. Similar decreases in MT numbers were seen whether closure occurs as the result of darkness or treatment with ABA (10 µ;M), hydrogen peroxide (1.0 mM), or sodium hydrogen carbonate (1.2 mM). During stomatal closure we observed no changes in microtubule (MT) numbers in adjacent epidermal cells with any of the above treatments. Therefore, since MT numbers in guard cells, but not other epidermal cells, show a decline in response to these varied stimuli, we hypothesize a causal link between decreasing numbers of guard cell MTs and stomate closure. In addition we used GFP labeled-EB1 protein (a marker for MT growth) to investigated rates of MT assembly in guard cells and adjacent epidermal cells. The length and number of GFP:EB1 comets seen at the growing ends of MTs remain unchanged during stomate closure. Since EB1 showed no changes apparent MT assembly rates during stomate closure, we conclude that MT numbers in guard cells decrease because of reduced MT stability rather than a reduced rate of MT assembly. "
(a) Dept Biology, Santa Clara University (b) Dept Plant Biology, Carnegie Institution

M1402 Stochastic dynamics of actin filaments in the cortical array of Arabidopsis epidermal cells
Staiger, Chris-presenter staiger@purdue.edu(a) Sheahan, Michael (b) Khurana, Parul (a) Wang, Xia (a) McCurdy, David (b) Blanchoin, Laurent (c)
"Eukaryotic cells harness the power of actin dynamics to create cytoskeletal arrays that stimulate protrusions and drive intracellular organelle movements. In plants, the actin cytoskeleton is generally understood to participate in cell elongation and responses to biotic and abiotic stimuli; however, a detailed description and molecular mechanism(s) underpinning filament nucleation, growth and turnover are lacking. We have used variable-angle epifluorescence microscopy (VAEM) to examine the organization and dynamics of the cortical cytoskeleton in growing and non-growing epidermal cells from Arabidopsis hypocotyls. Collectively, actin filaments in the cortical array are randomly oriented and surprisingly dynamic. Single actin filaments grow at rates of 1.7 micron/s, but are mostly short lived. Instead of depolymerization at their ends, actin filaments are disassembled by prominent severing activity. Incessant remodeling of the cortical actin array also features filament buckling and straightening events. We consider several mechanisms for the control of actin dynamics, including rapid polymerization from a large pool of profilin-actin, specific severing and capping activities, and myosin-driven filament-filament interactions. Aspects of these models have been tested with pharmacological agents, and future work will use reverse-genetics to further dissect the molecular mechanisms underlying actin dynamics. Our observations, the first to describe single actin filament behavior in plant cells, indicate a mechanism inconsistent with treadmilling, instead resembling the stochastic dynamics of a recently described biomimetic system for actin assembly in vitro."
(a) Dept of Biological Sciences, Purdue University (b) Plant Science Group, Newcastle University (c) iRTSV, CEA/CNRS/UJF, Grenoble

M1403 Class II formins and myosin XIs are required for tip growth
Vidali, Luis-presenter lvidali@bio.umass.edu(a) vanGisbergen, Peter (a,b) Guerin, Christophe (c) Franco, Paula (a) Li, Ming (a) Burkart, Graham (a) Augustine, Robert C. (a) Blanchoin, Laurent (c) Bezanilla, Magdalena (a)
"A striking form of cell growth occurs in the highly polarized protonemal cells of mosses and ferns, pollen tubes, and root hairs; where all the growth activity is concentrated to one end of the cell. The actin cytoskeleton has been known to be a central player in tip growth, but the underlying molecular mechanisms of this dependence remain unknown. In an effort to understanding these mechanisms we are investigating the role of actin binding proteins in tip growth. Using RNAi and transient complementation, we analyzed the role of formins and myosin XIs in tip growth. Formins are essential for the creation of actin-based structures responsible for a diverse array of processes in eukaryotes. Myosin XIs are plant-specific and most similar to myosin Vs from animals and fungi. In plants, myosin XIs are responsible for cytoplasmic streaming, but their role in polarized growth is not well understood. Vascular plants contain large formin and myosin XI gene families; this large number of isoforms has made it difficult to establish their physiological role. In contrast, moss has only 9 formins that group into three classes, and only two myosin XI genes. We analyzed the function of all formins in the moss *Physcomitrella patens* , and show that plants lacking class II formins or myosin XIs, are severely stunted and composed of spherical cells, with disrupted actin organization, and lacking tip growth. Silencing of all other formins results in normal cell morphology. We have also determined that the two class II formin genes as well as the two myosin XI genes are functionally redundant. We show that a class II formin and a myosin XI localize at the apex of growing cells and demonstrate that for the class II formins, the N-terminal PTEN-like domain mediates this localization. The PTEN-like domain is followed by the formin signature domains, FH1 and FH2, which are known to promote actin filament formation. Using transient complementation studies we show that only the class II FH1-FH2 domain rescues tip growth. To dissect the functional differences between these FH1-FH2 domains, we used *in vitro* polymerization assays to characterize them. We found that class II formins mediate exceptionally rapid rates of actin filament elongation, compared to class I or any other known formin. To further dissect myosin XI mechanism of action during polarized growth, we are also analyzing the specific effect of myosin XI loss-of-function in intracellular motility."
(a) University of Massachusetts (b) Wageningen Universiteit en Researchcentrum (c) French Atomic Energy Commission

M1404 "Myosin functions in organelle trafficking, F-actin organization, and plant development"
Dolja, Valerian V.-presenter doljav@science.oregonstate.edu(a) Prokhnevsky, Alex I (a) Peremyslov, Valera V (a)
"To understand the biological significance and mechanisms of the myosin-dependent cell dynamics, a series of the single, double, and triple gene knockouts of the class XI myosins of Arabidopsis was generated and analyzed. It was found that the rapid trafficking of Golgi stacks and peroxisomes is empowered by the myosins XI-K, XI-1, and XI-2, whereas mitochondria are transported primarily by myosins XI-K and XI-1. In addition to ~5-fold reduction of the organelle velocities, simultaneous inactivation of the myosins XI-K and XI-2 resulted in a dramatic rearrangement of the F-actin bundles in the leaf epidermal cells indicating that the myosins shape the tracks they run on. The double and triple gene knockouts xi-k/1 and xi-k/1/2 exhibited progressive reduction of the plant growth and fecundity that correlated with the cumulative reduction in organelle trafficking. Finally, myosins XI-K, XI-2, and XI-B were implicated in polarized elongation of the root hairs. Taken together, these data suggested that the myosin-driven trafficking of organelles and, perhaps, vesicles is required for the general growth and polarized elongation of the cells, as well as for the plant

development and reproduction."
(a) Oregon State University

Start	End	**Event**	Location
4:40 PM	6:20 PM	**Minisymposium 15: Plant Pathogen Interactions** - Chair: Elizabeth Fontes 4:40 - M1501: Elizabeth P.B. Fontes - *NIK-mediated antiviral signaling, a novel layer of innate plant defenses suppressed by the geminivirus nuclear shuttle protein* 5:05 - M1502: Birgit Schulze - *The BAK1-FLS2 receptor complex: Dynamics of heteromerization and phosphorylation in response to flagellin perception* 5:30 - M1503: Ping He - *Bacterial Effectors Target A Common Signaling Partner To Impede Host Immunity and Development* 5:55 - M1504: Anneke Prins - *The role of protease inhibitors in the hypersensitive response*	Moloka'I 315

M1501 "NIK-mediated antiviral signaling, a novel layer of innate plant defenses suppressed by the geminivirus nuclear shuttle protein"
Fontes, Elizabeth P.B.-presenter bbfontes@ufv.br(a) Carvalho, Claudine M. (a) Fietto, Luciano G. (a) Joao Paulo, Machado B. (a) Santos, Anesia A. (a)
"The NSP-interacting kinase (NIK) receptor-mediated antiviral signaling has been identified recently as a virulence target of the geminivirus nuclear shuttle protein (NSP). However, the NIK-NSP interaction does not fit into the elicitor-receptor model of resistance and hence the molecular mechanism that links this antiviral response to receptor activation remains obscure. Here we identified a ribosomal protein, rpL10, as a specific partner and substrate of NIK that functions as the immediate downstream effector of NIK-mediated signaling. Phosphorylation of cytosolic rpL10 by NIK redirects the protein to the nucleus where it may act to modulate viral infection. While ectopic expression of normal NIK or a hyperactive NIK mutant increases the accumulation of phosphorylated rpL10 within the nuclei, an inactive NIK mutant fails to redirect the protein to the nuclei of co-transfected cells. Likewise a mutant rpL10 defective for NIK phosphorylation is not redirected to the nucleus. Furthermore, loss of rpL10 function enhances susceptibility to geminivirus infection, resembling the phenotype of nik null alleles. We also provide evidence that geminivirus infection directly interferes with NIK-mediated nuclear relocalization of rpL10 as a counterdefensive measure. However, the NIK-mediated defense signaling neither activates RNA silencing nor promotes a hypersensitive response but inhibits plant growth and development. Therefore, the NIK antiviral signaling may represent a novel layer of the innate plant defenses that has the potential to affect basic compatibility functions. Although geminivirus NSP overcomes this layer of defense in Arabidopsis, the NIK1-mediated signaling response may be involved in restricting the host range of other viruses."
(a) Universidade Federal de Vicosa

M1502 The BAK1-FLS2 receptor complex: Dynamics of heteromerization and phosphorylation in response to flagellin perception
Schulze, Birgit-presenter birgit.schulze@unibas.ch(a) Mentzel, Tobias (a) Bittel, Pascal (a) Boller, Thomas (a) Felix, Georg (b) Chinchilla, Delphine (a)
"PAMP-triggered innate immunity, the first line of plant defense, is activated by recognition of pathogen associated molecular patterns (PAMPs). These are perceived by highly specific receptors at the plasma membrane, such as the flagellin receptor FLS2 (Flagellin sensing 2) [1]. Previously, we demonstrated that BAK1 interacts with FLS2 after binding of the ligand flg22 and is required for activation of physiological responses [2]. In the present work, we refine our kinetic analysis of receptor heteromerization and show that FLS2 can interact with BAK1 within less than 1 s after stimulation. While FLS2 is responsible for ligand binding, the kinase domains of both FLS2 and BAK1 are believed to be activated by phosphorylation leading to cellular signal transduction. Using in vivo labeling with [33P]phosphate, we characterized de novo phosphorylation events on FLS2 and BAK1 and followed the stability of the phosphorylated proteins over time. In Arabidopsis cell cultures both, FLS2 and BAK1, are phosphorylated within 15 s of treatment with flg22. Thus, de novo phosphorylation within this receptor complex clearly precedes activation of other signaling steps involved in the induction of innate immune responses. Funding by the SNF (31003A-120655 and 31003A-105852) and a post-doctoral grant from the Deutsche Akademie der Naturforscher Leopoldina (BMBF-LPD 9901/8-152) to BS are gratefully acknowledged. [1] Boller T., Felix G. 2009. Annu. Rev. Plant Biol. 60:379-406 [2] Chinchilla D., Zipfel C., Robatzek S., Kemmerling B., Nurnberger T., Jones J. D., Felix G., Boller T. 2007. Nature. 448:497-500. "
(a) Botanical Institute, University of Basel, Switzerland (b) Institute of Plant Biochemistry, ZMBP, University of Tuebingen, Germany

M1503 Bacterial Effectors Target A Common Signaling Partner To Impede Host Immunity and Development
He, Ping-presenter pinghe@tamu.edu(a,c) Shan, Libo (b,c) Sheen, Jen (c)
"Successful pathogens have involved diverse elegant virulence strategies to infect hosts. Many pathogenic bacteria deploy the type III secretion system to deliver virulence effectors into host cells to promote pathogenicity. The molecular actions of these effectors remain largely elusive. We performed a molecular cellular screen and identified two sequence-distinct effector proteins, AvrPto and AvrPtoB from a ubiquitous plant pathogen Pseudomonas syringae, as potent suppressors of host immune responses triggered by multiple microbe-associated molecular patterns (MAMPs). AvrPto and AvrPtoB target a receptor-like kinase BAK1, a shared signaling partner of diverse MAMP receptors and the plant hormone brassinosteroid receptor BRI1 in Arabidopsis. This targeting interferes with the ligand-dependent association of MAMP receptors with BAK1 during infection, and impedes plant immunity to nonpathogenic bacteria, and brassinosteroid-mediated plant development. The identification of BAK1 as a host target of AvrPto and AvrPtoB virulence uncovers a novel action of bacterial effectors in blocking signaling initiation from multiple receptor complexes. From an evolutionary point of view, it is parsimonious for pathogen effectors to target a common component involved in multiple plant signaling pathways. "
(a) Department of Biochemistry & Biophysics, and Institute for Plant Genomics and Biotechnology, Texas A&M University (b) Department of Biochemistry & Biophysics, and Institute for Plant Genomics and Biotechnology, Texas A&M University (c) Department of Molecular Biology, Massachusetts General Hospital, and Department of Genetics, Harvard Medical School, Boston, Massachusetts

M1504 The role of protease inhibitors in the hypersensitive response
Prins, Anneke-presenter A.Prins@exeter.ac.uk(a) Stevens, Conrad (a) Plume, Andrew (a) Grant, Murray (a)
"Pathogens that escape the plant's initial response to pathogen associated molecular patterns deploy effectors that contribute to pathogen virulence. Effector-triggered immunity is classically encoded by R proteins whose activation results in the hypersensitive response (HR). RPM1 is a typical NBS-LRR R protein that recognises the bacterial effectors AvrRpm1 and AvrB. Here we describe characterisation of RIN12, a protease inhibitor that

interact with RPM1 in yeast 2-hybrid and whose mis-expression modifies the HR. RIN12 overexpression causes delayed HR and enhanced bacterial growth; reduction in RIN12 leads to faster HR upon elicitation with AvrRpm1-expressing bacteria. The RIN12 protein shows structural homology to serine protease inhibitors, although the combining loop unusually contains a proline. *In vitro* expressed RIN12 inhibits subtilisin-type serine proteases Mutation of the combining loop abolishes both subtilisin activity and RPM1 interaction in yeast. These data are consistent with a 'guard hypothesis' model in which bacterial effector activity relieves RIN12 association with RPM1 enabling activation of the RPM1 signalling network. Paradoxically, RIN12 also exhibits bacterial defensin activity, which appears specific to bacteria that employ a type III secretion system. This RIN12 defensin function is independent from the protease inhibitory activity. *RIN12* transcripts are induced by PAMPs. These data suggest RIN12 may have evolved as part of a secondary host pathogen-triggered immune response to overcome effector-triggered susceptibility. The effector-triggered immunity role of RIN12 possibly evolved as a secondary function in R protein signalling. "
(a) University of Exeter

Start	End	**Event**	Location
4:40 PM	6:20 PM	**Minisymposium 16: Tropisms** - Chair: Gabriele Monshausen 4:40 - M1601: Miyo T. Morita - *SHOOT GRAVITROPISM 9, a novel RING finger protein, is involved in statolith dynamics by modulating interaction between F-actin and amyloplasts.* 5:05 - M1602: Takeshi Yoshihara - *LAZY1 belongs to a novel class of genes involved in gravitropic signal transduction in monocot and dicot plants* 5:30 - M1603: Gabriele B. Monshausen - *Basipetal migration of Ca2+ and pH waves during the graviresponse of Arabidopsis roots* 5:55 - M1604: Yutaka Miyazawa - *Identification of genes responsible for root hydrotropism in Arabidopsis roots*	Maui 316A

M1601 "SHOOT GRAVITROPISM 9, a novel RING finger protein, is involved in statolith dynamics by modulating interaction between F-actin and amyloplasts."
Morita, Miyo T-presenter mimorita@bs.naist.jp(a) Nakamura, Moritaka (a) Tasaka, Masao (a)
"Gravitropism is the directed growth forward or opposite direction of gravity. In higher plants, relative directional change of gravity is suscepted in the specialized cells (statocytes), and then the following processes are initiated. We have studied the molecular genetic mechanism of gravitropism of Arabidopsis inflorescence stems and demonstrated that endodermal cells are most likely to be the statocytes in Arabidopsis shoots. We have also reported that amyloplast sedimentation to the direction of gravity in the endodermal cells is important for gravity perception. The *sgr9 (shoot gravitropism 9)* mutant exhibits greatly reduced shoot gravitropism. In endodermal cells of *sgr9*, amyloplasts dynamically move around but did not sediment to the direction of gravity. The *SGR9* encodes a novel RING finger protein, which is expressed mainly in the shoot endodermis. The homology of RING finger domain between SGR9 and known ubiquitin E3 ligase suggests that SGR9 is also putative E3 ligase. Consistent to the expectation, mSGR9, which contains a point mutation at the putative essential site for E3 ligase activity in the RING finger domain, exerted a dominant negative effect on wild-type plant to mimic the *sgr9* mutant. In addition, mSGR9-GFP was localized to amyloplasts. It has been suggested that actin filaments (F-actin) is involved in amyloplast dynamics. To analyze the relationship between SGR9 and F-actin, we observed F-actin surrounding amyloplasts both in wild-type and in *sgr9*. Amyloplast surrounded by F-actin was more frequently observed in *sgr9* than in wild-type. In addition, inhibition of actin polymerization restored gravitropism and amyloplast sedimentation in *sgr9*. Taken together, SGR9 might modulate interaction between amyloplasts and F-actin on amylopalsts."
(a) Nara Institute of Science and Technology

M1602 *LAZY1* belongs to a novel class of genes involved in gravitropic signal transduction in monocot and dicot plants
Yoshihara, Takeshi-presenter yoshihara@wisc.edu(a,b) Spalding, Edgar P. (a) Iino, Moritoshi (b)
"Adult *lazy* mutants of rice (*Oryza sativa*) display a prostate growth habit. Coleoptiles of seedlings display defective circumnutation and gravitropism due to defective lateral auxin transport. Thus, LAZY1 appears to function at a step upstream of lateral auxin redistribution in gravity-directed processes that rely on differential growth. The *LAZY1* gene encodes a shoot-specific, possibly nuclear-localized protein lacking recognizable functional domains. The closest relative in *Arabidopsis thaliana* is a *LAZY1-like* gene (21% sequence identity) of unknown function. A question addressed by this work is whether the auxin distribution and growth control functions of *LAZY* genes are unique to monocots or if these roles evolved before monocot-dicot divergence. Arabidopsis plants expressing a *ProAtLAZY1-like:GUS* construct showed strong expression in adult shoots, especially in vascular tissues in rosette leaf blades and petioles, and where lateral inflorescence branches originate. In seedlings, the vasculature of cotyledons and upper hypocotyl showed strong expression. To identify the function of the *AtLAZY1-like* gene in Arabidopsis, RNA interference (RNAi) was used to suppress its expression. So far these RNAi lines revealed the following: Firstly, the inflorescence-stem gravitropism is weaker compared with the wild type (WT). Secondly, the angle between the main inflorescence shoot and the lateral shoots is much greater than that of WT. Gravitropism and nutation, which are affected in rice lazy mutants, are being examined in the Arabidopsis RNAi seedlings using custom image-processing tools. Such studies may demonstrate that the *AtLAZY1-like* gene is an ortholog of *OsLAZY1*, performing a similar role in monocot and dicot gravity signal transduction."
(a) University of Wisconsin (b) Osaka City University

M1603 Basipetal migration of Ca^{2+} and pH waves during the graviresponse of *Arabidopsis* roots
Monshausen, Gabriele B-presenter monshausen@wisc.edu(a) Miller, Nathan D (a) Murphy, Angus S (b) Spalding, Edgar P (a) Gilroy, Simon (a)
"During gravistimulation of roots, a signal perceived in root cap statocytes is likely translated into the generation of an auxin gradient across the root cap. This gradient is then thought to be propagated along the root axis through the concerted action of an array of auxin transporters. Using a combination of confocal vertical-stage and inverted-stage microscopy we show that gravistimulation also triggers the migration of an asymmetric wave of Ca^{2+} and pH along the Arabidopsis root axis. Furthermore, we provide evidence suggesting that the generation of these Ca^{2+} and pH waves is dependent on auxin fluxes: (i) auxin transport mutants show altered surface pH dynamics compared to wild type; (ii) adding auxin to the medium bathing a root results in an instantaneous increase in both cytosolic Ca^{2+} levels and in surface pH; (iii) localized application of auxin to the cap of vertically growing roots triggers a basipetally migrating wave of elevated Ca^{2+} and surface pH. Previous work has suggested a model where gravistimulation causes the differential accumulation of auxin on the lower flank of the root cap. These high auxin levels are then transported from the root tip to the root elongation zone to effect growth control. Our analyses suggest that such movement of auxin proceeds at a rate of ca 200-300 µ;m min^{-1} and triggers an increase in Ca^{2+} in cells on the lower flank of the root. This elevation in cytosolic Ca^{2+} in turn gives rise to rapid extracellular alkalinization which likely modulates growth and thus promotes root gravitropic curvature. Our observations indicate that the spatial and

temporal dynamics of Ca^{2+}-dependent auxin signaling play a central role in coordinating growth during such tropic responses. This work is supported by NSF (MCB- 0641288)."
(a) University of Wisconsin-Madison (b) Purdue University

M1604 Identification of genes responsible for root hydrotropism in *Arabidopsis* roots
Miyazawa, Yutaka-presenter miyazawa@ige.tohoku.ac.jp(a) Nobuharu, Fujii (a) Hideyuki, Takahashi (a)
"Roots display tropisms that control the direction of growth, hereby avoiding drought. It has also been hypothesized that roots display hydrotropism in response to moisture gradients, which could permit water acquisition more effectively than any other tropism. However, there have been surprisingly few studies. Recently, we established experimental systems using *Arabidopsis*, which has enabled us to use molecular genetics as a tool. Genetic screens based on the inability to develop hydrotropic root curvature allowed us to isolate a series of ahydrotropic mutants termed '*mizu-kussei (miz)*' (the words '*miz*' and '*kussei*' mean 'water' and 'tropism', respectively, in Japanese). Among six mutants, we recently succeeded in identifying the mutated genes for *miz1* and *miz2*, which are the only ahydrotropic mutants for which the responsible genes have been determined. Physiological and morphological analyses of these mutants showed that neither *miz1* nor *miz2* plants have defects in gravitropic responses, root elongation growth and root cap organization, while they completely lack the hydrotropic response. *MIZ1* encodes a protein of unknown function with a conserved domain at its C-terminus, which we termed MIZ domain. The MIZ domain is found only in the genomes of land plants, and it has no clear similarity to any characterized peptide sequence. The other gene, *MIZ2*, encodes a guanine-nucleotide exchange factor for ADP-ribosylation factor-type G proteins, which has been identified as GNOM. Further physiological analyses confirmed the indispensable role of GNOM-mediated vesicular trafficking in root hydrotropism. In this presentation, we summarize recent progress on the molecular mechanisms of root hydrotropism with special emphasis on *MIZ1* and *MIZ2*."
(a) Tohoku University

Start	End	**Event**	Location
4:40 PM	6:20 PM	**Minisymposium 17: Mineral Nutrition** - Chair: Li Li 4:40 - M1701: Li Li - *A broccoli COQ5 methyltransferase involved in ubiquinone biosynthesis mediates selenium volatilization* 5:05 - M1702: Jason D. Gillman - *Identification of the molecular basis of the seed low phytic acid phenotype in soybean line CX1834* 5:30 - M1703: Yi-Fang Tsay - *Mutation of the Arabidopsis NRT1.5 nitrate transporter causes defective root-to-shoot nitrate transport.* 5:55 - M1704: Narayanan N. Narayanan - *Functional Characterization of a Novel Iron Transporter, FEA1, from Chlamydomonas reinhardtii and its Application for Iron-Specific Metal Uptake in Plants*	Maui 316C

M1701 A broccoli COQ5 methyltransferase involved in ubiquinone biosynthesis mediates selenium volatilization
zhou, Xin (b) Yang, Yong (a) Thannhauser, Theodore W (a) Kochian, Leon V (a) Li, Li-presenter ll37@cornell.edu(a,b)
"Selenium is an essential micronutrient for animals and humans, but becomes toxic at high levels. To identify novel genes whose products are involved in plant selenium volatilization, a broccoli cDNA encoding COQ5 methyltransferase (BoCOQ5-2) in ubiquinone biosynthetic pathway was isolated via genomics approach. Its function was authenticated by complementing a yeast *coq5* mutant and by detecting increased cellular ubiquinone levels in *BoCOQ5-2* transformed bacteria. Proteomic analysis of differentially expressed proteins between bacteria expressing *BoCOQ5-2* and those containing the empty vector further supported its functional role in ubiquinone biosynthesis. BoCOQ5-2 was found to promote selenium volatilization in both bacteria and transgenic *Arabidopsis* plants. Bacteria expressing *BoCOQ5-2* produced an over 160-fold increase in volatile selenium compounds when they were exposed to selenate. Consequently, the *BoCOQ5-2* transformed bacteria had dramatically enhanced tolerance to selenate and contained reduced levels of total selenium in the cells. Transgenic *Arabidopsis* expressing *BoCOQ5-2* volatilized three times more Se than the vector only control plants when treated with selenite and exhibited significant tolerance to selenium. BoCOQ5-2 represents the first plant enzyme that is not known to be directly involved in sulfur/selenium metabolism, yet mediates selenium volatilization. This discovery opens up new prospective regarding our understanding of the complete metabolism of selenium and could lead to ways to modify selenium accumulator plants with increased efficiency in phytoremediation of selenium contaminated environments."
(a) Robert W. Holley Center for Ag & Health, USDA-ARS (b) Cornell University

M1702 Identification of the molecular basis of the seed low phytic acid phenotype in soybean line CX1834
Gillman, Jason D-presenter gillmanj@missouri.edu(a) Pantalone, Vince R (b) Bilyeu, Kristin (a)
"Plant seeds accumulate phosphorus in the form of *myo*-inositol-1,2,3,4,5,6 hexa*kis*phosphate, commonly referred to as phytic acid. Phytic acid is complexed with cationic mineral species in the form of phytate, which is not well digested or absorbed by monogastric species such as humans, poultry and swine. As a result, soybean has an effective deficiency of phosphorus and other minerals, despite high levels of these components in the seed. Excreted phytate can also contribute to phosphorus contamination of groundwater and eutrophication of freshwater lakes and streams. In maize, a recessive mutation in a conserved region within the *low phytic acid 1 (lpa1)* gene is responsible for the low phytic acid phenotype. We have identified recessive mutations in two soybean homologues of the maize *lpa1* gene in CX1834, a soybean line with a low phytic acid phenotype derived from EMS mutagenesis of a breeding line with normal phytate levels. In three populations analyzed, we identified complete association between homozygosity for mutant alleles of the two *lpa1* homologues and the low phytic acid phenotype. Molecular marker assays were designed that can be used to directly select for the mutant alleles that control the phenotype. The identification of the molecular basis for the low phytic acid phenotype will dramatically ease the introgression of the low phytic acid trait into elite soybean cultivars. The ultimate goal of such introgression is soybean-derived food and feed which require less nutrient supplementation, are more nutritious, and are more environmentally friendly."
(a) Agricultural Research Service, USDA (b) Dept. of Plant Sciences, University of Tennessee

M1703 Mutation of the *Arabidopsis NRT1.5* nitrate transporter causes defective root-to-shoot nitrate transport.
Lin, Shanhua (a) Tsay, Yi-Fang-presenter yftsay@gate.sinica.edu.tw(a)
"NRT1.5 is one of the 53 Arabidopsis NitRate Transporter *NRT1* (Peptide Transporter PTR) genes, of which two members, *NRT1.1 (CHL1)* and *NRT1.2*, have been shown to be involved in nitrate uptake. Compared to the wild type, the root-to-shoot ^{15}N- nitrate translocation of *nrt1.5* mutants were reduced. To confirm the function of NRT1.5, the nitrate contents in the xylem sap were measured. Consistent with the defect of the root-to-shoot nitrate translocation, in *nrt1.5* mutants, the nitrate contents of the xylem sap were lower than that of wild type. in situ hybridization showed that *NRT1.5* is expressed in root pericycle cells close to the xylem. In addition, localization of *NRT1.5* expression was analyzed by transgenic plants

carrying GUS gene under the control of the *NRT1.5* promoter. Consistent with the in situ hybridization result, GUS activity was detected in the parenchyma cells (more likely the pericycle cells) close to the xylem vessels. Subcellular localization showed that NRT1.5 is located in the plasma membrane. Functional analysis of cRNA-injected Xenopus oocytes showed that NRT1.5 is a low-affinity, pH-dependent bidirectional nitrate transporter. Taken together, these data suggested that NRT1.5 is located in the plasma membrane of root pericycle cells close to the xylem and responsible for nitrate efflux out of pericycle cell for root xylem loading of nitrate. The root-to-shoot nitrate transport is not completely eliminated in *nrt1.5* mutants suggesting that there are multiple nitrate xylem loading mechanisms and nitrate efflux mediated by a proton coupled nitrate transporter is one of them. "
(a) Institute of Molecular Biology, Academia Sinica

M1704 "Functional Characterization of a Novel Iron Transporter, *FEA1*, from *Chlamydomonas reinhardtii* and its Application for Iron-Specific Metal Uptake in Plants"
Narayanan, Narayanan N-presenter nnarayanan@danforthcenter.org(a) Chiu, WaiTing (a) Ihemere, Uzoma (a) Moon, Hangsik (b) Siritunga, Dimuth (c) Singh, Sareena (e) Oda, Saharu (e) Falcao, Vanessa (e) Rajamani , Sathish (d) Sayre, Richard T (a)
"The FEA1 protein is a unique algal soluble protein secreted into the periplasmic space of *Chlamydomonas reinhardtii* under iron deficient conditions or in the presence of toxic concentrations of cadmium. We demonstrate that the FEA1 protein compliments both yeast iron permease (*ftr1*) and uptake mutants (*fet3fet4*), suggesting that the FEA1 protein functions as an iron transporter. Based on protein structural predictions, we hypothesize that the FEA1 protein undergoes a transition from a soluble to a membrane protein following formation of a hairpin structure with two potential transmembrane spanning domains (TMS) that then insert into the membrane. The formation of the hairpin structure is dependent on the formation of either a disulfide or metal thiolate bond between two cysteines located at the periplasmic surface of the two predicted TMS. We provide evidence that this structural transition is necessary for iron transport activity. We show that the polar residues located in the TMS are critical for FEA1 function presumably for formation of an ion channel. A predicted cytoplasmically-localized nucleotide binding motif (P-loop) is also required for function. In plants, *FEA1* compliments the irt1 iron-uptake mutant of *Arabidopsis* indicating that the FEA1 protein functions in a variety of organisms. Transgenic *FEA1* wild-type plants have three- to four-fold higher root iron levels compared with wild-type plants when grown at high pH (8.5) indicating an enhanced ability to transport iron in calcareous soils. Furthermore, we demonstrate that the FEA1 transporter is Fe+2-specific. These features indicate that algae have efficient mechanisms for transporting iron under a variety of stress conditions that can impair iron-dependent metabolism and growth."
(a) Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132, USA (b) Syngenta Biotechnology, Inc., 3054 E. Cornwallis Road, Research Triangle Park, NC 27709, USA (c) Department of Biology, University of Puerto Rico, Mayaguez, Puerto Rico, 00680, USA (d) Department of Biology, Dartmouth College, Hanover, New Hampshire, 03755, USA (e) Department of Plant Cellular and Molecular Biology, The Ohio State University, Columbus, OH 43210, USA

Start	End	**Event**	Location
4:40 PM	6:20 PM	**Minisymposium 18: Photosynthesis** - Chair: Thomas Brutnell 4:40 - M1801: Sungsoon Park - *REP27, a thylakoid membrane protein functioning in the D1/32 kD reaction center protein turnover and PSII repair from photodamage* 5:05 - M1802: Shizue Matsubara - *Acclimation and adaptation of leaf carotenoid composition and biosynthesis in tropical plant species* 5:30 - M1803: Tammy L. Sage - *The functional anatomy of rice leaves: implications for refixation of photorespiratory CO2 and efforts to engineer C4 photosynthesis into rice.* 5:55 - M1804: Thomas P. Brutnell - *A systems approach to understanding C4 photosynthetic differentiation in maize*	313A

M1801 "REP27, a thylakoid membrane protein functioning in the D1/32 kD reaction center protein turnover and PSII repair from photodamage"
Park, Sungsoon-presenter sungsoon@nature.berkeley.edu(a) Dewez, David (a) Garcia-Cerdan, Jose (a) Lindberg, Pia (a) Melis, Anastasios (a)
"The goal of the research is to identify genes and proteins required for the photosystem-II (PSII) repair mechanism in oxygenic photosynthesis. Via DNA insertional mutagenesis in *Chlamydomonas reinhardtii*, *rep27*, a PSII repair aberrant strain was isolated. Molecular analysis of the *rep27* strain resulted in the cloning of REP27, a nuclear gene encoding a chloroplast-targeted protein containing two tetratricopeptide repeat motifs (TPR1 & 2), two transmembrane domains, and an extended C-terminal region. Cell fractionation and Western blot analysis localized the REP27 in the *C. reinhardtii* chloroplast thylakoids. A folding model for REP27 suggested N- and C-termini exposed to the chloroplast stroma. Truncated REP27 cDNA constructs were made for complementation of the *rep27* mutant, whereby TPR1, TPR2, both TPR1 and TPR2, or the C-terminal domain were deleted. *rep27*-complemented strains with the REP27 minus the TPR motifs showed elevated levels of D1, comparable to those in the wild type, but the PSII photochemical efficiency of these complemented strains was not restored, suggesting that the functionality of the PSII reaction center was not recovered. It is suggested that TPR motifs play a role in the functional activation of the newly integrated D1 protein in the PSII reaction center. *rep27*-complemented strains missing the REP27 C-terminal domain showed low levels of D1 protein, as well as low PSII photochemical efficiency, comparable to those in the *rep27* mutant. Therefore, the C-terminal domain is needed for a *de novo* D1 biosynthesis and/or assembly of D1 in the PSII template. We conclude that REP27 plays a dual role in the regulation of D1 protein turnover by facilitating co-translational biosynthesis-insertion and activation of the nascent D1 in the PSII repair process."
(a) University of California Berkeley

M1802 Acclimation and adaptation of leaf carotenoid composition and biosynthesis in tropical plant species
Matsubara, Shizue-presenter s.matsubara@fz-juelich.de(a) Krause, G. Heinrich (b,c) Aranda, Jorge (c) Virgo, Aurelio (c) Beisel, Kim G (a) Jahns, Peter (b) Winter, Klaus (c)
"The composition of carotenoid pigments, involved in two essential and contrasting processes of photosynthesis (light harvesting and photoprotection), is highly conserved among higher plants. One branch of the carotenoid biosynthetic pathway (b,b-branch) gives rise to b-carotene, zeaxanthin, antheraxanthin, violaxanthin and neoxanthin, whereas another branch (b,e-branch) leads to formation of lutein. While our understanding of biosynthesis and functions of different carotenoids in photosynthesis has greatly advanced for model plants, regulation of leaf carotenoid biosynthesis and accumulation can vary in different plant species, as exemplified by some tropical plants that contain a-carotene (a-Car) and lutein epoxide (Lx), two additional carotenoids synthesized in the b,e-branch. Based on their occurrence and sun-shade responses studied in several species, roles of a-Car and Lx in improving light harvesting, as opposed to those of b-carotene and zeaxanthin in photoprotection, have been

proposed. In this study we conducted a survey of photosynthetic pigments, including 86 tropical plant species from 64 families, to examine whether occurrence of a-Car and Lx represents convergent evolution, i.e. adaptive changes in unrelated species under similar ecological constraints. Further, relative investment in different carotenoids was compared between sun and shade leaves/species to infer their importance under contrasting light environments and to identify a general acclimatory pattern in leaf carotenoid biosynthesis in tropical plants. The results will be discussed in the context of trade-off between investment in light harvesting and leaf structural components, differential functions of b,b- and b,e-carotenoids, and enzyme evolution in the carotenoid biosynthetic pathway."
(a) Phytosphaere (ICG-3), Forschungszentrum Juelich (b) Heinrich-Heine-Universitaet Duesseldorf (c) Smithsonian Tropical Research Institute

M1803 The functional anatomy of rice leaves: implications for refixation of photorespiratory CO2 and efforts to engineer C4 photosynthesis into rice.
Sage, Tammy L-presenter tammy.sage@utoronto.ca(a) Sage, Rowan R (a)
"One mechanism to radically enhance global food stocks is to introduce C4 photosynthesis into C3 crops from warm climates, notably rice. To accomplish this, an understanding of leaf structure and function is essential. Chlorenchyma cell structure of rice and related warm-climate C3 grasses is distinct from cool temperate C3 grasses. In temperate C3 grasses, vacuoles occupy the majority of the cell, while chloroplasts, peroxisomes and mitochondria are pressed against the cell periphery. In rice, 66% of protoplast volume is occupied by chloroplasts, and chloroplasts/stromules cover >95% of the cell periphery. Mitochondria and peroxisomes occur in the cell interior and are intimately associated with chloroplasts/stromules. We hypothesize that the chlorenchyma architecture of rice enhances diffusive CO2 conductance and maximizes scavenging of photorespired CO2. The extensive chloroplast/stromule sheath forces photorespired CO2 to exit cells via the stroma, where it can be refixed by Rubisco. Deep cell lobing and small cell size, coupled with chloroplast sheaths, creates high surface area exposure of stroma to intercellular spaces thereby enhancing mesophyll transfer conductance. In support of this, rice exhibits higher mesophyll transfer conductance, greater stromal CO2 content, lower CO2 compensation points at warm temperature, and less oxygen sensitivity of photosynthesis than cool temperate grasses. Rice vein length per leaf, mesophyll thickness, and intercellular space volume are intermediate between most C3 and C4 grasses, indicating the introduction of Kranz anatomy into rice may not require radical changes in leaf anatomy; however, deep lobing of chlorenchyma cells may constrain efforts to engineer C4 photosynthesis into rice."
(a) University of Toronto

M1804 A systems approach to understanding C4 photosynthetic differentiation in maize
Li, Pinghua (a) Majeran, Wojchiech (b) Wang, Lin (a) Fernie, Alisdair (e) Myers, Chris (c) Liu, Peng (f) Turgeon, Robert (b) Sun, Qi (c) Nelson, Tim (d) van Wijk, Klaas (b) Brutnell, Thomas P-presenter tpb8@cornell.edu(a,b) Ponnala, Lalit (c) Tausta, Lori (d) Reidel, Edwin (b) Nunes-Nesi, Adriano (e) Friso, Giulia (b) Connolly, Brian M (b) Gandotra, Neeru (d) Kebrom, Tesfamichael (a)
"The maize leaf is an excellent model system to study photosynthetic development as a proximal-distal gradient from base (youngest) to tip (oldest). To exploit this system in understanding C4 photosynthetic differentiation we have conducted a detailed histological, physiological and molecular survey of a maize seedling leaf undergoing the sink-source transition. This survey was used to define four developmental zones of a 9 day old leaf: immature, transition, photosynthetic and mature. Quantitative proteome analysis of the leaf developmental zones, including isolated bundle sheath strands, was performed using large scale spectral counting by high sensitivity tandem mass spectrometry. We have generated over 100 million Illumina reads from cDNA libraries created from RNA isolated from various leaf segments and from laser capture microdissected bundle sheath and mesophyll cells. These data are being integrated with a survey of over 50 primary metabolites and detailed EM sections of the leaf that were taken from the same developmental zones. Collectively, these studies revealed major shifts in primary and secondary metabolism and protein biogenesis associated with leaf development, as well as C4 cell-specific differentiation of photosynthesis and carbon metabolism. The tools that are being developed to interrogate these datasets and the major biological findings will be discussed. "
(a) Boyce Thompson Institute, Cornell University (b) Department of Plant Biology, Cornell University (c) Computational Biology Service Unit, Cornell University (d) Department of Molecular, Cellular and Developmental Biology, Yale University (e) Max Planck Institute for Molecular Plant Physiology (f) Department of Statistics, Iowa State University

MINISYMPOSIA/TALKS – WEDNESDAY, JULY 22

Start	End	Event	Location
8:30 AM	10:10 AM	**Minisymposium 19: Jasmonates** - Chair: Juergen Engelberth 8:30 - M1901: Juergen Engelberth - *Activity profiling of green leafy volatiles* 8:55 - M1902: Tayana V. Savchenko - *Role of fatty acid-based signaling in coordinating plant stress responses* 9:20 - M1903: Abraham J.K. Koo - *Wound-Induced Systemic Synthesis of Bioactive Jasmonates in Arabidopsis* 9:45 - M1904: John C. Withers - *Structure-Function Analysis of Coronatine-Mediated Formation of the COI1:JAZ Receptor Complexes and their Contribution to the Pathogenicity of Pseudomonas syringae*	Lana'I 314

M1901 Activity profiling of green leafy volatiles
Engelberth, Juergen-presenter jurgen.engelberth@utsa.edu(a)
"Green leafy volatiles (GLVs), which are generally emitted by plants in response to mechanical damage and insect herbivory have been found in recent years to play an important role in inter- and intra-plant signaling. Plants receiving these volatile signals generally appear to prime their defenses resulting in a stronger and faster response when under actual attack. To further study the effects of GLVs on plants we employed analytical and molecular techniques to gain further insights into the regulatory networks activated by these compounds. A structure/function analysis of various GLVs and related compounds in corn revealed the structural requirements for GLV activity measured as jasmonic acid accumulation. In contrast we also found that small α, β-unsaturated carbonyls like acrolein do significantly inhibit JA biosynthesis induced by mechanical wounding, insect elicitor treatment, and GLV exposure. However, the activation of jasmonic acid by GLVs appears to be limited to monocotyledonous plants. A comparative analysis of GLV responses in a variety of different plant species on a metabolic level showed that many plants respond to this treatment by rapid changes in the free fatty acid composition. Additionally, we studied early responses to GLVs on a genomic level by microarray analysis. While the results confirmed previous findings with regard to GLV-induced defense gene expression, several new groups of genes affected by this treatment were detected comprising a distinct set of transcription factors, genes involved in lipid- and fatty acid signaling, as well as genes involved in direct and indirect defense responses. The results will provide new insights into GLV-induced signaling processes and will be discussed in the context of plant defense signaling, gene networks, and the consequences for defense priming. "
(a) UTSA, Dept. Biology

M1902 Role of fatty acid-based signaling in coordinating plant stress responses
Savchenko, Tatyana V.-presenter savchenko@ucdavis.edu(a) Walley, Justin (a) Chehab, Wassim (a) Pye, Matthew (b) Kliebenstein, Dan (d) Liu, Junyan (c) Bostock , Richard (b) Hammock, Bruce (c) Dehesh, Katie (a)
"Fatty acids and fatty acid-metabolites are not only major structural and metabolic constituents of the cell, but they also function as modulators of signal transduction pathways. To determine the mechanisms of action of fatty acids and their metabolites in stress signaling we have focused on the oxylipin pathway. The relative levels of oxylipins provide a species-specific signature and help determine the ability of plants to adapt to various developmental and environmental stimuli. Among the several branches of this pathway, we have concentrated on the *AOS* branch of the oxylipin pathway to explore its critical role in plant stress responses via production of jasmonates (JAs). To elucidate the role of fatty acid-mediated signaling in the oxylipin pathway network we have employed transgenic plants that produce minor but easily detectable levels of eicosapolyenoic acids, specifically eicosadienoic acid (EDA, C20:2 $\Delta^{11,14}$) and arachidonic acid (AA, C20:4 $\Delta^{5,811,14}$). We have established that this perturbation of the *in vivo* fatty acid composition of membrane lipids profoundly alters the expression levels of JA-biosynthetic and JA-responsive genes, and modifies the levels of JAs. The physiological consequences of these alterations are modified plant resistance to a number of pathogens examined. These findings suggest that *in vivo* perturbation of fatty acid or fatty acid-derived metabolites, directly or indirectly, regulates *AOS* pathway gene expression. "
(a) University of California, Davis Plant Biology Department (b) University of California, Davis Plant Pathology Department (c) University of California, Entomology Department (d) University of California, Davis Plant Sciences Department

M1903 Wound-Induced Systemic Synthesis of Bioactive Jasmonates in Arabidopsis
Koo, Abraham J.K.-presenter koojeon1@msu.edu(a) Gao, Xiaoli (b) Jones, A. Daniel (b) Howe, Gregg A. (a,b)
"The plant hormone jasmonoyl-L-isoleucine (JA-Ile) and its likely receptor, COI1, regulate wound-induced systemic changes in gene expression by promoting the degradation of JAsmonate ZIM-domain (JAZ) repressors. In Arabidopsis thaliana, it is not known whether wounding activates the synthesis of bioactive jasmonates (JAs) in systemic undamaged leaves or whether these signals are synthesized at the site of wounding and subsequently transported to systemic tissues. To address this question, we a developed liquid chromatography-tandem mass spectrometry procedure to measure 18 different JA derivatives, including the JA precursor 12-oxophytodienoic acid (OPDA), jasmonic acid, and various JA-amino acid conjugates. Systemic increases in the level of JA-Ile were detected within 5 min of mechanical wounding and were accompanied by a decrease in OPDA. Results from experiments conducted with a transgenic line in which the capacity for JA synthesis can be spatially manipulated with a dexamethasone-inducible promoter showed that the systemic JA-Ile burst requires JA production in systemic unwounded leaves but not in damaged leaves. Petiole excision experiments showed that the wound signal responsible for systemic JA-Ile synthesis exits the wounded leaf within 2 min of tissue damage. Based on these results, we suggest that wound-induced systemic responses in Arabidopsis are mediated by a rapid long-distance signal that activates de novo JA-Ile synthesis in undamaged leaves. "
(a) Michigan State University, DOE-Plant Research Laboratory (b) Michigan State University, Department of Biochemistry and Molecular Biology

M1904 Structure-Function Analysis of Coronatine-Mediated Formation of the COI1:JAZ Receptor Complexes and their Contribution to the Pathogenicity of *Pseudomonas syringae*
Withers, John C-presenter withersj@msu.edu(a,b) Mecey, Christy (a,b) Melotto, Maeli (c,d) He, Sheng Yang (a,b)
"The bacterial phytotoxin coronatine, produced by several pathovars of the foliar pathogen *Pseudomonas syringae*, contributes to suppression of plant immune responses both locally and systemically. Coronatine is a structural mimic of the plant hormone jasmonoyl-isoleucine and modulates host transcriptional responses to infection by binding to the SCF^{COI1} ubiquitin ligase and promoting the degradation of jasmonate ZIM-domain (JAZ) transcriptional repressors. Degradation of JAZ proteins leads to the induction of the jasmonate (JA)-response pathway, which regulates many aspects of plant growth, development, and response to pathogen attack or wounding. Receptor complex formation in the presence of coronatine occurs between the conserved C-terminal Jas domain of JAZ repressors and the leucine-rich repeat (LRR) domain of COI1. We have shown that coronatine,

like JA-Ile, is capable of promoting interaction between multiple JAZ proteins and COI1 and that conserved residues within the C-terminal Jas domain of JAZ1 and JAZ9 are crucial for ligand-dependent formation of JAZ-COI1 complexes. To further investigate the importance of specific amino acids, site-directed mutagenesis was used to generate a comprehensive series of point mutations in the Jas domain of JAZ9 and the LRR domain of COI1. Additional JAZ9 mutants that disrupt the interaction with wild-type COI1 were identified, and wild-type JAZ proteins were screened against a suite of COI1 mutants. Here, we identify specific amino acids within both the C-terminal Jas domain of JAZ9 and the LRR domain of COI1 that are necessary for coronatine-mediated complex formation, providing molecular insights into ligand-dependent formation of a major plant hormone receptor complex."
(a) Michigan State University (b) DOE-Plant Research Laboratory (c) University of Texas - Arlington (d) Department of Biology

Start	End	**Event**	Location
8:30 AM	10:10 AM	**Minisymposium 20: Crop Improvement** - Chair: David Christopher 8:30 - M2001: Mark E. Westgate - *Elemental processes controlling soybean seed composition* 8:55 - M2002: Kelly M. Gillespie - *Elevated carbon dioxide and ozone concentrations alter soybean antioxidant metabolism* 9:20 - M2003: Abul K. Mandal - *Development of a new variety of rice for effective prevention of people and their environment from arsenic contamination* 9:45 - M2004: Kristie O. Matsumoto - *An extended AE-rich N-terminal trunk in secreted pineapple cystatin enhances inhibition of bromelain and is post-translationally removed during fruit ripening*	Kaua'I 311

M2001 Elemental processes controlling soybean seed composition
Westgate, Mark E.-presenter westgate@iastate.edu(a) Rotundo, Jose (a) Cianzio, Silvia (a)
"Complex quantitative traits governed by many genes can be dissected into more elemental processes. The final concentration of protein in soybean seeds (SPC), for example, is determined by a combination of accumulation rates and durations of the major storage components: protein, oil and carbohydrate. As such, similar values for SPC can result from a variety of developmental and metabolic strategies within the maternal and zygotic tissues. To identify elemental processes that might determine high SPC, we examined genetic variation in seed development traits in a population of 100 F2:3 progeny lines constructed from parents differing widely in seed composition. We identified two developmental strategies that achieved the same high level of SPC within this population of segregating lines. One subset maintained protein content constant while decreasing accumulation of other seed components. A second subset increased seed protein accumulation. These lines are being screened for SSR markers associated with high SPC to identify genomic regions determining these two unique strategies. In a related study, we evaluated the association between seed composition and assimilate supply per seed. In both experimental lines and elite varieties, high SPC was associated with near saturating levels of assimilate supply per seed during seed filling. The more favorable source-sink ratio, however, was due to reduced seed set. We hypothesize different suites of genes determine these alternative strategies to achieve high SPC, which are not necessarily linked to those associated with reduced oil synthesis or seed yield. If so, identifying genes controlling these high SPC strategies may allow breeders to overcome the commonly observed negative correlation between SPC and grain yield."
(a) Iowa State University

M2002 Elevated carbon dioxide and ozone concentrations alter soybean antioxidant metabolism
Gillespie, Kelly M-presenter kramig@life.uiuc.edu(a) Xu, Fangxiu (a) Rogers, Alistair (b) Leakey, Andrew DB (a) Ort, Donald R (a) Ainsworth, Elizabeth A (c)
http://www.life.uiuc.edu/ainsworth
"One important mechanism by which plants sense and respond to their environment is through redox control. Oxidative damage at the cellular level can feed forward to decrease leaf photosynthesis and therefore canopy and ecosystem productivity. How rising atmospheric carbon dioxide ($[CO_2]$) and tropospheric ozone ($[O_3]$) will alter oxidative stress and resultant antioxidant metabolism in the future is largely unknown. Our goal is to understand and integrate the molecular, biochemical and physiological responses of soybeans to those climate change factors, using the Soybean Free-Air gas Concentration Enrichment (SoyFACE) site. SoyFACE enriches the $[CO_2]$ and $[O_3]$ to levels predicted for 2050 under fully open-air conditions without disturbing the microclimate. We investigated antioxidant metabolism at the genomic and biochemical scales in upper canopy soybean leaves throughout two growing seasons using Affymetrix soybean microarrays and high-throughput assays of ascorbate, phenolic content, total antioxidant capacity, lipid peroxidation and enzyme activity of six antioxidant enzymes. One challenge of this experiment is interpreting the results in a biologically meaningful way. In order to meet this challenge, we have adapted the Mapman visualization software, originally written for *A. thaliana*, for use with soybean. Our results indicate total antioxidant capacity was lower in soybeans grown at elevated $[CO_2]$ and higher in soybeans grown at elevated $[O_3]$, which was mirrored in leaf total phenolic content. Elevated $[CO_2]$ also improved the redox potential of the ascorbate pool. We are integrating these results with changes in antioxidant transcripts and enzymes to provide a mechanistic analysis of the response of the soybean antioxidant system to two factors of global change."
(a) Institute for Genomic Biology , University of Illinois at Urbana Champaign (b) Environmental Sciences Department, Brookhaven National Laboratory (c) USDA Photosynthesis Research Unit, Univeristy of Illinois at Urbana Champaign

M2003 Development of a new variety of rice for effective prevention of people and their environment from arsenic contamination
Mandal, Abul K-presenter abul.mandal@his.se(a) Mirtahery, BentolHoda (a) Nahar, Noor (a,b) Lundh, Dan (a)
http://www.his.se/mana
"Arsenic poisoning through consumption of cultivated crops is a severe health problem in many countries in South Asia especially in India, Bangladesh, Burma and Thailand. For instance, in Bangladesh more than 30 millions people are affected from rice-derived arsenic contamination leading to severe damage of kidney, liver, lungs, bladder etc and many other neurological and vascular disorders. To solve this severe problem we aim to generate a genetically modified variety of rice either by inhibiting and/or activating native gene(s) responsible for arsenic uptake. Alternatively, we can insert foreign genes responsible for arsenic metabolism 'in planta'. For identification and characterization of genes responsible for uptake or metabolism of arsenics in planta we have employed data mining, an in silico analysis based on searching of the existing genomic databases. Data mining experiments resulted in identification of four candidate genes that are proposed to be involved either in uptake, transport or cellular localization of arsenic in plants. However, there is only one candidate gene that might be involved in arsenic metabolism in rice. As an alternative to in silico analysis we have also screened available T-DNA insertion mutants for identification of the candidate genes. Results obtained in both in silico analyses and screening of T-DNA insertion mutants were then utilized for cloning of the candidate genes. To date we have cloned and characterized

two candidate genes ADC1 and ADC25 from the genomic DNA of Arabidopsis thaliana by PCR using database sequences as primers. We are now studying these genes in heterologous systems such as the yeast or E. coli and the results will be discussed. Vectors containing the target genes will be constructed for transformation of rice. "
(a) University of Skovde, Sweden (b) University of Rajshahi, Bangladesh

M2004 An extended AE-rich N-terminal trunk in secreted pineapple cystatin enhances inhibition of bromelain and is post-translationally removed during fruit ripening
Matsumoto, Kristie O.-presenter kokazaki@hawaii.edu(a) Neuteboom, Leon W. (a) Christopher, David A. (a)
"Phytocystatins are potent inhibitors of cysteine proteinases that participate in senescence, seed biogenesis, organ development and, defense against pests and pathogens in plants. Besides kiwi fruit cystatin, no other cystatin has been found to effectively inhibit the cysteine proteinase, bromelain, of pineapple (*Ananas comosus*). Here we demonstrate that a novel pineapple cystatin, *Ac*CYS1, completely inhibits bromelain. *Ac*CYS1 is unique from other cystatins in that it contains an extended N-terminal trunk (NTT) of 63 residues rich in Ala and Glu. A signal peptide that precedes the NTT is processed *in vitro* by canine microsomal membranes giving rise to a 27 kDa species, which is also immunodetected *in vivo*. *Ac*CYS1 mRNA was ubiquitously present in roots, leaves and fruit, with highest abundance in fruit. Immunofluorescence and immunoelectron microscopy indicate that *Ac*CYS1 was present in the apoplasm. Immunoblot analysis identified a distinct 27 kDa protein in fruit, roots, leaves, and a 15 kDa species in mature ripe fruit. We show that ripe fruit extracts proteolytically remove the NTT of *Ac*CYS1 *in vitro* to produce the 15 kDa species. The presence of the AE-rich NTT completely inhibited stem bromelain, whereas its deletion decreased inhibition to 80%. Surface plasmon resonance assays determined that the NTT increased the affinity of *Ac*CYS1 with bromelain. The calculated K_i values with and without the NTT were 0.69 nM and 1.3 nM, respectively. We propose that removal of the NTT enhances the proteolytic activity of bromelain during fruit ripening and senescence. "
(a) University of Hawaii

Start	End	**Event**	Location
8:30 AM	10:10 AM	**Minisymposium 21: Non-coding Regulatory RNAs** - Chair: Lila Vodkin 8:30 - M2101: Liang Song - *Characterization of pri-miRNA structures important for efficient miRNA processing in Arabidopsis thaliana* 8:55 - M2102: Lorenz Buelow - *Bioinformatic prediction of target genes for proposed small activating RNAs in Arabidopsis thaliana* 9:20 - M2103: Melissa D. Lehti-Shiu - *Abundant novel small protein and non-coding RNA genes in the Arabidopsis thaliana genome* 9:45 - M2104: Lila Vodkin - *Flux in the coding and small RNA transcriptomes during soybean seed and seedling development*	Moloka'I 315

M2101 Characterization of pri-miRNA structures important for efficient miRNA processing in *Arabidopsis thaliana*
Song, Liang-presenter lxs926@psu.edu(a) Fedoroff, Nina (b)
"MicroRNAs (miRNAs) are small, non-coding RNAs that are involved in various aspects of plant development and physiological responses. MiRNAs are processed sequentially from pri-miRNAs and pre-miRNAs. The structural features of pri-miRNA that are important for efficient miRNA processing have yet to be identified. To address this question, we introduced a series of mutations that alter the secondary structure of two pri-miRNAs, pri-miR171a and pri-miR167a. The mutated and wild-type pri-miRNAs were over-expressed in *Arabidopsis* and the phenotypes of the transgenic plants were analyzed. Over-expression of the wild-type pri-miRNA resulted in moderate to severe phenotypic effects, such as reduced shoot branching caused by over-expression of miR171a, or reduced silique length caused by over-expression of miR167a. When the base-pairing at the base of the miRNA-containing stem-loop was fully abolished, the transgenic plants were similar to those transformed with the vector alone. When the bulges and mismatches at the base of the miRNA-containing stem-loop were replaced by perfectly matched base pairs, the transgenic plants exhibited less severe phenotypes compared to those over-expressing wild-type pri-miRNAs. In contrast, removal of the bulges near the terminal loop in the miRNA-containing stem-loop did not significantly reduce the severity of the phenotypes observed upon over-expression of the wild-type pri-miRNA. Consistent with the phenotypic effects, lower levels of mature miRNA were observed in plants expressing pri-miRNA structural variants causing less severe phenotypes. Overall, these observations suggest that a stem with bulges and mismatches at the base of the miRNA-embedded hairpin is the most important structural feature for optimal miRNA processing."
(a) Plant Biology Program, The Pennsylvania State University (b) Huck Institute of Life Sciences and Biology Department, The Pennsylvania State University

M2102 Bioinformatic prediction of target genes for proposed small activating RNAs in *Arabidopsis thaliana*
Buelow, Lorenz-presenter l.buelow@tu-bs.de(a) Hehl, Reinhard (a)
http://www.tu-braunschweig.de/ifg/ag/hehl
"Recent studies in mammalian systems suggest that small RNA molecules may be involved in transcriptional activation. To identify putative target genes for proposed activating small RNAs (asRNA) in *A. thaliana*, we used the AthaMap database recently updated by positional information of small RNA target sites. These were derived from a massive parallel signature sequencing approach of the small RNA transcriptome. A total of 403,173 genomic positions of small RNAs have been mapped in the *A. thaliana* genome (Buelow *et al.*, 2009, Nucleic Acids Res. 37: D983-986). The small RNAs were derived from a seedling and an inflorescence library enabling the identification of putative target sites for inflorescence-specific small RNA molecules. Correlation with inflorescence-specific microarray expression data identified several possible target genes of predicted asRNA molecules."
(a) Institute of Genetics, Technical University of Braunschweig, Germany

M2103 Abundant novel small protein and non-coding RNA genes in the *Arabidopsis thaliana* genome
Lehti-Shiu, Melissa D-presenter lehtishi@msu.edu(a) Moghe, Gaurav (a) Yin, Shan (a) Chen, Yani (a) Boniface, Jordan R (a) Juntawong, Piyada (b) Bailey-Serres, Julia (b) Shiu, Shin-Han (a)
http://shiulab.plantbiology.msu.edu
"Transcription evidence indicates that there are likely thousands of novel genes that are presumed to be non-coding. However, their protein-coding potential is rarely verified experimentally. Using computational approaches we have identified thousands of potential small open reading frames (sORFs) in the *Arabidopsis thaliana* genome that are conserved across species and/or have evidence of transcription [Hanada et al. (2007) Genome Research 17:632]. To verify sORF translation on a large scale, we are sequencing mRNAs that are associated with polyribosome complexes and are therefore likely actively translated. We are also verifying the translation of several sORFs directly by monitoring the expression of sORF-YFP fusion proteins *in planta*. Together, these approaches as well as recently published proteomics studies have allowed us to demonstrate the translation of a

class of proteins that, despite their potential importance as small signaling peptides, have been virtually overlooked. In a parallel line of work, we have identified >1600 novel non-coding RNA (ncRNA) genes in *A. thaliana*. These genes do not have similarity to known ncRNAs or protein-coding sequences; therefore, they represent a completely unstudied set of molecules. As a first step towards their functional characterization, we are verifying that these ncRNAs are in fact non-coding by looking for their absence in polyribosome complexes as well as testing their ability to be translated *in planta*. By rigorously verifying the protein-coding potential of putative ncRNAs, and of predicted sORFs, we will get insight into the properties that distinguish protein-coding from non-coding RNA genes. This information can be applied towards more accurate gene annotation."
(a) Michigan State University (b) University of California, Riverside

M2104 Flux in the coding and small RNA transcriptomes during soybean seed and seedling development
Vodkin, Lila-presenter l-vodkin@illinois.edu(a) Jones, Sarah (a) Varala, Kranthi (a) Hudson, Matthew (a) Zabala, Gracia (a) Campos, Edhilvia (a) Hunt, Matt (a) Tuteja, Jigyasa (a) Calla, Bernarda (a) Radwan, Osman (a) Clough, Steven (a) Win, Hling (a)
"Soybean cotyledons and seed coats undergo major developmental transitions during seed development. Changes in the transcriptome from a few days after flowering through maturation and in the cotyledons during early seedling growth have been revealed using microarrays. In addition, we have explored the flux of small RNA populations for several stages and tissues of soybean seed and seedling development by using Illumina Sequence-by-Synthesis deep sequencing yielding over 50 million reads. The small RNAs are being characterized by enumerating the species present and alignment of each to the curated miRNA Sanger database and to the non-redundant nucleotide and protein databases of NCBI. Many match known miRNAs from other organisms in the database, while many others appear to be novel siRNAs or miRNAs. We have also developed statistical techniques to identify differentially expressed small RNAs. Similar to the coding transcriptome, the small RNAome reveals many organ, tissue specific and developmental shifts in the population of small RNAs. Finally, we are correlating the changes in small RNA populations to those of the mRNAs as elucidated by microarray analyses and additionally by digital gene expression high throughput sequencing during normal seed development and in selected mutant isolines. The information is being used to identify possible targets of the siRNA and miRNAs. Naturally occurring soybean siRNAs that control the activity of chalcone synthase in a tissue specific manner only during seed coat development are examples of endogenous small RNAs that produce a physiological effect and visible trait (inhibition of pigmentation) in soybean. Supported by CRI and SDBC programs of Univ. of Illinois, USDA, USB, and ISA. "
(a) University Of Illinois

Start	End	**Event**	Location
8:30 AM	10:10 AM	**Minisymposium 22: Intracellular Signalling** - Chair: Cheolmin Yoo 8:30 - M2201: Xing-guo Lan - *A J domain protein that physically interacted with ARC1 is involved in pollination response in Brassica stigma* 8:55 - M2202: Yan Zhang - *Lipid Raft-Mediated Internalization of Arabidopsis Pollen-Specific Receptor Kinase PRK2a Regulates Polarized Growth of Pollen Tubes through Spatiotemporal Activation of small GTPase ROP* 9:20 - M2203: Gregory L. Richter - *Mechanically induced Ca2+transients may play a role in curve-associated lateral root initiation.* 9:45 - M2204: Cheolmin Yoo - *Altered Root Hair Polarity of the Arabidopsis thaliana agd1 Mutant is Associated with Defects in Various Components of the Tip Growth Machinery*	Maui 316A

M2201 A J domain protein that physically interacted with ARC1 is involved in pollination response in *Brassica* stigma
Lan, Xing-guo-presenter lanxingguo1979@nefu.edu.cn(a) Li, Yu-hua (a) Kawabata, Saneyuki (b)
"Self-incompatibility (SI) is a physiological strategy of many flowering plants to prevent inbreeding. In *Brassica*, the *S*-haplotype specific interaction between the pollen-borne SP11/SCR and the stigmatic SRK receptor activates an intracellular signaling pathway in the stigmatic papilla cell. Arm repeat containing 1 (ARC1), a stigmatic U-box protein with E3 ubiquitin ligase activity, functions as a positive mediator of SI signaling. Upon self-pollination, ARC1 is activated by SRK and then promotes the ubiquitination and degradation of unknown compatibility factors in the pistil, which in turn results in pollen rejection. To determine potential targets of ARC1, we used yeast two-hybrid library screening to identify stigmatic proteins that interact with ARC1. JDP1, a J domain-containing protein, was indentified and interacted specifically with ARC1 but failed to interact with two different *Arabidopsis* U-box E3 ligases, AtPUB14 and AtPUB17. The interaction between ARC1 and JDP1 was confirmed through *in vitro* pull down assays and subcellular colocalization analysis. Domain-mapping studies revealed this interaction was mediated by the C-terminal region of JDP1. In pollination, the ubiquitination level of stigma proteins was increased substantially after incompatible pollination compared with compatible pollination. However, the JDP1 protein level of stigmas is noticeable reduced after incompatible pollination compared with compatible pollination, and had a high peak within 45-60 min after compatible pollination, suggesting an important role of JDP1 in compatible pollination. Taken together, these data suggest that JDP1 interacted with ARC1 is involved in pollination response in *Brassica*. "
(a) College of Life Sciences, Northeast Forestry University (b) Graduate School of Agricultural and Life Sciences, University of Tokyo

M2202 Lipid Raft-Mediated Internalization of Arabidopsis Pollen-Specific Receptor Kinase PRK2a Regulates Polarized Growth of Pollen Tubes through Spatiotemporal Activation of small GTPase ROP
Zhang, Yan-presenter mpizyanzhang@berkeley.edu(a,b) He, Junmin (b,c) McCormick, Sheila (a,b)
"Plant-specific Rho GTPases, ROPs, control pollen tube polarity through their spatiotemporal activation, for which a feedback loop composed of RopGAPs and RhoGDIs was reported. However, little was known about a feed-forward loop that should operate in parallel. Here we provide evidence that endocytosis of an Arabidopsis receptor kinase, PRK2a, feed-forward regulates spatiotemporal activation of ROPs. PRK2a co-localized with endosome markers in ring-shaped cytosolic compartments when over-expressed or when pollen was treated with membrane trafficking inhibitors. A sorting motif (YXXφ) in the carboxy-terminus of PRK2a is essential for its interaction with a μ;2 adaptin subunit in yeast and for its sorting/recycling in pollen tubes. Despite the involvement of an adaptin complex, clathrin-coated pits were not required for internalization of PRK2a because over-expression of the C-terminus of clathrin heavy chain did not show dominant negative effects on PRK2a internalization. Instead, PRK2a endocytosis was mediated by a population of detergent-resistant membranes (DRMs). In mammalian cells, over-expression of a non-phosphorylatable version of human Caveolin1 ($HsCav1_{Y14F}$) inhibits receptor internalization. Over-expression of $HsCav1_{Y14F}$ in pollen tubes resulted in apical plasma membrane retention of PRK2a and depolarized tube growth indicative of ectopic activity of ROPs. DRM-mediated PRK2a internalization may in turn be regulated by ROP activity, because over-expression of dominant-negative or constitutively active ROP5 disturbed distribution of Lyn24GFP, a fluorescent probe labeling a certain DRM population. A feed-forward loop through endocytic trafficking of PRK2a provides a fine-tuned mechanism for spatiotemporal control of ROP during directional tube growth."

(a) Plant Gene Expression Center, United States Department of Agriculture/Agricultural Research Service (b) Department of Plant and Microbial Biology, University of California at Berkeley (c) School of Life Sciences, Shaanxi Normal University, Xi'an 710062, China

M2203 Mechanically induced Ca^{2+}transients may play a role in curve-associated lateral root initiation.
Richter, Gregory L-presenter grichter@psu.edu(a) Monshausen, Gabriele B (b) Giroy, Simon (b,a)
"Development of the root system represents a morphogenetic program where the positioning of new lateral organs occurs through the periodic recruitment of pericycle cells to become founder cells of a new lateral root (LR) primordium. While the hormone auxin appears intimately involved in specifying LR formation, it remains unclear why some pericycle cells are specified to initiate a LR while others are not. Here we show that mechanical forces can act as one of the triggers for founder cell formation and so entrain the pattern of LR production to the environment. We observed that transient physical bending of the root was capable of eliciting LR formation to the convex side of the curve. Such mechanical stimulation triggered a Ca^{2+} transient within the pericycle, which was associated with the recruitment of cells to a LR founder cell fate. The initial establishment of the mechanically induced LR primordium was independent of an auxin supply from the shoot and was not disrupted by mutants in a suite of auxin transporters and receptor/response elements. Mechanical forces have long been proposed to act as plant morphogenetic factors, however the cellular elements that translate mechanical force to a developmental signaling cascade have remained obscure. Our observations indicate that in the case of mechanical induction of LR formation, the program of organogenesis may be triggered by mechanically elicited Ca^{2+} changes that can even suppress the requirement for many auxin-related elements normally involved in founder cell recruitment. Thus, the plant mechano-sensitive Ca^{2+} signaling system provides a potentially widespread mechanism whereby external and endogenous mechanical forces could be translated into morphogenetic programs during plant growth and development."
(a) Pennsylvania State University, Huck Institutes of the Life Sciences (b) University of Wisconsin, Department of Botany

M2204 Altered Root Hair Polarity of the *Arabidopsis thaliana agd1* Mutant is Associated with Defects in Various Components of the Tip Growth Machinery
Yoo, Cheol-Min-presenter cyoo@noble.org(a) Blancaflor, Elison B. (a)
http://www.noble.org/PlantBio/Blancaflor/index.html
"Knockouts to *AGD1*, encodes a class I ADP-ribosylation factor-GTPase activating protein, result in root hairs with wavy and bifurcated tip growth. To understand the role of AGD1 in the polar growth of root hairs, we used live cell imaging approaches to evaluate how various components of the tip growth machinery are affected in the mutant. The growing *agd1* root hairs showed bundles of endoplasmic microtubules and actin filaments extending into the extreme tip. The wavy phenotype and pattern of cytoskeletal distribution in root hairs of *agd1* partially resembled that of an armadillo-repeat containing kinesin (*ARK1*) mutant. We found that cytoplasmic calcium ($[Ca^{2+}]_{cyt}$) tip oscillations, RabA4B and Rop2 targeting and vacuolar membrane dynamics were modified in root hairs of *agd1*. Tip focused $[Ca^{2+}]_{cyt}$ gradients was shown to persist in *agd1* root hairs, but the frequency of the oscillations was significantly dampened in the root hairs. RabA4B occasionally dissipates at the tips of *agd1* root hairs. In addition, the shifting of RabA4B and ROP2 localization was observed to coincide with a change in root hair growth direction. Organelle trafficking as revealed by a Golgi marker was slightly inhibited and Golgi stacks frequently protruded into the extreme root hair apex of *agd1* mutants. Transient expression of GFP-AGD1 labeled punctate bodies that partially colocalized with the endocytic marker, FM4-64, while ARK1-YFP associated with microtubules. Brefeldin A rescued the phenotype of *agd1* indicating that the altered activity of an AGD1-dependent ARF contributes to the various phenotypes. We propose that AGD1 and ARK1 are components of converging signaling pathways that impact cytoskeletal organization and membrane trafficking to specify growth orientation in Arabidopsis root hairs."
(a) The Samuel Roberts Noble Foundation, Plant Biology Division

Start	End	**Event**	Location
8:30 AM	10:10 AM	**Minisymposium 23: Vegetative Development** - Chair: Kathy Barton 8:30 - M2301: Kathryn Barton - *Using oppositely acting transcription factors to identify components of the ad/abaxial network of Arabidopsis* 8:55 - M2302: Derek W.R. White - *PEAPOD limits and coordinates vascular procambium activity and stomatal density in Arabidopsis.* 9:20 - M2303: Maureen C. McCann - *Functions of rhamnogalacturonan-I in plant growth* 9:45 - M2304: Eric Engstrom - *Arabidopsis orthologs of the Petunia HAM mutant regulate meristem indeterminacy, organ generation and growth in both the shoot and the root.*	Maui 316C

M2301 Using oppositely acting transcription factors to identify components of the ad/abaxial network of *Arabidopsis*
Barton, Kathryn-presenter kbarton@stanford.edu(a) Reinhart, Brenda J. (a) Kerstetter, Randall (b) Huang, Tengbo (b) Wenkel, Stephan (a)
"The asymmetric distribution of cell and tissue types from the top to the bottom of the leaf is critical to leaf function. The upper domain of the leaf with its tightly packed, chlorophyll rich palisade layer of cells is specialized for light capture while the lower domain with its loosely packed spongy mesophyll is specialized for gas exchange. Asymmetric differences are established along the ad/abaxial dimension of the leaf primordium while the primordium is still closely associated with the meristem. The ad/abaxial regulatory network controls the specification of ad/abaxial polarity within the leaf primordium. In addition to controlling the asymmetric development of the leaf blade this network also controls branching in the shoot and the root and vascular development. The KANADI and REVOLUTA transcription factors work in opposite directions in the ad/abaxial network: KANADI promotes abaxial development while REVOLUTA promotes adaxial development. We have used inducible versions of these transcription factors and time course experiments to identify downstream targets in an effort to complete our understanding of the ad/abaxial network. Because these factors act in opposite directions, it is possible to leverage more information from these studies than would be possible by analyzing each factor in isolation. In this way, we have been able to identify targets regulated by both factors acting in opposite directions. One of the key findings of this work is that the ad/abaxial network patterns hormone signaling. We will discuss aspects of hormone signaling in the brassinolide, auxin and ABA pathways that are patterned by the ad/abaxial network. "
(a) Department of Plant Biology, Carnegie Institution (b) Waksman Institute, Rutgers University

M2302 *PEAPOD* limits and coordinates vascular procambium activity and stomatal density in *Arabidopsis*.
White, Derek W. R.-presenter derek.white@agresearch.co.nz(a)

"Vascular plants can vary their size in response to different environmental conditions by regulating the growth of leaves, stems and roots. Although this is a common observation there is only limited information about the genetic mechanisms controlling and coordinating plant secondary growth. Much of higher plant secondary growth is determined by the activity of populations of dispersed stem cells (DSC), most notably the vascular procambium/cambium and the shoot epidermal meristemoid mother cells (MMC) that initiate the stomatal lineage. The densities of both the vascular tissue and stomata play significant roles in the water transpiration stream that is critical to the growth of higher plants. In *Arabidopsis* deletion of the *PEAPOD* (*PPD*) locus results in enlarged domed-shaped leaves (1). This excess leaf growth in *ppd* mutant plants is due to increased proliferation of the MMCs that develop into the stomata guard and leaf pavement cells of the epidermis. The loss-of-function mutant also has thickened roots, hypocotyls and stems due to higher levels of procambium/cambium activity producing greater vascular growth throughout the plant. The *PPD* locus is composed of two orthologs, *PPD1* and *PPD2*, which encode novel members of the *TIFY* gene family. Over expression of *PPD* reduces procambium/cambium activity and MMC activity resulting in reductions in both vascular growth and stomatal density. The *PPD* genes therefore act as repressors of dispersed stem cell activity during organ development, coordinating tissue growth by limiting vascular and stomatal density. *PPD* homologs are present in a wide range of eudicot plants, conifers and lycophytes, all plants that have a vascular cambium,but absent from plants without vascular cambium. White, D.W.R. (2006) PNAS 103:13238-13243 "
(a) AgResearch, Grasslands Research Centre

M2303 Functions of rhamnogalacturonan-I in plant growth
McCann, Maureen C.-presenter mmccann@purdue.edu(a) Zhou, Shaohua (a) Penning, Bryan (a) Carpita, Nicholas C. (a)
"The cell wall constrains the sizes and shapes that plant cells achieve. The synthesis of cellulose microfibrils is essential for plant growth and the orientations in which microfibrils are deposited determine the directions in which cells can expand. However, we have obtained evidence that another cell wall polysaccharide, rhamnogalacturonan-I (RG-I), has crucial functions in plant and organ growth. Seven genes (MYST genes) in Arabidopsis have sequence similarity to microbial rhamnogalacturonan lyases (RG-lyases), secreted enzymes that cleave RG-I in plant cell walls. For one gene family member, AtMYST6, we have confirmed that the gene encodes a functional RG-lyase by heterologous expression in E. coli. A genetic functional analysis of AtMYST6 shows that the MYST6 protein is required for root hair development and over-expression of AtMYST6 increases root growth and alters root system architecture, overcoming constraints on normal root growth. These constraints may be biophysical as the substrate for RG-lyase, RG-I, may act to constrain root cell expansion, or physiological as the reaction products may act as signal molecules. Expression of a second MYST family member, MYST4, is required for normal plant stature: knockdown of MYST4 transcription results in severe dwarfing of the aerial parts of the plant. The key challenge is to reconcile the activity of RG-lyase in the cleavage of RG-I molecules with the dramatic growth phenotypes in our gain-of-function and loss-of-function lines. Structural variants of RG-I with different side-chains are developmentally regulated but their functions in cell wall architecture and in plant growth are not known. We hypothesize that endogeneous RG-lyases regulate biophysical properties of cell walls by modulating RG-I structure. "
(a) Purdue University

M2304 "Arabidopsis orthologs of the Petunia HAM mutant regulate meristem indeterminacy, organ generation and growth in both the shoot and the root."
Engstrom, Eric-presenter emengs@wm.edu(a) Hu, John (a) Orlova, Evguenia (a) Bowman, John (b)
"Maintenance of indeterminacy is fundamental to the generation of plant architecture, and a central component of the plant life-strategy, woody perennials being capable of growing for thousands of years. The Petunia HAIRY MERISTEM (HAM) gene, a member of the GRAS family of transcriptional regulators, promotes meristem indeterminacy by undefined non cell-autonomous signaling mechanisms. ham mutants exhibit arrest of lateral organ formation and differentiation of the meristem as stem. No equivalent phenotypes are have been reported to date in Arabidopsis, nor have phenotypes resulting from loss-of-function alleles of Arabdopsis HAM orthologs (AtHAMs). Here we report that Arabidopsis mutants homozygous for probable null alleles of three AtHAMs exhibit phenotypes of, axillary shoot meristem arrest, abnormal phyllotaxis, meristem identity defects, reduced shoot growth, partial male infertility, and reduced length of the main root, and reduction in the size of the root apex. Gain-of-function mutants, resulting from fusion of a YFP protein to the C-terminal end of the AtHAM At2g45160, exhibit the complementary phenotype of multiple shoots, while gain-of-function mutants resulting from disruption of the microRNA binding site exhibit the phenotypes of reduced shoot and root growth. Phylogenetic analysis of GRAS genes from P. patens, S. moellendorffii, O. sativa and Arabidopsis, suggest that HAM genes are closely related to the DELLA subfamily, and that At4g36710 is a HAM gene that lacks the microRNA binding site conserved among all other members of the HAM subfamily. "
(a) The College of William and Mary (b) Monash University, Clayton Campus

Start	End	**Event**	Location
8:30 AM	10:10 AM	**Minisymposium 24: Cell Walls** - Chair: Allan Showalter 8:30 - M2401: Markus Pauly - *The substitution pattern of plant cell wall cross-linking glycans is determined by apoplastic glycosidases* 8:55 - M2402: Allan M. Showalter - *Identification and characterization of hydroxyproline β-galactosyltransferase activity involved in arabinogalactan-protein biosynthesis in tobacco and Arabidopsis* 9:20 - M2403: Patrick T. Martone - *'Lignified' seaweeds: mechanical consequences of cell wall elaboration in a red alga* 9:45 - M2404: Daniel L. Mullendore - *A new method to investigate the cell wall of living cells by high-resolution scanning electron microscopy*	O'ahu 313A

M2401 The substitution pattern of plant cell wall cross-linking glycans is determined by apoplastic glycosidases
Pauly, Markus-presenter paulymar@msu.edu(a) Gunl, Markus (a) Souza, Amancio (a) Neumetzler, Lutz (b) Florian, Kraemer (a)
"Among plant cell wall polymers cross-linking glycans play a major role in maintaining the structural integrity of the wall by their tight association with cellulose microfibrils. More importantly, their metabolism is thought to be essential resulting in cell elongation and thus plant growth. Unlike cellulose, crosslinking glycans (also known as hemicelluloses) contain sidechains of various length making them water soluble and thus enzyme accessible. Although many glycosyltransferases and glycansynthases have been characterized in recent years, it is not known what determines the heterogeneity of the sidechain structures. Our data presented here provides strong evidence that this heterogeneity is determined by apoplastic glycosidases and not by the biosynthetic machinery in the Golgi-apparatus. We used a semi-automated forward genetic approach facilitating oligosaccharide mass profiling (OLIMP) to identify Arabidopsis mutants with altered cross-linking glycan structures, specifically xyloglucan, the most dominant crosslinking glycan in the primary walls of dicots. So far, 36 mutants have been identified containing xyloglucans with different degrees of carbohydrate

substitutions including patterns of O-acetylation as well as novel hitherto unknown structures. Positional cloning of two of the mutants indicated a mutation in two different apoplastic glycosidases acting on specific xyloglucan substituents. The characterisation of those mutants in terms of cell wall polysaccharide structure but also functional aspects on cross-linking glycan metabolism as well as effects on plant growth and development will be presented. "
(a) DOE-Plant Research Laboratory (b) Max-Planck Institute for molecular Plant Physiology

M2402 Identification and characterization of hydroxyproline β-galactosyltransferase activity involved in arabinogalactan-protein biosynthesis in tobacco and Arabidopsis
Liang, Yan (a) Faik, Ahmed (a) Tan, Li (b) Kieliszewski, Marcia K (b) Showalter, Allan M-presenter showalte@ohio.edu(a)
http://www.plantbio.ohiou.edu/epb/faculty/faculty/ams.htm
"Arabinogalactan-proteins (AGPs) are involved in a wide range of processes in plants, including plant growth and development, cellular morphology, programmed cell death and plant-microbe interactions. The sugar side chains of AGPs are attached to hydroxyproline (Hyp) residues in the protein backbone through *O*-glycosidic bonds and account for more than 90% of the molecular mass of these glycoproteins. This study seeks to understand the biochemistry and molecular genetic basis of AGP glycosylation in order to elucidate the biosynthetic pathway and function of AGPs. We previously expressed a synthetic gene encoding an AGP core protein motif of fifty-one [Ala-Pro] repeat units in tobacco BY-2 cells and obtained the $[Ala\text{-}Hyp]_{51}$ peptide glycosylated with arabinogalactan side chains, indicating that tobacco BY-2 cells have a complete AGP glycosylation system. An AGP galactosyltransferase (GalT) assay was developed using microsomal membranes from tobacco BY-2 cells as the enzyme source and HF-deglycosylated $[Ala\text{-}Hyp]_{51}$ peptide ($[AO]_{51}$) as the substrate acceptor, in the presence of UDP-[^{14}C]Galactose as the sugar donor. In addition, chemically synthesized $[Ala\text{-}Hyp]_7$ peptide ($[AO]_7$) was also used as the substrate acceptor in place of $[AO]_{51}$. Hyp: β-GalT activity was detected in the assay with both peptide substrate acceptors and was verified by structural analysis of the products using reverse phase-high performance liquid chromatography analysis, sugar analysis and linkage analysis. Similar Hyp: β-GalT activity was also detected in microsomal membranes from Arabidopsis suspension cells. Purification of the Hyp: β-GalT enzyme from Arabidopsis cells is currently underway to allow for proteomic analysis and identification/testing of candidate Hyp: β-GalT genes."
(a) Ohio University, Department of Environmental & Plant Biology, Molecular & Cellular Biology Program (b) Ohio University, Department of Chemistry and Biochemistry, Molecular & Cellular Biology Program

M2403 'Lignified' seaweeds: mechanical consequences of cell wall elaboration in a red alga
Martone, Patrick T-presenter pmartone@interchange.ubc.ca(a) Estevez, Jose M (b)
http://www.botany.ubc.ca/martone/
"The recent discovery of secondary cell walls and lignin in the intertidal red alga *Calliarthron cheilosporioides* has raised many questions about the adaptive significance and evolutionary history of these traits. In land plants, lignified cell walls mechanically stabilize upright growth and facilitate hydraulic transport. In *Calliarthron*, thick lignified cell walls strengthen tissues, helping fronds resist breaking under waves crashing on the shore. In this study, we performed standard material testing techniques to further explore the mechanical properties of this unique algal tissue. Engineering stress-strain analyses reveal that *Calliarthron* tissue is stronger and stiffer than other algal tissues, but not as stiff as terrestrial plant tissues. *Calliarthron* tissues are also highly extensible, able to stretch more than twice their original length, unlike lignified terrestrial tissues which generally cannot stretch more than 1-3%. The addition of secondary walls makes *Calliarthron* tissues stronger and tougher, absorbing more than ten-times the energy per volume as most woody or algal tissues before breaking. This mechanical augmentation coincides with a doubling of cellulose content within the walls and may be unrelated to lignin content. Surprisingly, as *Calliarthron* cell walls get thicker, they also get weaker per unit area, suggesting that either primary walls deteriorate over time or that secondary walls are made of weaker materials than primary walls."
(a) Department of Botany, University of British Columbia (b) Laboratorio de Fisiologia y Biologia Molecular, IFIByNE (CONICET), University of Buenos Aires

M2404 A new method to investigate the cell wall of living cells by high-resolution scanning electron microscopy
Mullendore, Daniel L.-presenter mullendore@wsu.edu(a) Knoblauch, Michael (a)
"Most three-dimensional investigations of cell wall structure using SEM and AFM deal with non-living woody tissue; where the plant clears cytoplasmic debris from cell walls during programmed cell death. However, investigation of living cells requires cytoplasm removal to expose the cell wall. Common methods to clear the cytoplasm include hypochloride and triton X-100. Due to the action of free radicals, fragile, thin cell walls often collapse before the cytoplasm is cleared. We have developed a method employing a progressive enzymatic digest of the cytoplasm to expose the thin cell wall of living cells (e.g. parenchyma, cambium etc.) while maintaining ultrastructure. Cell wall structures like plasmodesmata and cellulose fibrils are easily visualized. We have investigated the three dimensional structure of sieve plates and occlusion of sieve plate pores by callose in response to injury. It has been proposed that callose can occlude pores in a matter of seconds. Our data indicate that callose is deposited in the sieve pores at a rate of 16nm per minute. This may be sufficient to occlude smaller sized sieve pores and plasmodesmata within minutes. However, many plants contain sieve plate pores with diameters of 1 μ;m or more. These large sieve pores require additional occlusion mechanisms to prevent excess loss of assimilates in an injury event. Our data have a major impact on the understanding of phloem physiology and plant-insect interactions. Furthermore, this method allows for high-resolution investigations of cell walls in a number of other cell types."
(a) Washington State University, School of Biological Sciences

Start	End	**Event**	Location
10:40 AM	12:20 PM	**Minisymposium 25: Cell to Cell Communication** - Chair: David Jackson 10:40 - M2501: David P. Jackson - *Regulation of KNOTTED1 cell-to-cell trafficking by a chaperonin protein* 11:05 - M2502: Jae-Yean Kim - *Dof transcription factors: to move or not to move, that is the question* 11:30 - M2503: Linqu Han - *New insights into the CLAVATA signal transduction pathway* 11:55 - M2504: Gad Miller - *Reactive oxygen species mediate a rapid systemic signal in Arabidopsis thaliana.*	Lana'I 314

M2501 Regulation of KNOTTED1 cell-to-cell trafficking by a chaperonin protein.
Jackson, David p-presenter jacksond@cshl.edu(a) Xu, Morgan (a) Wang, Jing (a) Benitez Alfonso, Yoselin (a)
"Cell-to-cell communication plays critical roles in specifying cell fate and coordinating development in multi-cellular organisms. A new paradigm for

such communication in plants is the selective trafficking of transcription factors through plasmodesmata (PDs), channels that traverse the cell wall and connect all plant cells. We have taken an unbiased genetic strategy to dissect the mechanism of PD trafficking. The maize KNOTTED1 (KN1) homeodomain protein was the first plant protein found to selectively traffic through PD, and its trafficking appears to be important for its function in stem cell maintenance. A gain-of-function trafficking assay in Arabidopsis was developed to demonstrate that the C-terminal region of KN1 is necessary and sufficient for trafficking in vivo. This system provides a simple and tractable model to understand how proteins traffic and to isolate mutants defective in trafficking. As a proof of concept for our strategy, a mutant with attenuated KN1 trafficking has been identified as a chaperonin gene. This chaperonin appears essential for PD trafficking of some but all non-cell-autonomous proteins, and biochemical evidence suggests a physical association between chaperonin and KN1. Proteins are thought to undergo partial unfolding during PD translocation, which makes the discovery of this chaperonin particularly exciting. A functional characterization of chaperonins, the first ever factor so far known to be critical for KN1 PD trafficking will further our understanding of developmental regulation and mechanisms of selective cell-to-cell trafficking. In addition, it may give mechanistic insights into this elaborate protein folding machinery, which is not well understood in any system at a molecular level. "
(a) Cold Spring Harbor Laboratory

M2502 "Dof transcription factors: to move or not to move, that is the question"
Kim, Jae-Yean-presenter kimjaeyean@gmail.com(a) Rim, Yeonggil (a) Lucas, William J. (b) Munawar , Ahmad (a) Cho, Won Kyong (a) Chu, Hyosub (a) Jo, Yeonhwa (a) Zhao, Xuping (a) Jeon, Che Ok (a) Kim, Hye-Jin (a) Hong, Jong-Chan (a)
"Plant cells developed a sophisticated signaling pathway through intercellular symplasmic channels termed plasmodesmata. Over the past decade, intercellular trafficking of transcriptional factors (TFs) has emerged as a novel mechanism of cell-to-cell communication in plant development. This movement through plasmodesmata occurs either via a selective pathway or a gated, non-selective pathway. To identify non-cell-autonomous transcription factors (NCATFs), we performed a genome-wide screen using the GAL4-UAS activation system in Arabidopsis. For this purpose, we used the CS9094 GAL4 enhancer trap line which drives the expression of GFPer in the cortex and endodermal layers of root tip. Among about 300 transgenic lines carrying a UAS-TF-mCherry construct, 75 TF T1 lines showed detectable mCherry fluorescent signal, and mCherry fluorescent signal in 27 lines was detected outside of the expression domain. Hence, approx. 37% of the tested TFs, including 19 members of 64 TF families, showed potential intercellular TF trafficking. As a model to study the trafficking mechanism and function of NCATFs, we selected the Dof family, a plant specific class of TFs. This family has a highly conserved Dof domain which recognizes an AAAG motif as the essential sequence element within the target DNA-binding site. The Dof domain performs the dual function of DNA binding and protein."
(a) Gyeongsang National University (b) University of California

M2503 New insights into the CLAVATA signal transduction pathway
Han , Linqu -presenter hanl@umich.edu(a) Clark , Steven (a)
"In Arabidopsis, almost all aerial organs are developed from the shoot apical meristem (SAM). A functional SAM is maintained through a delicate balance between the restriction of stem cell proliferation and the promotion of organ primordia differentiation. In Arabidopsis, the stem cell population in the SAM is specified by a negative feedback loop which consists of WUSHEL (WUS) and CLAVATA genes. The CLAVATA (CLV) genes including CLV1, CLV2 and CLV3 are signaling proteins which are shown to function together in the stem cells to restrict its proliferation by repressing WUSHEL expression. It has been proposed that stem cells in the SAM secrete the putative CLV3 signaling ligand into extracellular space where it is perceived by a receptor complex compromising a receptor-like kinase CLV1 and a receptor-like protein CLV2. The CLV3 ligand has been shown to bind the ectodomain of CLV1 in vitro while the mechanism by which the CLV3 ligand activates CLV1 remains unknown. Recent genetic studies have identified multiple receptor-like kinases including CORYNE (CRN), BAM1 and BAM2, which genetically interact with the CLV1 or CLV2 to specify stem cell population. The BAM1 and BAM2 share a similar protein structure with CLV1 while the CRN is a membrane-associated kinase with a very short extracellular domain. Despite of the extensive genetic interactions demonstrated among these receptors, detailed biochemical analysis of these receptor proteins have been made difficult due to their limited expression in small number of cells. Here we took advantage of a heterologous protein expression system and conducted a preliminary biochemical study of these receptors. "
(a) The University of Michigan

M2504 Reactive oxygen species mediate a rapid systemic signal in Arabidopsis thaliana.
Miller, Gad-presenter gadmiller@gmail.com(a) Schlauch, Karen (a) Tam, Rachel (a) Cortes, Diego (b) Torres, Miguel A (c) Shulaev, Vladimir (b) Dangl, Jeffery L (d) Mittler, Ron (a,e)
"Cell-to-cell communication and long distance signaling play an important role in the response of plants to pathogens, pests, mechanical wounding and extreme environmental conditions. Here, we uncover a rapid systemic signal that travels at a rate of ~8 cm min-1 and is dependent on the presence of the respiratory burst oxidase homolog D (RbohD) gene. Signal propagation is accompanied by the accumulation of reactive oxygen species (ROS) in the extracellular spaces between cells, and can be inhibited by the suppression of ROS accumulation at locations distant from the initiation site. The systemic signal can be triggered by wounding, heat, cold, high light and salinity stresses. Our results reveal a profound and general role played by ROS in mediating rapid, self-propagating systemic signals in plants. "
(a) Department of Biochemistry University of Nevada, Reno (b) Virginia Bioinformatics Institute, Virginia Tech (c) Department of Biotechnology, Madrid Technical University (d) Department of Biology, University of North Carolina (e) Department of Plant Sciences, Hebrew University of Jerusalem

Start	End	**Event**	Location
10:40 AM	12:20 PM	**Minisymposium 26: Transcriptional and Post-transcriptional Regulation** - Chair: Christoph Peterhansel 10:40 - M2601: Wen-hui Shen - *Histone methylation and histone ubiquitylation in regulation of gene transcription, plant growth and development* 11:05 - M2602: Christoph Peterhansel - *Signal integration on chromatin: The histone language of photosynthetic gene expression in maize* 11:30 - M2603: Yoo-Sun Noh - *The RNA-Binding Protein ELF9 Directly Reduces SOC1 Transcript Levels through Nonsense-Mediated mRNA Decay in Arabidopsis* 11:55 - M2604: Q. Quinn Li - *Calcium signaling and the role of a polyadenylation factor in plants response to environment*	Kaua'I 311

M2601 "Histone methylation and histone ubiquitylation in regulation of gene transcription, plant growth and development"
Shen, Wen-Hui-presenter wen-hui.shen@ibmp-ulp.u-strasbg.fr(a) Berr, Alexandre (a) Menard, Rozenn (a) Molitor, Anne (a) Gao, Juan (a) Meyer, Denise (a)

http://ibmp.u-strasbg.fr/
"Histone methylation and histone monoubiquitylation are two types of epigenetic memory marks in eukaryotes. Both types of modifications can act either in activation or in suppression of transcription. Reverse genetic analysis in Arabidopsis unravels critical roles for regulators of histone methylation and/or histone monoubiquitylation in several processes of plant growth and development. Our studies demonstrated that H3K36 di- and tri-methylation together with H2B ubiquitylation are involved in activation of expression of FLOWERING LOCUS C (FLC) and its homologue MAF genes. This regulatory pathway is essential for the control of flowering time. Our work on POLYCOMB group (PcG) genes showed that a PRC1-like complex containing LHP1, AtRING1a and AtRING1b acts in conjunction with the PRC2-catalyzed H3K27 methylation in suppression of Class I KNOX genes (STM, BP/KNAT1, KNAT2 and KNAT6). This regulatory pathway plays important roles in the maintenance of proper stem cell activity within the shoot apical meristem (SAM). We will show and discuss our recent data to highlight roles of histone methylation and histone ubquitylation in gene transcription, plant growth and development as well as in plant responses to environmental stimuli, including abiotic and biotic stresses. References: LIU, Z., et al. (2009) Plant J., accepted. XU, L., et al. (2009) Plant J. 57:279-288. XU, L. and SHEN, W.-H. (2008) Curr. Biol. 18:1966-1971. XU, L., et al. (2008) Mol. Cell. Biol. 28:1348-1360. LIU, S.M., et al. (2007) Plant J. 52:914-926. ZHU, Y., et al. (2006) Plant Cell 18:2879-2892. ZHAO, Z., et al. Nature Cell Biol. 7:1256-1260. DONG, A., et al. (2005) Plant Physiol. 138:1446-1456. "
(a) IBMP du CNRS

M2602 Signal integration on chromatin: The histone language of photosynthetic gene expression in maize
Peterhansel, Christoph-presenter cp@botanik.uni-hannover.de(a) Horst, Ina (a) Dreesen, Bjoern (c) Offermann, Sascha (b)
http://www.botanik.uni-hannover.de
"Photosynthetic gene expression is regulated by a wealth of stimuli that are integrated into a single promoter response. We use transcriptional control of genes controlling C4-metabolism in maize as a model to study information storage and read-out on chromatin. Histones, the building blocks of the nucleosome, can be modified in various ways. Many of these modifications have been associated with gene activity or repression, respectively. Our analyses reveal that positional and environmental stimuli control specific promoter modifications independent of whether the gene is finally activated: Three acetylation sites are modified on the core promoter upon illumination whereas most others are constitutively modified. This effect is regulated by light-dependent inactivation of a histone deacetylase [1,2]. Trimethylation of histone H3 lysine 4 is controlled by developmental information and set in a tissue-specific manner long before genes are activated [3]. This code is restricted to the proximal promoter region, whereas histone modifications on distal promoters follow a more simple charge neutralization model [2]. Pseudogene copies are repressed by a DNA methylation signal and dimethylation of histone H3 lysine 9. Both markers are precisely limited to a short region closely behind the transcription start site. We currently extend our studies to circadian control of gene regulation and genome-wide analyses of stimulus-dependent histone modifications. These results will allow new insights into the vocabulary of the histone language in plants. [1] Offermann et al (2006) Plant Physiology 141: 1078-1088, [2] Offermann et al (2008) Genetics 179: 1891-1901 [3] Danker et al (2007) Plant Journal 53: 465-474"
(a) Leibniz University Hannover (b) Washington State University (c) RWTH Aachen University

M2603 The RNA-Binding Protein ELF9 Directly Reduces *SOC1* Transcript Levels through Nonsense-Mediated mRNA Decay in Arabidopsis
Noh, Yoo-Sun-presenter ysnoh@snu.ac.kr(a) Song, Hae-Ryong (a) Song, Ju-Dong (a) Cho, Jung-Nam (a) Amasino, Richard M (c) Noh, Bosl (b)
"*SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) is under a complex transcriptional regulatory network that allows for the integration of multiple floral regulatory inputs from photoperiods, gibberellin, and *FLOWERING LOCUS C*. However, the posttranscriptional regulation of *SOC1* has not been explored. Here we report that EARLY FLOWERING 9 (ELF9), an Arabidopsis RNA-binding protein, directly targets the *SOC1* transcript and reduces *SOC1* mRNA levels, possibly through a nonsense-mediated mRNA decay (NMD) mechanism. The fully spliced *SOC1* transcript is up-regulated in *elf9* mutants as well as in mutants of NMD core components. Further, a partially spliced *SOC1* transcript containing a premature termination codon increases more significantly than the fully spliced transcript in *elf9* in an ecotype-dependent manner. A Myc-tagged ELF9 protein (MycELF9) directly binds to the partially spliced *SOC1* transcript. Previously known NMD target transcripts of Arabidopsis are also up-regulated in *elf9* and recognized directly by the MycELF9. *SOC1* transcript levels are also increased by the inhibition of translational activity of ribosome. Thus, the *SOC1* transcript is one of direct targets of ELF9, which appears to be involved in NMD-dependent mRNA quality control in Arabidopsis."
(a) Seoul National University (b) Gyeongsang National University (c) University of Wisconsin

M2604 Calcium signaling and the role of a polyadenylation factor in plants response to environment
Li, Q. Quinn-presenter liq@muohio.edu(a) Xu, Ruqiang (a) Liu, Man (a) Addepalli, Balu (b) Hunt, Arthur G (b)
http://www.polyA.org
"Processing of mRNA is recognized as an important gene expression regulatory hub due to its role in dramatically increase of transcriptome and proteome complexity in higher eukaryotes. Among such processing events is the alternatively polyadenylation of mRNA that produce diverse transcript ends, many of which may result in different protein coding capacity. We have previously characterized an Arabidopsis polyadenylation factor, Cleavage and Polyadenylation Specificity Factor subunit 30 (CPSF30) that is part of the plant polyadenylation apparatus. A T-DNA insertion in the first exon of the gene for CPSF30 (*OXT6*) led to an increased tolerance to oxidative stress. Interestingly, we found that the CPSF30 is a calmodulin binding protein as well as an RNA binding protein, and its activity can be modulated by redox. Moreover, calmodulin inhibits the RNA binding activity of CPSF30, indicative of a role of calcium signaling. Further characterization of the *oxt6* mutant revealed that it also differently responds to other environmental stimuli, e.g. heat stress, hormones, flowering time, and even lateral root development. Early analysis of mRNA polyadenylation profile of *oxt6* demonstrates potential change of alternative polyadenylation profile. More interestingly, *OXT6* gene also encodes another larger protein (C30Y) that contains most of CPSF30, but has a C-terminal extension that contains a domain homologous to a protein implicated in mRNA splicing. Since C30Y also contains the calmodulin binding domain, its role in environmental and developmental responses would also be impacted by calcium signaling. Further analysis of the calmodulin binding domain mutants on both proteins and their responses to environmental stimuli are on going. "
(a) Miami University, Ohio (b) University of Kentucky

Start	End	Event	Location
10:40 AM	12:20 PM	**Minisymposium 27: Bioenergy Crops** - Chair: Frank Dohleman, ASPB Ambassador 10:40 - M2701: Leyla T. Hernandez-Gomez - *Development of Dunaliella strains for enhanced biofuel feedstock production.* 11:05 - M2702: Rui Zhou - *A functional genomics approach to understanding and remodeling plant cell walls of bioenergy crops* 11:30 - M2703: Jaemo Yang - *Controlled silencing of 4-Coumarate:Coenzyme A Ligase alters lignocellulose composition.* 11:55 - M2704: Frank G. Dohleman - *Sixty percent more productive than maize in the Midwest! How does Miscanthus do it?*	Moloka'I 315

M2701 Development of *Dunaliella* strains for enhanced biofuel feedstock production.
Hernandez-Gomez, Leyla T.-presenter leylathg@yahoo.com(a) Lemos, Mark S. (a) Albion, Rebecca L. (a) Shintani, David K. (a) Harper, Jeff F. (a) Cushman, John C. (a)
"Halophytic unicellular green algae within the genus *Dunaliella* could serve as alternative feedstocks for the generation of biofuels, because they contain high amounts of triacylglycerols (TAGs) and starch, which could be extracted, processed and refined to meet the global demand for transportation fuels. The overall goal of our project is to develop *Dunaliella* strains that contain elevated TAGs or starch content in order to increase their feedstock potential for biodiesel or ethanol production, respectively, and to reduce production costs. In order to create an algal strain with improved traits, *Dunaliella* cells were chemically mutagenized using ethyl methyl sulfonate (EMS) and mutant cells were selected reiteratively, by continuous percoll density gradient centrifugation, for cells populations containing high oil/starch amounts. After only 10 rounds of density selection there were significant qualitative differences observed in buoyant density between mock selected and reiteratively selected cell populations. Furthermore, the selected populations displayed quantitative increases in either TAG or starch production illustrating that alterations in feedstock characteristics can be derived in relatively short time frames. In addition, a stable transformation strategy to establish genetic manipulation tools is being developed that will allow for the engineering of algal strains with improved feedstock traits."
(a) University of Nevada, Reno

M2702 A functional genomics approach to understanding and remodeling plant cell walls of bioenergy crops
Chen, Fang (a,c) Wang, Huanzhong (a,c) Zhao, Qiao (a,b) Shen, Hui (a,c) Zhou, Rui-presenter rzhou@noble.org(a) Jackson, Lisa (a) Shadle, Gail (a) Hernandez, Tim (a,c) Qi, Liying (a) Dixon, Richard A (a,c)
http://www.noble.org
"The composition and structure of lignified cell walls has a significant impact on the value of plant-derived raw materials. We have previously demonstrated clear relationships between lignin content/composition and the efficiency of saccharification by both chemical pre-treatment and enzymatic hydrolysis using stably transformed alfalfa lines. It is clear that lignification is critical in plant support, water transport and, in some cases, disease resistance. Therefore, technology for tissue-specific modification of lignin biosynthesis is highly desirable. To obtain a genetic and biological understanding of the mechanisms that regulate cell wall biosynthesis, we developed a method for the large scale screening of M. truncatula transposon insertion lines for discovery of novel genes affecting spatial patterns and amounts of lignin deposition in Medicago (~10,000 plants with multiple insertions). Twenty five particularly interesting mutant lines were obtained with altered patterns of lignification and cell wall structure. Some of these mutants have intact vascular systems but have altered fiber cell walls and lignin content with no visible defects in plant growth and development. Cloning and characterization of the genes affected in these mutants will enable us to confirm their biological functions and provide a better understanding of the biochemical and regulatory pathways that determine cell wall phenotypes. Candidate genes were also selected from microarray and comparative genomic studies in M. truncatula and switchgrass. We anticipate that this approach will ultimately provide a technical platform for the remodeling of plant cell wall structure of bioenergy crops. "
(a) The Samuel Roberts Noble Foundation (b) Oklahoma Bioenery Center (c) DOE BioEnergy Science Center

M2703 'Controlled silencing of 4-Coumarate:Coenzyme A Ligase alters lignocellulose composition.'
Yang, Jaemo-presenter jyang@danforthcenter.org(a) Roger, Beachy N (a)
"Lignocellulose is touted as the primary source of renewable energy in the coming years. An important challenge to achieving this goal is that most biomass, such as corn stover, switchgrass, woody plants and other feedstocks, are not easily converted to fermentable substrates. Typically, lignin, a phenolic polymer, impedes the degradation of cell wall polysaccharides to fermentable sugars. In order to reduce lignin levels during or before full maturation we generated transgenic Arabidopsis plants containing genes that confer constitutive or inducible silencing of the 4CL gene that plays a key role in lignin biosynthesis. While the 4CL gene was highly expressed in WT plants, the amount of 4CL mRNA was greatly reduced in transgenic plants in which the 4CL gene was silenced by RNAi. As a result the stems of the transgenic plants exhibited 25% reduction in lignin with concomitant 20% increase in cellulose. To determine if it was possible to alter lignocellulose composition at specific times in plant development we applied an inducible gene expression system to 4CL-silencing. We treated the gene-inducing ligand to three different growing stages: at bolting, in immature stages (5 ~ 7 cm high), and at intermediate stages (10 ~ 15 cm high). The result was similar to the result in plants with constitutive knock-down of 4CL: i.e., the stems of induced plants exhibited increased cellulose content and reduced amount of total lignin when compared with non-induced stems. Interestingly, even the bolting stems from plants induced during the intermediate stage exhibited altered biochemical composition of the cell wall. Our results suggest that it will be possible to alter lignocellulose composition in some plants without affecting normal growth and development. "
(a) Donald Danforth Plant Science Center

M2704 Sixty percent more productive than maize in the Midwest! How does Miscanthus do it?
Dohleman, Frank G.-presenter dohleman@illinois.edu(a) Long, Stephen P (a)
http://www.miscanthus.uiuc.edu
"An economically and energetically favorable bioenergy crop must be able to produce large quantities of biomass with minimal inputs. The C_4 species Miscanthus (*Miscanthus* x *giganteus*) and maize (*Zea mays*) are being used or considered as energy crops. Until now their productivity has not been directly compared. In side-by-side large scale field trials in the Corn Belt of central Illinois, USA, Miscanthus was 60% more productive than maize ($p<0.0001$). The total productivity of a crop species is determined by the product of the total amount of solar radiation which is incident on an area of land (Q_{tot}) efficiencies of light interception (ε_i) and conversion into biomass (ε_c). Understanding the basis for higher productivity in Miscanthus will

show how corn and other C4 crops could be engineered to increase yield. Averaged over two complete growing seasons, ε_i for Miscanthus was 61% higher than in maize ($p<0.0001$) accounting for the difference in biomass accumulation. This was because Miscanthus developed a green leaf canopy earlier and maintained it later than maize. Conversion efficiency was not different between species ($p=0.5$). In 2007 and 2008, the diurnal course of photosynthesis was measured on sunlit and shaded leaves of each species on 26 dates throughout the two growing seasons. The daily integral of photosynthetic CO2 uptake was up to 60% higher in maize, during mid-summer, however when integrated across the two complete growing seasons, there was no difference in leaf-level assimilation ($p=0.4562$). Green Leaf Area Index (GLAI), was measured destructively on the same dates as gas exchange, and when integrated over two full growing seasons, GLAI was more than double for Miscanthus when compared to maize ($p<0.0001$). By combining information on photosynthesis of different leaf layers with canopy size and structure, total photosynthesis of both crops were calculated. Canopy photosynthesis across both growing seasons was 44% higher in Miscanthus than in maize ($p<0.0001$), corresponding closely with the difference in peak biomass. Finally to determine the basis for the superior leaf-level photosynthesis in maize at mid-season, light and CO2 responses were derived to determine *in vivo* biochemical limitations. These showed that in mid-season maize has a higher maximum velocity of PEP carboxylation (V_{pmax}) and a higher velocity of PEP regeneration (V_{pr}), as well as a higher light saturated rate of photosynthesis (A_{sat}) and higher maximum quantum efficiency of CO_2 assimilation ($\Phi_{CO2\ max}$). These biochemical differences, however, are compensated by a larger leaf area in Miscanthus, and its ability to maintain photosynthetically competent leaves at the cooler temperatures of late spring and fall."
(a) University of Illinois

Start	End	**Event**	Location
10:40 AM	12:20 PM	**Minisymposium 28: Rhythms** - Chair: Shu-Hsing Wu 10:40 - M2801: Shu-Hsing Wu - *Two new clock proteins, LWD1 and LWD2, regulate Arabidopsis photoperiodic flowering* 11:05 - M2802: Norihito Nakamichi - *Pseudo-response Regulator 9, 7 and 5 are Repressors of CCA1 and LHY Transcription in Arabidopsis Circadian Clock* 11:30 - M2803: Sergei A. Filichkin - *Diurnal and circadian transcript profiling defines functionally conserved key clock regulated genes among arabidopsis, rice, and poplar* 11:55 - M2804: C. Robertson McClung - *Natural Allelic Variation In Circadian Clock Function In Brassica rapa*	Maui 316A

M2801 "Two new clock proteins, LWD1 and LWD2, regulate Arabidopsis photoperiodic flowering"
Wu, Shu-Hsing-presenter shuwu@gate.sinica.edu.tw(a,b) Wu, Jing-Fen (a) Wang, Ying (a,b)
"The 'light' signal from the environment sets the circadian clock to regulate multiple physiological processes for optimal rhythmic growth and development. One such process is the control of flowering time by photoperiod perception in plants. In Arabidopsis, the flowering time is determined by the correct interconnection of light input and signal output by the circadian clock. The identification of additional clock proteins will help to better dissect the complex nature of the circadian clock in Arabidopsis. Here we show LWD1/LWD2 as new clock proteins involved in photoperiod control. The *lwd1lwd2* double mutant has an early flowering phenotype, which could be complemented by either *LWD1* or *LWD2*. LWD1 and LWD2 share greater than 90% sequence similarity and function redundantly, yet their expression is independent of each other. The early flowering phenotype is contributed by the significant phase shift of *CO* and, therefore, an increased expression of *FT* before dusk. Clock genes tested have a short period length in the *lwd1lwd2* double mutant. Our data imply that LWD1/LWD2 proteins function in close proximity to or within the circadian clock for photoperiodic flowering control. We will describe the in depth characterization of LWD1/LWD2 in the aspects of their expression kinetics, subcellular localization and their impact on clock genes."
(a) Institute of Plant and Microbial Biology, Academia Sinica (b) Graduate Institute of Life Science, National Defense Medical Center and Academia Sinica

M2802 "PSEUDO-RESPONSE REGULATOR 9, 7 and 5 are Repressors of CCA1 and LHY Transcription in Arabidopsis Circadian Clock"
Nakamichi, Norihito-presenter nnakamichi@psc.riken.jp(a) Kiba, Takatoshi (a) Sakakibara, Hitoshi (a)
http://labs.psc.riken.jp/brt/English/index.html
"Circadian clocks regulate daily fluctuations of many physiological events in organism. In plant, an interlocking transcriptional/translational of 24h-feedback loop of clock-associated genes is thought to be clock core. PSEUDO-RESPONSE REGULATOR9 (PRR9), PRR7, PRR5, TIMING OF CAB EXPRESSION 1 (TOC1, also called PRR1), closest paralog of MYB transcription factors CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), play pivotal role in the interlocking feedback loop. Genetic study have suggested that PRR9, PRR7, and PRR5 somehow repress morning genes CCA1 and LHY, whereas activate evening gene TOC1. However, the molecular function of PRR proteins is mostly unknown. Here we demonstrate that nuclear entry of PRR5 can immediately repress CCA1 and LHY expression. Reporter assay indicated that PRR proteins repress CCA1 promoter activity. In addition, chromatin-immunoprecipitation analysis indicated the interaction between PRR proteins and upstream region of CCA1 and LHY genes during daytime in vivo. On the other hand, PRR proteins neither associated to TOC1 promoter region in vivo, nor attenuate TOC1 promoter activity. These results establish PRR9, PRR7, and PRR5 as repressors for morning genes CCA1 and LHY during daytime, which is essential to proper clock function. "
(a) RIKEN Plant Science Center

M2803 "Diurnal and circadian transcript profiling defines functionally conserved key clock regulated genes among arabidopsis, rice, and poplar."
Filichkin, Sergei A. -presenter filichks@onid.orst.edu(a) Priest, Henry (a) Michael, Todd P. (b) Breton, Ghislain (c) Kay, Steve (c) Chory, Joanne (d) Mockler, Todd C. (a)
"To interrogate diurnal gene expression under light/temperature cycles in rice, Arabidopsis, and poplar we used whole-genome oligonucleotide microarrays. Transcript abundance was phased to all hours over the day, with most genes peaking before dawn or dusk. Collectively, photocycles and thermocycles controlled a large proportion (~40 %) of both the rice and the poplar transcriptomes. We have identified clusters of co-expressed genes for each phase of the day in all three species. A total of 41-46% of predicted Arabidopsis-poplar-rice orthologs cycled under diurnal conditions. Approximately 21-36% of the putative Arabidopsis-poplar-rice orthologs were phased to within 3 hrs of each other, suggesting that daily phasing of gene expression is strongly conserved in higher plants. After cycling genes were identified, the promoters of genes on the individual gene lists for each 1 hour phase of the day were analyzed using ELEMENT to identify significantly over-represented 3-8mer DNA words. The Z-score profiles for several well characterized (ME, GBOX, EE, GATA) and novel (TBX, SBX) diurnal/circadian associated cis-regulatory promoter elements were conserved among Arabidopsis, poplar, and rice. Identification of orthologous motifs in promoters of co-cycling gene clusters suggested that the diurnal/circadian regulatory network is conserved among all three species and likely in other higher plants. "

(a) Oregon State University, Corvallis, OR 97331 (b) Rutgers University, Waksman Institute, Piscataway, NJ 08855 (c) University of California, San Diego, CA 92093 (d) The Salk Institute and HHMI, La Jolla, CA 92037

M2804 Natural Allelic Variation In Circadian Clock Function In *Brassica rapa*.
Lou, Ping (a) Xie, Qiguang (a) Xu, Xiaodong (a) Brock, Marcus T. (b) Edwards, Christine E. (b,b) Weinig, Cynthia (b) McClung, C. Robertson-presenter mcclung@dartmouth.edu(a)
http://www.dartmouth.edu/~rmcclung/index.html
"The endogenous circadian clock confers an internal periodicity that synchronizes an organism with the environmental period imposed by Earth's rotation; when internal and external periods diverge, performance measures such as net photosynthesis decline dramatically. Crop species encounter widely differing environmental conditions, including variable daylength and temperature. Thus, variation in circadian clock function has implications for agricultural productivity, especially among crops grown across wide latitudinal ranges. To date, studies of the plant circadian clock have emphasized *Arabidopsis thaliana*. We have extended this study to the crop plant, *Brassica rapa*. There is considerable variation in clock function among cultivated *Brassica rapa* accessions. To identify genes responsible for this natural variation we have analyzed a set of Recombinant Inbred Lines to identify Quantitative Trait Loci (QTL) for period, amplitude and temperature compensation of the circadian rhythm in leaf movement and for flowering time, as well as for a number of morphometric parameters, including, size of floral organs, and hypocotyl length. We are generating Heterogeneous Inbred Families to identify the genes responsible for these QTL. We also are testing candidate loci in transgenic Arabidopsis and *B. rapa*. We have developed a transgenic *B. rapa* hypocotyl system that expresses a light- and temperature-entrained circadian clock, as measured with clock-regulated reporter gene fusions. Our results suggest that there is considerable potential for the modification of circadian clock function as well as floral traits in *B. rapa* crops grown under agroecologically relevant daylength and temperature settings. Supported by National Science Foundation grant IOB-0517111. "
(a) Dartmouth College (b) University of Wyoming

Start	End	**Event**	Location
10:40 AM	12:20 PM	**Minisymposium 29: Protein Trafficking** - Chair: Bonnie Bartel 10:40 - M2901: Tishiaki Mitsui - *Plastid-targeting of rice alpha-amylase glycoprotein from the Golgi apparatus through the secretory pathway* 11:05 - M2902: Naxhiely Martinez - *Interdependence of the PEX5 and PEX7 peroxisome-targeting receptors in Arabidopsis thaliana* 11:30 - M2903: Sundaram Kuppu - *The Arabidopsis ankyrin repeat-containing protein 2A is an essential molecular chaperone for the biogenesis of a class of membrane-bound proteins and it plays an important role in plant growth and development* 11:55 - M2904: Rosa Lopez-Marques - *Lipid pumps required for endocytosis and formation of secretory vesicles*	Maui 316C

M2901 Plastid-targeting of rice alpha-amylase glycoprotein from the Golgi apparatus through the secretory pathway
Mitsui, Tishiaki-presenter t.mitsui@agr.niigata-u.ac.jp(a) Kitajima, Aya (a) Asatsuma, Satoru (a) Okada, Hisao (a) Hamada, Yuki (a) Kneko, Kentaro (a) Toyooka , Kiminori (c) Matsuoka, Ken (b,c) Nakano , Akihiko (d,e)
"The well-characterized secretory glycoprotein, rice alpha-amylase isoform I-1 (AmyI-1), was localized within the plastids, and proved to be involved in the degradation of starch granules in the organelles of rice cells. In addition, a large portion of AmyI-1 fused to green fluorescent protein (AmyI-1-GFP), expressed transiently, also co-localized with a simultaneously-expressed fluorescent plastid marker in onion epidermal cells. The plastid targeting of AmyI-1 was inhibited by both dominant negative and constitutive active mutants of AtARF1 and AtSAR1, which arrest the ER-to-Golgi traffic. In cells expressing fluorescent trans-Golgi and plastid markers, they frequently co-localized when co-expressed with AmyI-1. Three-dimensional time-lapse imaging and electron microscopy of high-pressure frozen/freeze-substituted cells clearly demonstrated that contact and subsequent absorption of the Golgi-derived membrane vesicles with cargo into plastids occur within the cells. The transient expression of a series of carboxy-terminal truncated AmyI-1-GFP fusion proteins in the onion cell system showed that the region from Trp301 to Gln369 is necessary for plastid targeting of AmyI-1. Furthermore, the results obtained by site-directed mutations for Trp302 and Gly354, located on surface opposite sides of AmyI-1 protein, suggest that multiple surface regions are necessary for plastid targeting. Based on these results, we propose that Golgi-to-plastid traffic is involved in the transport of glycoproteins to plastids, and that plastid targeting is accomplished in a sorting signal-dependent manner."
(a) Niigata University (b) Kyushu University (c) RIKEN Plant Science Center (d) RIKEN Advanced Science Instititute (e) University of Tokyo

M2902 Interdependence of the PEX5 and PEX7 peroxisome-targeting receptors in *Arabidopsis thaliana*
Martinez, Naxhiely-presenter nax@rice.edu(a) Woodward, Andrew (b) Bartel, Bonnie (a)
"Peroxisomes are single membrane-bound organelles that function to compartmentalize certain metabolic reactions critical to plant and animal development. The import of proteins from the cytoplasm into the organelle matrix depends on more than a dozen peroxin (PEX) proteins, with PEX5 and PEX7 serving as receptors that shuttle proteins bearing a peroxisome targeting sequence (PTS) into the organelle. PEX5 is the PTS1 receptor, PEX7 is the PTS2 receptor, and in both plants and mammals, PEX7 depends upon PEX5 binding to deliver PTS2 cargo into the peroxisome. In this study, we characterized *Arabidopsis thaliana pex7* mutants isolated through forward and reverse genetic screens in physiological and biochemical assays. We found a *pex7* missense mutation, *pex7-2*, that disrupts PEX7-cargo binding and PEX7-PEX5 interactions in yeast, as well as PEX7 accumulation in plants. Moreover, we observed an unexpected decrease in PEX5 accumulation in *pex7* mutants. We examined localization of various peroxisomally targeted GFP derivatives in the *pex7* mutants and, surprisingly, we observed defects not only in PTS2 import, but also in PTS1 protein import. Together, our data suggest that PEX5 and PTS1 import depend on PEX7 for function in *Arabidopsis*. This work was supported by the NSF (MCB-0745122) and by an NIH predoctoral fellowship (F31-GM081911)."
(a) Rice University (b) Southwestern University

M2903 The Arabidopsis ankyrin repeat-containing protein 2A is an essential molecular chaperone for the biogenesis of a class of membrane-bound proteins and it plays an important role in plant growth and development
Kuppu, Sundaram-presenter sundaram.kuppu@gmail.com(a) Shen, Guoxin (a) Venkataramani, Sujatha (b) Narendra, Savitha (c) Qiu, Xiaoyun (a) Kolli, Sasi (a) Zhang, Hong (a)
"Peroxisomes are eukaryotic organelle without its own genome; consequently all peroxisomal proteins are post-translationally targeted to

peroxisomes. Biogenesis of peroxisomal matrix proteins is better understood, whereas biogenesis of peroxisomal membrane-bound proteins is less understood. The Arabidopsis ankyrin repeat-containing protein 2A (AKR2A) was found to interact with the peroxisomal membrane-bound ascorbate peroxidase 3 (APX3), and this interaction involves the C-terminal sequence of APX3, i.e. a transmembrane domain plus a few basic amino acid residues, that resembles the mPTS, a targeting signal for some peroxisomal membrane-bound proteins. The specificity of the AKR2A-APX3 interaction hints at a possibility that AKR2A regulates APX3s biogenesis, because binding of AKR2A to APX3s mPTS could prevent APX3 from forming aggregates after biosynthesis. Analysis of three AKR2A mutants indicates that AKR2A is required for APX3s stability in plant cell. Furthermore, reduced expression of AKR2A by using RNA interference technique also leads to reduced steady-state level of APX3 and significantly reduced APX3 targeting to peroxisomes in plant cells. In addition, AKR2A mutants display abnormal phenotypes and delayed flowering, indicating that AKR2A plays important roles in plant growth and development. Given the fact that AKR2A also binds specifically to an mPTS -like sequence in several chloroplast outer membrane proteins, AKR2A appears to be a general chaperone in plant cells. The AKR2A-binding mPTS should therefore be re-defined as a membrane protein targeting signal, and AKR2A is an essential chaperon that binds specifically to the mPTS in a group of membrane-bound proteins and regulates their biogenesis in plant cells. "
(a) Texas Tech University, Lubbock, Texas, 79409 (b) University of Chicago, Chicago, IL 60637 (c) Penn State University, University Park, PA 16802

M2904 Lipid pumps required for endocytosis and formation of secretory vesicles
Palmgren, Michael G (a) Lopez-Marques, Rosa-presenter rlo@life.ku.dk(a) Poulsen, Lisbeth R (a) McDowell, Steve C (b) Harper, Jeff F (b) Okkeri , Juha (c) Schulz, Alexander (a) Licht, Dirk (c) Pomorski, Thomas (c)
"Vesicle budding in eukaryotes depends on the activity of lipid translocases that generate membrane bilayer lipid asymmetry and in this way initiate changes in membrane curvature which lead to budding. We present evidence that in a higher plant, a member of the P4-ATPase subfamily (*aminophospholipid ATPase3; ALA3*) is involved in lipid flipping across Golgi membranes and is required for initial formation of secretory vesicles. The *ALA3* gene of *Arabidopsis thaliana* show highest activity in root tips. Plants carrying mutations in *ALA3* show impaired growth of roots and shoots. The growth defect is accompanied by failure of the root cap to release border cells involved in the secretion of slime required for root penetration of the soil. Further, *ala3* mutants are devoid of slime vesicles, which normally bud off from the Golgi and contain secreted polysaccharides and proteins. ALA3 function requires interaction with members of a novel family of membrane proteins, ALIS1 to ALIS5 (for ALA-Interacting Subunit). *In planta*, like ALA3, ALIS1 localizes to the Golgi and is expressed in root peripheral columella cells. We propose that the ALIS1 protein is a beta-subunit of ALA3 and that this protein complex forms an essential part of the Golgi machinery required for secretory processes during plant development. We have subsequently identified ALA2, which also interacts with ALIS1. The ALA2/ALIS1 complex resides in the plasma membrane and is involved in flipping phosphatidylserine from the outer to the inner plasma membrane leaflet. We propose that the ALA2/ALIS1 complex is required for invagination of the plasma membrane during initial endocytosis."
(a) University of Copenhagen (b) University of Nevada (c) Humboldt University, Berlin

Start	End	**Event**	Location
10:40 AM	12:20 PM	**Minisymposium 30: Programmed Cell Death & Senescence** - Chair: Julie Stone 10:40 - M3001: Julie M. Stone - *Sphingolipids and programmed cell death in Arabidopsis thaliana* 11:05 - M3002: Gerald A. Berkowitz - *Leaf senescence signaling: Ca2+ accumulation mediated by Arabidopsis cyclic nucleotide gated channel2 acts through nitric oxide to repress senescence programming* 11:30 - M3003: Judy A. Brusslan - *A bioinformatics/genetic approach to identify chloroplast proteases that degrade chloroplast proteins during leaf senescence in Arabidopsis* 11:55 - M3004: Susheng Gan - *Plant senescence: a paradigm of translational plant sciences*	O'ahu 313A

M3001 Sphingolipids and programmed cell death in *Arabidopsis thaliana*
Lin, Jiusheng (a,b) Stone, Julie M-presenter jstone2@unl.edu(a,b)
"Classically viewed as membrane structural components, sphingolipids also modulate cell proliferation and death. The fungal toxin fumonisin B1 (FB1) perturbs sphingolipid metabolism and induces plant programmed cell death (PCD). The FB1-resistant mutant *fbr6* is disrupted in a gene encoding an SBP domain DNA-binding transcription factor. Double mutants between *fbr6* and selected *accelerated cell death acd* mutants, the single mutants, and wild-type controls were tested for FB1 sensitivity. *fbr6* effectively suppressed the enhanced sensitivity of *acd5* to FB1-induced cell death and ROS accumulation, but failed to suppress these in *acd1* and *acd2*. Because *ACD1* and *ACD2* encode proteins involved in the metabolism of chlorophyll breakdown products and *ACD5* encodes a ceramide kinase, FBR6 target geneslikely regulate sphingolipid metabolism/signaling. Sphingolipid profiles for wild-type, *fbr6*, *acd5*, and *acd5 fbr6* mutant plants were determined. FB1 treatment clearly caused a shift in sphingolipids pools, as predicted given its function as a competitive inhibitor of ceramide synthase (sphinganine N-acyl transferase). There was a shift from very long chain fatty acids (VLCFAs; C20 to C26) to shorter chain C16 FAs, but this shift was circumvented by the fbr6 mutation. FB1 also caused significant accumulation of saturated LCBs and LCB-Ps. The *fbr6* mutant, however, accumulated very different levels and types of LCBs and LCB-Ps relative to wild-type plants, and these were also suppressed in the *acd5 fbr6* double mutant. Sphingolipidomics profiling link FBR6-mediated gene expression modulation to sphingolipid-dependent signal transduction pathways providing new information on the mechanisms by which plants control cell death."
(a) University of Nebraska, Department of Biochemistry (b) University of Nebraska, Center for Plant Science Innovation

M3002 Leaf senescence signaling: Ca^{2+} accumulation mediated by Arabidopsis cyclic nucleotide gated channel2 acts through nitric oxide to repress senescence programming
Ma, Wei (a) Smigel, Andries (a) Walker, Robin K (a) Moeder, Wolfgang (b,c) Qi, Zhi (a) Yoshioka, Keiko (b,c) Berkowitz, Gerald A-presenter gerald.berkowitz@uconn.edu(a)
"Ca^{2+} and nitric oxide (NO) are essential molecules involved in plant senescence signaling cascades. Previous studies suggest that Ca^{2+} and NO may act as negative regulators deferring senescence. In some signaling pathways, NO generation can be dependent on cytosolic Ca^{2+}. The Arabidopsis mutant *dnd1* lacks a functional plasma membrane-localized cation channel (CNGC2). Using this mutant, we recently demonstrated this channel affects plant response to pathogens through a signaling cascade involving Ca^{2+} modulation of NO generation; the pathogen response phenotype of *dnd1* can be complemented by application of an NO donor. At present, the interrelationship between Ca^{2+} and NO generation in plant cells during leaf senescence remains unclear. Here, we use *dnd1* plant to present genetic evidence indicating that Ca^{2+} uptake and NO production play pivotal

roles in plant leaf senescence. Leaf Ca^{2+} accumulation is reduced in *dnd1* leaves compared to wild type. Importantly, many early senescence-associated phenotypes (such as loss of chlorophyll, expression level of senescence associated genes, H_2O_2 generation, lipid peroxidation, tissue necrosis, and salicylic acid levels) were more prominent in *dnd1* leaves compared to wild type. Application of an NO donor effectively rescues many *dnd1* senescence related phenotypes. We also identify a new phenotype associated with CNGC2 loss-of-function; *dnd1* plants are less sensitive than wild type to (exogenous) NO. Our work demonstrates that the CNGC2 channel is involved in Ca^{2+} uptake during plant development. Work presented here suggests that this function of CNGC2 may mediate downstream 'basal' NO production during the course of plant development, and that this NO generation acts as a negative regulator during plant leaf senescence signaling."
(a) Agricultural Biotechnology Laboratory,Department of Plant Science, University of Connecticut , 1390 Storrs Rd., Storrs, CT 06269-4163, USA (b) Department of Cell and Systems Biology, University of Toronto, 25 Willcocks Street, Toronto, Ontario M5S 3B2, Canada (c) Center for the Analysis of Genome Evolution and Function, University of Toronto, 25 Willcocks Street, Toronto, Ontario M5S 3B2, Canada

M3003 A bioinformatics/genetic approach to identify chloroplast proteases that degrade chloroplast proteins during leaf senescence in Arabidopsis
Brusslan, Judy A.-presenter bruss@csulb.edu(a) Belanger, Eileen M. (a) Belletto, John V. (a) Hoiness, Robert D. (a) Kimoto, Maryann (a) Rodriguez, Francisco (a) Tai, Yu-tout (a) van de Wetering, Scott W. (a) Velis, Brenda L. (a)
http://www.csulb.edu/~bruss
"Chloroplast protein degradation during leaf senescence is a major contributor to nitrogen allocation since chloroplast proteins comprise 75% of cellular nitrogen. We have demonstrated that chloroplasts remain distinct organelles late into the senescence process, and thus some protein degradation within the chloroplast, independent of SAVs and RCBs, is likely. Two bioinformatics approaches were used to identify 18 chloroplast proteases that have increased expression during senescence. One approach started with all protease genes (MEROPS) while the other (Virtual Plant) started with all genes up-regulated in senescent tissue. To determine if these genes play a role in leaf senescence, a three-pronged approach is being taken. First, protease-green fluorescent protein (GFP) fusions are being expressed in plants. For the first four proteases studied, three were shown to be in the chloroplast. Second, real-time qPCR is being used to confirm up-regulation of protease mRNAs during senescence. Data for the first seven proteases indicate that expression is increased in older leaf tissue. The third approach is to isolate Arabidopsis lines with T-DNA insertions in protease genes. These mutants are currently being analyzed for protein content during senescence using immunoblots. Rubisco large subunit was quantified relative to Lhcb1 midway through senescence in WT and mutant lines. For the first seven mutant lines tested, two serine proteases, *s41-3* and *s33a-1* and one metalloprotease, *m41a-2*, displayed increased Rubisco LSU protein content (120-130%) during senescence when compared to WT. These results suggest that some of the chloroplast proteases identified using bioinformatics approaches are contributing to chloroplast protein degradation during leaf senescence."
(a) Department of Biological Sciences, California State University Long Beach

M3004 Plant senescence: a paradigm of translational plant sciences
Westbrook, Jessica (a) Gan, Susheng-presenter sg288@cornell.edu(a)
"Plants exhibit both mitotic and post-mitotic senescence. Leaf senescence is a typical post-mitotic senescence while cease of cell proliferation in early fruitlet formation and shoot apical meristem arrest are examples of mitotic senescence. Leaf senescence limits crop yield and biomass accumulation and contributes to much postharvest loss of vegetables and ornamental plants. Mitotic senescence in fruitlets limits fruit size. We have studied the regulatory mechanisms underlying senescence in Arabidopsis, and have translated knowledge gained from the model system into crops for agricultural improvement. For example, we identified a master regulator that controls leaf senescence in Arabidopsis, and based on this finding, we cloned and manipulated the orthologs in various crops including soybean. Soybean plants display a significantly delayed leaf senescence phenotype and more than 10% increase in seed yield. By delaying mitotic senescence in fruitlets, cell numbers are increased in Arabidopsis, tobacco and tomato fruits, respectively, and the size of tomato fruits is significantly increased. These translational research on plant senescence is approaching commercialization. "
(a) Cornell University, Department of Horticulture

POSTER SESSIONS

Poster Location
Posters will be located in the Exhibit Hall in the Hawaii Convention Center. Find your final poster number by looking up your name in the author index in the back of the program. Posters are numbered by category.

Poster Setup
Saturday July 18, 2009 starting at 9 am
Posters will be organized in rows by category. Put your poster on the half side of the board below where your final poster number is located in the corresponding session category. The maximum size allocated for each poster is 4' x 4'. Be sure to use a type size and font that will be easily readable. Poster pins will be provided. Each side can hold two 4' x 4' posters. No electrical or multi-media displays. Do not move the boards or your poster number. If you did not submit your abstract for poster online before the closing of the online site, place your poster in the Late/Moved section in an open space.

Poster Display Times
All posters will be displayed daily at the Hawaii Convention Center until the last event ends in the center. The hall will be closed after the last event of the day in the center for security reasons.
Photographing, video taping, or recording of any kind will be PROHIBITED of the posters unless a poster author is present and provides specific permission.

Poster Attendance during the Exclusive Poster Session
Please attend your poster during the exclusive poster session times below according to your final poster number.

Exclusive Poster Sessions
Sunday July 19 Poster Session – Box lunch provided
1:00 PM - 2:00 PM Even Poster Numbers
2:00 PM - 3:00 PM Odd Poster Numbers
Monday, July 20 Poster Session
9:00 PM – 10:00 PM Odd Poster Numbers
10:00 PM – 11:00 PM Even Poster Numbers
Tuesday, July 21 Poster Session 12:20 PM – 2:30 PM
All Posters

Poster Removal
All posters must be removed by 2 pm, Wednesday, July 22, 2008. Posters not removed will be discarded.

Poster Numbering
Abstract numbers are preceeded with the poster session number (i.e. P15) and then run from 001 increasing numerically. Each category has its own set of numbers starting from 001 preceeded with the session number. For example, the 33rd poster in the Root Biology session will be numbered P01033. Check the author index for your final poster number. Minisymposium speakers, place your poster under the poster abstract number rather than their minisymposium abstract number.

PSA POSTER/TALK CATEGORIES
P01 Algal Biotechnology
P02 Algal Cellular and Molecular Biology
P03 Algal Coral Reef Ecology
P04 Algal Ecology and Population Biology
P05 Algal Phylogenetics and Taxonomy
P06 Algal Physiology and Biochemistry
P07 Algal Species Concepts in Molecular Ecology

ASPB POSTER/MINI CATEGORIES

P08 Abiotic Stress
P09 Agriculture & Crop Breeding
P10 Bioenergy Crops & Biofuels
P11 Cell Cycle & Division
P12 Cell-to-Cell & Long-Distance Signaling
P13 Cellular Growth
P14 Cellular Imaging Technologies
P15 Cell Walls
P16 Chromatin
P17 Climate Change Biology
P18 Comparative Genomics
P19 Cytoskeleton Structure & Dynamics
P20 DNA Replication, Recombination & Repair
P21 Dormancy
P22 Ecophysiology
P23 Education & Outreach
P24 Embryogenesis
P25 Emerging Model Systems
P26 Emerging Technologies
P27 Environmental Physiology
P28 Epigenetics
P29 Evolution of Development & Physiology
P30 Gene Regulation Mechanisms
P31 Genome Evolution
P32 Heavy Metals & Phytoremediation
P33 Herbicide Physiology
P34 Hormone Biology
P35 Intracellular Signaling
P36 Lipids
P37 Medicinal Plant Biology
P38 Membrane Biology & Transport
P39 Metabolic Engineering
P40 Mineral Nutrition
P41 Modeling & Computational Biology
P42 Organelle Biology
P43 Organismal Evolution
P44 Photoreceptors, Light Signaling & Photomorphogenesis
P45 Photosynthesis & Respiration
P46 Plant Biotechnology & Risk Assessment
P47 Plant Herbivore Interactions
P48 Plant Pathogen Interactions
P49 Plant Symbiont Interactions
P50 Pollen Biology
P51 Primary Metabolism
P52 Protein Modification & Turnover
P53 Protein Targeting & Vesicular Trafficking
P54 Quantitative Traits
P55 Reactive Oxygen, Nitric Oxide & Redox Regulation
P56 Reproductive Development
P57 Rhizosphere
P58 Rhythms
P59 Root Biology
P60 Secondary Metabolism & Natural Products
P61 Seed Biology
P62 Small Regulatory RNAs
P63 Systems Biology
P64 Translational Research, From Bench to Field
P65 Tropical Plant Biology
P66 Tropisms
P67 Vascular Biology
P68 Vegetative Development
P69 Water Relations

POSTER ABSTRACTS

SESSION P01 – ALGAL BIOTECHNOLOGY

P01001 – See PSA Plenary 1 on Monday, July 20 – Algal Biotechnology

P01002 - See PSA Plenary 1 on Monday, July 20 – Algal Biotechnology

P01003 – See PSA Talk on Saturday, July 18 – Applied Phcology I

P01004 – See PSA Talk on Monday, July 20 – Applied Phycology II

P01005 – See PSA Talk on Saturday, July 18 – Applied Phycology I

P01006 – See PSA Talk on Saturday, July 18 – Applied Phycology I

P01007 – See PSA Talk on Saturday, July 18 – Applied Phycology I

P01008 – See PSA Talk on Monday, July 20 – Applied Phycology II

P01009 Isolation and characterization of marine phytoplankton as a next generation biofuel
Johnson, Zackary I-presenter zij@hawaii.edu(a) Bidigare, Robert R (a) Brown, Susan L (a) Bruyant, Flavienne (b) Cochlan, William (c,d) Cullen, John J (b) Huntley, Mark E (a,d) Redalje, Donald G (e) de Scheemaker, Gabriel (f)
http://www2.hawaii.edu/~zij
"Global energy demands continue to rise, but global fossil fuel production is struggling to keep up. Further, fossil fuels contribute to the increase in global atmospheric carbon dioxide and are a major driver of global climate change. To address these concerns, Cellana BV is pursuing the use of marine phytoplankton as a next generation, low carbon-profile biofuel. Marine phytoplankton have three major advantages over other biofuels including (1) they do not use freshwater (2) they do not require arable land and (3) phytoplankton can grow much faster than traditional plant-based biofuels. Working with Cellana, our group has isolated phytoplankton strains from diverse locations and some have significant lipid content. Using an efficient experimental strategy, we have analyzed promising candidates from this collection and discovered a wide range of growth rates and lipid compositions in conditions simulating environmental scenarios associated with commercial applications. Here we describe our screening process and show that some phytoplankton have characteristics that make them excellent candidates for commercial-scale production suggesting that marine phytoplankton are the leading contenders for large scale production of next generation biofuels."
(a) University of Hawaii (b) Dalhousie University (c) Romberg Tiburon Center for Environmental Studies, San Francisco State University (d) Cellana LLC (e) The University of Southern Mississippi (f) Cellana BV

P01010 Relationship between fatty acid and squalene biosyntheses in microalga Aurantiochytrium sp.
Jiang, Y-presenter yjiang@hkbu.edu.hk(a,b) G.Q., Chen (a,b) T., Aki (d) F., Chen (c)
"Polyunsaturated fatty acids (PUFAs) have shown a wide range of important functions in biological systems and been widely used as food and feed industries. However the products of peroxidation or spontaneous oxidation are toxic to cells and may signal cell death. Squalene, a triterpene hydrocarbon, has proved to be a strong antioxidant to protect the oxidative stability of PUFA in vitro. A PUFA-producing microalga Aurantiochytrium sp. BR-MP4-A1 was found having the ability to biosynthesize a large amount of squalene and has the potential for the co-production of PUFA and squalene. In this microalga, acetyl-CoA is used as the common carbon source for the syntheses of PUFA and squalene. Therefore these two pathways may compete for common pools of substrates. In this study, two cholesterogenesis inhibitors - terbinafine and zaragozid acid as well as two fatty acid inhibitors - sethoxydim and cerulenin were used to inhibit the biosyntheses of squalene and fatty acids, respectively. The inhibition on the syntheses of fatty acid and squalene as well as their influence on each other were evaluated. It was found that the supplementation of terbinafine at 600 um could stimulate the accumulation of squalene. By adding sethoxydim and cerulenin, the syntheses of total fatty acids were inhibited and the inhibition was in a dose-dependent manner. It was found that the block of sterol biosynthesis could not have significant influence on fatty acid accumulation. However, the inhibition on fatty acid biosynthesis could induce a significant accumulation of squalene (increased about 40% compared to the control) in Aurantiochytrium sp. BR-MP4-A1. Considering the high content of fatty acids synthesized from acetyl-CoA in this strain, the carbon saved from squalene synthetic pathway through the application of cholesterogenesis inhibitors could not be high enough to influence the fatty acid biosynthesis. (This research was supported by Research Grant Council of HKSAR) Enhanced Accumulation of Astaxanthin under Stress Condition Induced by UVB irradiation G.Q. Chen1,2, N.K. Mak1, F. Chen3, Y. Jiang1,2 1Department of Biology and 2Kwong Living Trust Food Safety and Analysis Laboratory, Hong Kong Baptist University, Hong Kong; 3School of Biological Sciences, The University of Hong Kong, Hong Kong; Astaxanthin is a high-value ketocarotenoid with strong antioxidant capacity and growing application in food, aquaculture and pharmaceutical industries. The green microalga Haematococcus pluvialis is one of its best potential producers. In recent years, research interest has been shown to find specific stresses that lead to enhanced accumulation of astaxanthin in this model microalga. Ultraviolet B (UVB) irradiation is effective in inducing lipid peroxidation as well as the generation of reactive oxygen species (ROS) in microorganisms. Microalgae are the basic component of aquatic biological system and develop different systems to mitigate UVB-induced ROS damage. A notable mitigating strategy is the accumulation of UV-B absorbing compounds such as carotenoids. Carotenoids play critical structural and functional roles in the photosynthesis and have strong antioxidant activities. In general, low-level UVB exposure increases the synthesis of carotenoids, whereas excessive ROS production may cause oxidative damage and lead to cell death finally. In this study, the vegetative cell of H. pluvialis was exposed to UV-B light with the exposure intensities at 0, 3, 5, 7, 9 w/m2 and exposure time of 0, 20, 40, 60, 80, 100 min followed by 72-hr cultivation under high light condition in order to investigate the impact of UV-B on cell growth and the biosynthesis of astaxanthin within H. pluvialis. It was found that when UV-B intensity was 5 w/m2 and exposure time was 60 min, the cellular astaxanthin concentration doubled and the cell dry weight increased 17% compared to the control (without UV-B irradiation treatment) and other treatment conditions. (This research was supported by Faculty Research Grant of HKBU) "

(a) Dept Biology, Hong Kong Baptist University (b) Kwong Living Trust Food Safety and Analysis Laboratory, Hong Kong Baptist University (c) School of Biological Sciences, The University of Hong Kong, Hong Kong (d) Department of Molecular Biotechnology, Hiroshima University, Japan

P01011 Relationship between Fatty Acid and Squalene Biosyntheses In Microalga *Aurantiochytrium* sp.

Jiang, Y-presenter yjiang@hkbu.edu.hk(a,b) Chen, G.Q. (a,b) Chen, F. (c)

"Polyunsaturated fatty acids (PUFAs) have shown a wide range of important functions in biological systems and been widely used as food and feed industries. However the products of peroxidation or spontaneous oxidation are toxic to cells and may signal cell death. Squalene, a triterpene hydrocarbon, can protect the oxidative stability of PUFA in vitro. A PUFA-producing microalga *Aurantiochytrium* sp. BR-MP4-A1 was found having the ability to biosynthesize a large amount of squalene and has the potential for the co-production of PUFA and squalene. In this microalga, acetyl-CoA is used as the common carbon source for the syntheses of PUFA and squalene. Therefore these two pathways may compete for common pools of substrates. In this study, two cholesterogenesis inhibitors - terbinafine and zaragozid acid as well as two fatty acid inhibitors - sethoxydim and cerulenin were used to inhibit the biosyntheses of squalene and fatty acids, respectively. The inhibition on the syntheses of fatty acid and squalene as well as their influence on each other were evaluated. It was found that the supplementation of terbinafine at 600 um could stimulate the accumulation of squalene. By adding sethoxydim and cerulenin, the syntheses of total fatty acids were inhibited and the inhibition was in a dose-dependent manner. The inhibition on fatty acid biosynthesis could induce a significant accumulation of squalene (increased about 40% compared to the control). However, the block of sterol biosynthesis could not have significant influence on fatty acid accumulation in *Aurantiochytrium* sp. BR-MP4-A1 (This research was supported by Research Grant Council of HKSAR). "

(a) Dept Biology, Hong Kong Baptist University (b) Kwong Living Trust Food Safety & Analysis Lab, Hong Kong Baptist University (c) School of Biological Sciences, The University of Hong Kong

P01012 "Microalgal Biotechnology, Its Science and Applications"

Chen, Feng-presenter sfchen@hkusua.hku.hk(a)

"Microalgae are ubiquitous and represent a genetic and metabolic diversity. Microalgae have been found to contain large quantities of high-value products, including chemicals, pharmaceuticals and nutraceuticals, and there is renewed interest in microalgae as a source of biofuels due to their ability to accumulate large amounts of lipids. As a result, much attention has been attracted to effective mass cultivation of microalgae, leading to the emergence of an important area, microalgal biotechnology. One important aspect of microalgal biotechnology is to develop functional foods and nutraceuticals from microalgae either by using the whole cells or by extracting functional ingredients from the algae. Consequently the development of a cost-effective process for the large-scale production of microalgal biomass is of vital importance. Traditionally algae are considered photosynthetic organisms requiring carbon dioxide as carbon source and sunlight as energy source. While this method has been practiced for many decades, the yield possibly obtained therefrom, however, is meager, which greatly hinders the application of microalgae. Such a bottleneck can be solved by developing a heterotrophic process taking advantage of the properties of certain algae being able to grow rapidly on organic carbon-containing substrates in the dark. In this talk, the development of a high-cell density process for microalgal mass culture coupled with the novel separation and purification technique as well as the use of metabolic engineering to produce functional foods and nutraceuticals by microalgae or by transgenic plant containing a unique microalgal gene, together with their theoretical basis, will be presented. The biotechnological potential of microalgae will be highlighted and discussed. "

(a) School of Biological Sciences, The University of Hong Kong

SESSION P02 – ALGAL CELLULAR AND MOLECULAR BIOLOGY

P02001 – See PSA Talk on Monday, July 20 – Applied Phycology II

P02002 – See PSA Talk on Monday, July 20 – Algal Cellular & Molecular Biology

P02003 – See PSA Talk on Monday, July 20 – Algal Cellular & Molecular Biology

P02004 Generation and analysis of the ESTs from *Porphyra seriata*
Choi, Dong-Woog-presenter dwchoi63@chonnam.ac.kr(a) Kim, EuiCheol (a) Park, Hong-Sil (a) Jung, YoungJa (a) Park, Hong-Seok (b) Hwang, Mi Sook (c)
"Porphyra is one of the major economic marine red alga used as sources of food. Recently Porphyra has been recognized as a useful model plant for fundamental and applied studies in marine sciences. Although more than one hundred species of Porphyra have been reported from all over the world, only several species including *P. yezoensis*, *P. tenera*, *P. seriata* and *P. dentate* have been cultivated in aquaculture industries of Japan and Korea. To create a Porphyra gene resource, we are generating ESTs from two Porphyra species. At the first step, about 4,000 ESTs were generated from P. seriata and 1,000 ESTs from P. tenera. To generate ESTs from P. seriata, we construct two cDNA libraries from whole plant grown at normal growth condition and under heat stressed condition. Comparison of the ESTs from two cDNA libraries show that several unknown cDNAs are abundant in heat stressed plant tissue. Porphyra ESTs and comparison data will be presented."
(a) Chonnam National University (b) Korea Research Institute of Bioscience and Biotechnology (c) Marine Algae Research Institute

P02005 – See PSA Talk on Monday, July 20 – Algal Cellular & Molecular Biology

P02006 – See PSA Talk on Monday, July 20 – Algal Cellular & Molecular Biology

P02007 – See PSA Talk on Monday, July 20 – Applied Phycology II

P02008 – See PSA Talk on Saturday, July 18 – Applied Phycology I

P02009 – See PSA Bold Talk on Saturday, July 18

P02010 – See PSA Bold Talk on Saturday, July 18

P02011 Differential Expression of *lhcf*5 and *lhcf*6 Genes between the Male and the Female Gametophytes of *Laminaria japonica*
Zhou, Zhi-Gang-presenter zgzhou01@163.com(a) Bi, Yan-Hui (a) Zou, Dan-Yan (a) Ouyang, Long-Ling (a)
"The relative transcription of *lhcf*5 and *lhcf*6 genes between male and female gemetophytes of *Laminaria japonica* grown under different kinds of light, light intensities and the conditions favorable for gametogenesis was estimated by using real-time quantitative PCR. The *lhcf*5 and *lhcf*6 genes were transcripted little at dark. Their transcription reached a maximum under 40 μ;mol photons m^{-2} s^{-1} in males, whereas they did under 80 μ;mol photons m^{-2} s^{-1} in females. When light iradiance was higher than the most moderate one, the transcription of *lhcf*5 and *lhcf*6 decreased while light intensity increased. Compared to the illumination with white light, red light could significantly promote the relative transcription of the two genes for a both short (3 d) and long (7 d) time. Blue light had a positive effect on promoting transcription of the two genes in males, on the contrary, it played an inconspicuous role in females. When transferred to the conditions favorable for gametogenesis from vegetative growth, both males and females showed remarkably increased transcription of *lhcf*5 and *lhcf*6 genes on Day 1. Except for this, *lhcf*6 and *lhcf*5 genes were transcripted on Days 4 and 12 (*lhcf*6) or 14 (*lhcf*5) in females and on Days 8 and 14 in males whereas their transcription on the other days was lower than the vegetative growth. It is deduced that the transcription of *lhcf*5 and *lhcf*6 genes is helpful to cellular substance and energy storage and construction of chloroplast of the gametophytes both beneficial for cell division and vegetative growth. The differential expression between the female and the male gametophytes is possibly regulated by the different transcription elements present upstream in *lhcf*5 and *lhcf*6 genes."
(a) Shanghai Ocean University

P02012 The plastid thief *Dinophysis acuminata* has nuclear-encoded genes for plastid maintenance and metabolite exchange
Hughes, Jennifer L-presenter hughesj@email.arizona.edu(a) Hackett, Jeremiah D (a)
http://www.eebweb.arizona.edu/Faculty/Hackett/Home.html
"Endosymbiosis has been a fundamental process in eukaryotic evolution, giving rise to both mitochondria and plastids. Kleptoplastidy, the temporary acquisition of plastids from prey, may represent an early stage of endosymbiosis and serve as a model for understanding how these organelles are established. The dinoflagellate *Dinophysis acuminata* contains a kleptoplast from a cryptophyte alga. However, unlike kleptoplasts found in other organisms, it acquires the plastid second-hand from the kleptoplastidic ciliate *Myrionecta rubra*. *D. acuminata* retains this stolen plastid for months, despite the absence of plastid genes encoded in the cryptophyte nucleus. We sequenced cDNA from *D. acuminata* to determine if plastid genes are encoded in the nucleus. Five proteins with plastid-targeting peptides, psbM, psbU, ferredoxin, a light harvesting protein and a triose-phosphate transporter are encoded in the nuclear genome of *D. acuminata*. Phylogenetic analysis show that only psbM is derived from a cryptophyte, where as the others come from other dinoflagellates or haptophytes. These results shed light on how *Dinophysis* maintains the stolen organelle and how metabolites are exported to the host cell. Our findings support the hypothesis that establishment of metabolite exchange is an important early step in endosymbiosis."
(a) Department of Ecology and Evolutionary Biology, University of Arizona

P02013 One of three Cu-transporting P-type ATPases is required in Cu-deficient Chlamydomonas for the biosynthesis of a plasma membrane ferroxida
Hong-Hermesdorf, Anne-presenter ahhong@chem.ucla.edu(a) Chen, Jen-Chih (b) Kropat, Janette (a) Merchant, Sabeeha S (a)
"Cu is an essential micronutrient due to its role as an enzymatic cofactor in respiratory as well as photosynthetic pathways. *Chlamydomonas*

reinhardtii represents a convenient system to study Cu homeostasis for its unique ability to replace the important Cu-containing photosynthetic player plastocyanin by the Fe-containing cytochrome c6 under Cu-limiting conditions. Thus, much less Cu is required for survival. Cu response defect 2 (*crd2*) was identified in a screen for *C. reinhardtii* mutants with Cu-dependent growth. It leads to a Cu-conditional Fe deficiency, exemplifying the tight interconnection between the homeostasis of various trace metals. In Cu-replete medium, *crd2* cells grow like the wildtype, but significantly slower under Cu-limiting conditions. The phenotype can be enhanced by lowering iron concentrations and is suppressed by supplementation with excess Fe (Eriksson et al., 2004). The mutation was mapped to the locus of CTP1, a Cu translocating P-ATPase. CTP1 gDNA was shown to complement the Cu deficiency phenotype of *crd2* (Kropat, unpublished). We hypothesize that CTP1 is required for loading Cu into the active site of the FOX1 gene product, which participates in a Cu-dependent Fe assimilation pathway. The goal of this project is to refine the gene model for CTP1 and to pinpoint the exact mutation in CTP1 leading to the *crd2* phenotype. Furthermore we seek to clarify the function of CTP1 in the network of Cu and Fe homeostasis. We suggest that CTP1 plays a role in the secretory pathway, while CTP2 and CTP3 might be homologous to AtPAA1 and AtPAA2, which function in the chloroplast. Eriksson, M., Moseley, J.L., Tottey, S., Del Campo, J.A., Quinn, J., Kim, Y. and Merchant, S. (2004) Genetics, 168, 795-807. Kropat, J. et al (unpublished). "

(a) Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095-1569 (b) Institute of Biotechnology, National Taiwan University, Taipei 106, Taiwan

P02014 Regulation of thiamine biosynthesis in Chlamydomonas reinhardtii

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http://www.plantsci.cam.ac.uk/Smith/Index.html

"The active form of thiamine (vitamin B1), thiamine pyrophosphate (TPP) is a cofactor for enzymes in central metabolism like pyruvate dehydrogenase, α-ketoglutarate dehydrogenase and transketolase. Many studies on the regulation of thiamine biosynthesis have been done especially in bacteria and fungi. Recently, it has been found that in bacteria many genes involved in the biosynthesis of cofactors such as TPP are regulated by riboswitches, a sequence in the mRNA to which the cofactor binds influencing the secondary structure of the transcript. In the green alga Chlamydomonas reinhardtii, we have found that TPP riboswitches are found in THI4 and THIC, the first genes on each branch of the thiamine biosynthesis pathway and that binding of TPP affects splicing of the transcripts. In the pyr1 mutant of C. reinhardtii, there is a mutation in the THI4 riboswitch so that the alternative splicing does not occur. The effect of pyrithiamine, an inhibitor, on the expression of the THI genes in wild type and pyr1 mutant cells has been investigated. Characterization of various thiamine-dependent mutants has been carried out in order to see the effect of the addition of the vitamin on the expression of genes known to be controlled by riboswitches. Site directed mutagenesis has been carried out to determine if alteration of the riboswitch sequence interferes with binding of the cofactor and also the alternative splicing activity."

(a) Department of Plant Sciences, University of Cambridge

P02015 "Occurrence of chimeras with mosaic patterns in archeospore germlings of the marine crop *Porphyra yezoensis* Ueda (Bangiales, Rhodophyta)"

Niwa, Kyosuke-presenter kyousuke_niwa@pref.hyogo.lg.jp(a,b) Abe, Tomoko (b)

"The marine crop *Porphyra yezoensis* Ueda (nori) has a dimorphic life cycle with gametophytic haploid blades and sporophytic diploid filaments referred to as conchocelis phase. Since this species undergoes meiosis during the first two divisions of germinating conchospores which are released from mature conchocelis, the four-celled germling resembles an ordered tetrad. Therefore, it is known that conchospores from heterozygous conchocelis develop into sectored gametophytic blades (chimeras), but archeospores from haploid blades do not usually grow to chimeric blades. In the present study, we report new chimeras with mosaic patterns consisting of wild-type and green mutant colors. Interestingly, new chimeric blades developed from archeospores that were released from a blade piece containing a cell cluster of green color induced by heavy-ion beam irradiation. Furthermore, cell clusters of wild type, green mutant, and their mosaic pattern were cut out from the chimeric blade, and the blade pieces of different colors were cultured separately. From each of the blade pieces archeospores were released. Archeospores from the wild-type blade piece and from the green mutant blade piece developed into only wild-type blades and green mutant blades, respectively. However, archeospores from the blade piece with mosaic pattern developed into wild-type blades, green mutant blades, and chimeric blades with mosaic patterns of the two colors, although the frequency of the chimeras was low. Because each gametophytic cell possesses a single chloroplast (cp), it is difficult to explain occurrence of the new chimeras as mutation of cpDNA. Further genomic investigations, including transposon, are need to be done to account for the genetic mechanism."

(a) Fisheries Technology Institute, Hyogyo Prefectural Technology Center for Agriculture, Forestry and Fisheries (b) RIKEN Nishina Center

P02016 Cloning and characterization of two novel chloroplastic glycerol-3-phosphate dehydrogenases of *Dunaliella viridis*

He, Yunxia (a) Meng, Xiangzong-presenter xzmeng@sibs.ac.cn(b) Fan , Qianlan (a) Sun, Xiaoliang (a) Xu, Zhengkai (a,b) Song, Rentao (a)

"*Dunaliella*, a unicellular green alga, has unusual ability to survive dramatic osmotic stress through accumulating high concentration of intracellular glycerol as compatible solute. The chloroplastic glycerol-3-phosphate dehydrogenase (GPDH) has been considered to be the key enzyme to produce glycerol for osmoregulation in *Dunaliella*. In this study, we cloned two most prominent GPDH cDNAs (*DvGPDH1* and *DvGPDH2*) from *Dunaliella viridis*, encoding two polypeptides of 695 and 701 amino acids respectively. Unlike higher plant GPDHs, both proteins contained extra phosphoserine phosphatase (SERB) domains at N-terminus in addition to C-terminal GPDH domains. Such bi-domain GPDHs represented a novel type of GPDH, and were found existed exclusively in chlorophyte lineage. Transient expression of EGFP fusion proteins in tabacco leaf cells demonstrated that both DvGPDH1 and DvGPDH2 were localized in chloroplast. Overexpression of *DvGPDH1* or *DvGPDH2* could complement yeast GPDH mutant (*gpd1Δ*), but not yeast SERB mutant (*ser2Δ*). In vitro assay with purified DvGPDH1 and DvGPDH2 also showed apparent GPDH activity, but no SerB activity. Surprisingly, unlike chloroplastic GPDHs from plants, DvGPDH1 and DvGPDH2 can utilize both NADH and NADPH as coenzyme, and exhibited significantly higher GPDH activity when NADH was used as coenzyme. Q-PCR analysis revealed that the expression of both genes exhibited a transient transcriptional induction upon hyper salinity shock, followed by a negative down-regulation. The possible mechanism of glycerol synthesis regulation by GPDHs under salt stress in *Dunaliella* was discussed."

(a) School of Life Sciences, Shanghai University (b) Institute of Plant Physiology & Ecology, Chinese Academy of Sciences

P02017 The physiological function of chloroplast carbonic anhydrase in a marine diatom

Kikutani, Sae-presenter scbc1118@kwansei.ac.jp(a) Yamazaki, Yukiko (a) Hara, Satoshi (b) Hisabori, Toru (b) Matsuda, Yusuke (a)

"The β-type carbonic anhydrase, PtCA1 was previously localized as clustered particles on the girdle lamellae in the chloroplast of the marine diatom *Phaeodactylum tricornutum*. PtCA1 has been thought to play an important role in inorganic carbon concentrating mechanism (CCM). Cluster formation of PtCA1 occurs only in the chloroplast, strongly suggesting that particle formation requires some stromal factors. But the mechanisms of cluster formation and physiological function of PtCA1 are yet to be elucidated. Transformants which express PtCA1::GFP fusion independently of CO_2 concentrations were grown in 5% CO_2 and photosynthetic parameters were determined. Dissolved inorganic carbon (DIC) concentrations required for

achieving half-maximum rate of photosynthesis ($K_{1/2}$[DIC]) in transformants were lower than that of wild type cells grown in 5% CO_2. Stimulations of photosynthetic affinity to DIC in 5%-CO_2 grown cells with trace expressions of exogenous PtCA1::GFP fusion indicate the participation of PtCA1 to the CCM in the chloroplast of *P. tricornutum*. To investigate the chloroplastic environment which is crucial for the function of PtCA1, his-tag fusion of PtCA1 was produced in *E. coli* BL21(DE3) and purified by Ni-sepharose column. The activity of purified PtCA1::His^6 was measured by potentialmetric method, and compared in the absence or the presence of *Arabidopsis thaliana* thioredoxin (Trx). As a result, PtCA1 activity was significantly stimulated in the presence of both Trx and DTT. These results strongly suggest that the function of PtCA1 is supported under reducing condition of the stroma in the light via an aid of Trx. "
(a) School of Bioscience, Kwansei-Gakuin University (b) Chemical Resources Laboratory, Tokyo Institute of Technology

P02018 Isolation of uracil requiring mutants and phenotypic complementation by single gene transformation using a marine diatom
Sakaguchi, Toshiro-presenter brj21090@kwansei.ac.jp(a) Matsuda, Yusuke (a)
"Stable nuclear transformation technique recently established with two marine diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* accommodate only very limited selection systems. This in turn limits the capacity of diatom transformation systems. The purpose of this research is to isolate auxotrophic mutants of marine diatoms and to establish the phenotypic complementation system of the genetic lesion. Mutagenesis were induced to the marine diatom *P. tricornutum* by treatment with 1mg/mL ENU (*N*- ethyl -*N*- nitrosourea)solution for 30 min. Then, they were cultured on ASW (artificial sea water) agar plate containing 100 mg/L 5-FOA (5-fluoro orotic acid) and 50 mg/L uracil. 5-FOA resistant clones were inoculated to ASW agar plate containing 300 mg/L 5-FOA and 50 mg/L uracil and stable clones of uracil auxotrophic mutant of *P. tricornutum*was isolated. Uracil auxotrophic mutant was designated as RURF (Requiring Uracil Resistant FOA) according to their phenotypic characteristics. Tolerance RURF1 mutant to 5-FOA was 140 fold greater than that of wild type. Total RNAs were extracted from the Wild-type and RURF cells and the expression levels of the umps (uridine monophosphate syntase) gene were determined by semiquantitative RT-PCR. As a result, expression level of *umps* in RURF1 mutant was significantly lower than that of in wild-type cells. The *umps* gene isolated from *P. tricornutum* and human Hep3G cells was transformed into RURF1 by microprojectile bombardment. Revertant phonotype which was able to grow without uracil and sensitive to 5-FOA were successfully obtained from both transformations. These results clearly show that uracil auxotrophy was complemented by single gene transformation and can be utilized for a new selection marker system for marine diatoms."
(a) Department of Bioscience, Kwansei-Gakuin University

P02019 Molecular cloning and characterization of ammonium transporter in the dinoflagellate *Alexandrium tamarense*
Kobiyama, Atsushi-presenter kobiyama@kitasato-u.ac.jp(a) Okiyama, Shinkichi (a) Yamada, Yuichiro (a) Ogata, Takehiko (a)
"*Alexandrium tamarense* is the causative agent of paralytic shellfish poisoning, and its biological profile has been well studied. To date, many studies on the relationship between the nitrogen source and growth or toxin production have been performed. It has been reported that *A. tamarense* can grow using ammonium as nitrogen source. However, the molecular mechanism underlying the transportation of ammonium in *A. tamarense* has not been identified. In this study, to clarify the ammonium transport molecule, we attempted to isolate and characterize the ammonium transporter (AMT) in *A. tamarense*. cDNA cloning of *AMT* from *A. tamarense* was performed by the 3'- and 5'-rapid amplification of cDNA ends (RACE) method. The results of cDNA cloning enabled us to determine the nucleotide sequence of the *AMT* gene that encoded 453 amino acid residues. There were 3 types of clones which had different amino acid sequences and an amino acid identity of 20~40% with that of other organisms. The transmembrane domain prediction program indicated that *A. tamarense*AMT has 11 transmembrane domains, and its C-terminal domain is located in the cytoplasm. Then, we tried to investigate the ammonium transportability of *A. tamarense* AMT. The 3 types of cDNAs of the *A. tamarense AMT* were inserted into an expression vector of the fission yeast *Schizosaccharomyces pombe* and transferred into an amt-deficient yeast strain. The transgenic yeasts were grown on a medium containing NH_4Cl as the only nitrogen source. After transformation, however, the yeast strain transformed with only 1 of the 3 AMT types could grow on the medium. Therefore, it was speculated that this AMT functioned in ammonium transport in *A. tamarense*."
(a) Kitasato University

P02020 - **See PSA Plenary 1 on Monday, July 20 – Algal Biotechnology**

P02021 The mechanism of high-CO_2 responsive gene expression in a unicellular green alga *Chlamydomonas reinhardtii*
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http://www.sakura.cc.tsukuba.ac.jp/~plmet302/
"Atmospheric CO_2 concentration is increasing quite dynamically. Response of photosynthetic organisms to such change is very important topics to be clarified. On the other hand, high-CO_2 environment actually exists in the soil, ground water and so on. A green alga *Chlamydomonas reinhardtii* is one of organism that have been studied on the acclimation to low- and high-CO_2 conditions. The CO_2-concentration mechanism is well studied with this alga as a low-CO_2-inducible mechanism. In contrast to low-CO_2, the acclimation mechanism to high-CO_2 is not studied yet. A unicellular green alga *Chlamydomonas* has a strong adoption ability from air-level to over 10% (v/v) high-CO_2 conditions. We previously reported that *Chlamydomonas* synthesizes a periplasmic 43 kDa protein (H43) *de novo* under high-CO_2 conditions. H43 was expressed under not only high-CO_2 conditions above 0.3%(v/v) but also ambient CO_2 conditions in the presence of acetate and H43 expression was induced by responding to extracellular, but not to intracellular, CO_2 concentration. DIDS, a membrane-impermeable modifies of proteins, inhibited the H43 expression. These results indicate that high-CO_2 sensing mechanism involves signal mediation via membrane surface protein in *Chlamydomonas*[1)]. Using such high-CO_2-responsible protein as a reporter, we studied on the molecular mechanism of high-CO_2 sensing. As a result, the *H43* gene expression is up-regulated at the transcriptional level by the mediation of a high-CO_2 responsive cis-acting element in the upstream sequence of *H43* gene by sensing high-CO_2 conditions. We also will discuss on a possible CO_2-responsive mechanism in *Chlamydomonas*. [1)]Hanawa et al., Plant Cell Physiol. 48 (2): 299-309 (2007) "
(a) Grad. Schl. Life & Environmtl. Sci., Univ. Tsukuba, Japan (b) IPOD, AIST, Japan

P02022 The *Ectocarpus* genome project
Gachon, Claire-presenter cmmg@sams.ac.uk(a)
http://www.sams.ac.uk
"Brown algae are predominant primary producers in temperate marine coastal ecosystems. A survey of several species led to *Ectocarpus siliculosus* being proposed as a genetic and genomic model for this group of organisms. A genome sequencing project for this organism was initiated at Genoscope in 2004, involving the generation of about three and a half million sequencing reads from genomic libraries, plus an additional 91,000 cDNA reads. A draft assembly of the 200 Mbp genome was completed in the summer of 2007, making *Ectocarpus* the first-ever fully sequenced seaweed. Protein coding genes in the genome were predicted at the VIB in Ghent using the automatic annotation program Eugene. An international consortium of annotators led by Mark Cock in the French Roscoff Marine Laboratory is currently annotating the genome manually. The presentation will describe some of the major steps of this project and some of the complementary projects that are being carried out. The focus will be on new

insights into brown algal physiology and ecology. "
(a) Scottish Association for Marine Science

P02023 Regulation of nitrogen assimilation in marine diatoms: Is RNA turnove important?
Brown, Kathryn L (a) Robertson, Deborah L-presenter debrobertson@clarku.edu(a)
"The assimilation of nitrate and ammonium is a key factor regulating primary productivity in both aquatic and terrestrial ecosystems. Ammonium repression of nitrate assimilation in diatoms is well documented however, the response is variable and the molecular mechanisms mediating the repression are not well characterized. Using real-time quantitative PCR, mRNA levels for *nia* (nitrate reductase), *nii*A (Fd-nitrite reductase) , *nir*B (NAD(P)H-nitrite reductase), *gln*II (chloroplast-localized GSII) and *gln*III (cytosolic glutamine synthetase) were measured following the addition of actinomycin D or actinomycin D and NH_{4+}. The abundance of *nia*, *nii*A, and *gln*II decreased more rapidly in treatments receiving both actinomycin D and NH_4^+ than actinomycin D alone. In contrast, transcript levels of actin and *nir*B were not significantly different between the two treatments while NH_4^+ addition slowed the decline of *gln*N mRNA. Our results suggest that NH_4^+ either (1) inhibits the transcription of *nia*, *nii*A, and *gln*II or (2) decreases *nia*, *nii*A, and *gln*II transcript stability. While independent measurements of transcription and degradation rates are required to distinguish our two hypotheses, we propose that mRNA turnover may be a key step in the regulation of nitrogen assimilation in diatoms"
(a) Clark University

P02024 Species-specific multiplex PCR assays for the detection and quantification of *Prymnesium parvum* Carter (Haptophyta) in natural bloom samples
Manning, Schonna R-presenter stresskitten@mail.utexas.edu(a) La Claire, John W (a)
"The toxic, bloom-forming alga, *Prymnesium parvum*, is responsible for massive fish mortalities worldwide. Sensitive, rapid methods of detection are needed to improve management strategies. Multiplex PCR assays were developed for the detection and quantification of *P. parvum* wherein suites of primers simultaneously amplify four species- and gene-specific products using isolated genomic DNA or whole cells. Oligonucleotides were designed *a priori* for an isolate of *P. parvum* collected in Texas, but strains from South Carolina, Maine, the United Kingdom and Norway were tested to assess the range of applicability. With conventional PCR, amplification products were easily resolved by gel electrophoresis, generating a diagnostic banding pattern. Gene-specific molecular beacons were also designed for use with real-time quantitative PCR (qPCR). Both multiplex PCR methods were capable of detecting as few as 1 or 2 cells (approximately 0.50 to 1.0 pg DNA) in 50 cycles. The species- and gene-specificity of the assays were evaluated using isolates (and mixtures) of *P. parvum*, closely related haptophytes and outgroup species. The diagnostic banding pattern in electrophoresis gels and real-time trace profiles were exclusive to reactions containing *P. parvum* with no interference from nonspecific template. Cell number estimations using qPCR to evaluate environmental samples from natural bloom events were close to mean values obtained from hemocytometer counts. This presents a significant improvement in DNA-based detection technology, enhanced by the rapid and simultaneous confirmation of four species-specific products, and the ability to specifically detect several widely-separated geographic isolates of *P. parvum*, including the conspecific, *P. parvum f. patelliferum*."
(a) University of Texas at Austin

P02025 Glycoconjugate organization of *Enteromorpha (=Ulva) flexuosa* and *Ulva fasciata* (Chlorophyta) zoospores
Michael, Teena S-presenter teena@hawaii.edu(a)
"Ecologically successful algae that colonize natural and artificial substrates in the marine environment have distinct strategies for opportunistic dispersal and settlement. The objective of this research was to visualize molecular architecture of zoospores from *Enteromorpha (=Ulva) flexuosa* (Wulfen) J. Agardh and *Ulva fasciata* Delile, that co-occur but alternate in dominance on an intertidal bench. Multiple fluorescent-lectins were used to stabilize and probe for diverse zoospore glycoconjugates (GC) that could be involved in cell and substrate interactions. Epifluorescence microscopy revealed distinct cellular and extracellular polymeric substance (EPS) domains of GC relative to settlement morphologies. Glycoconjugates were similar for both species with: 1) α-D mannose and/or glucose moieties localized on flagella, the anterior domes and anterior regions, the plasma membranes and EPS; 2) α-fucose was localized on flagella and anterior regions; 3) N or α,β-N acetylglucosamine was localized on flagella, the anterior regions and EPS; and 4) N-acetylgalactosamine and/or galactose moieties were localized on each domain excluding the plasma membrane. Glycoconjugate distributions shifted with morphological changes that followed initial adhesion. Some differences in glycoconjugates were also observed for each species. TEM of *E. flexuosa* zoospores following carbohydrate-stabilizing fixations and gold-conjugated lectin probes resolved GC with α-D mannose and/or glucose, and/or N-acetylglucosamine at the plasma membranes, ER and diverse vesicles of the anterior pole, EPS, and discontinuous regions or knobs associated with flagellar surfaces. The distinct distribution and diversity of zoospore GC may be central to recognition and attachment on diverse substrata by these algae."
(a) Chaminade University

P02026 "A 26 kD protein initially isolated from *Phaseolus vulgaris* embryo axes based on phosphotyrosine binding, is present in the dinoflagellate alga *Symbiodinium kawagutii* and has membrane association but not highly hydrophobic properties."
Castillo-Medina, Raul E. (a) Villanueva, Marco A.-presenter marco@cmarl.unam.mx(a)
"SH2 domains are important sites for the interaction of transduction proteins in signaling cascades through phosphotyrosine binding. We have previously isolated a 26 kD protein from *Phaseolus vulgaris* embryo axes on sepharose-phosphotyrosine columns and this protein had short peptide sequence similarity to a desiccation-related protein. Antibodies against an octapeptide of this protein cross-reacted with a 26 kD protein in extracts of the dinoflagellate alga *Symbiodinium kawagutii* indicating the presence of a homologue in this organism. Analysis of the distribution of the protein in differentially extracted fractions revealed that it is membrane associated and it has both ionically-bound and intrinsic-membrane properties. However, phase partition assays on Triton X-114 detergent, enriched the protein in the aqueous phase indicating low hydrophobicity. Heat stress did not produce an increase in the expression of the protein and remarkably, the *S. kawagutii* protein did not associate to sepharose-phosphotyrosine columns. These results indicate that: a) although the protein is closely related inasmuch as the antibody against a short octapeptide sequence from the bean homologue can specifically recognize the algal protein with an identical molecular weight, it appears to display dissimilar features in terms of its phosphotyrosine binding; and b) it appears to associate to the membrane in two different manners which may depend on other interactive partners. This work was supported by grant IN200409-3 from DGAPA-UNAM. We also thank Claudia Morera for technical help. "
(a) Unidad Academica Puerto Morelos, Instituto de Ciencias del Mar y Limnologia-UNAM, Puerto Morelos, Quintana Roo, Mexico

P02028 Transcriptome analysis of the saxitoxin-producing dinoflagellate *Alexandrium tamarense* using next generation sequencing technology.
Hackett, Jeremiah D-presenter hackettj@email.arizona.edu(a) Hughes, Jennifer L (a)
http://www.eebweb.arizona.edu/Faculty/Hackett/Home.html

"Dinoflagellate algae are important primary producers in marine environments and have a significant ecological impact through the production of toxins and formation of harmful algal blooms (HABs). These algae also have several physiological traits that distinguish them for other algae, such as reduced plastid genomes, form II Rubisco and large nuclear genomes with a unique structure. To investigate the genetic basis of toxin production and other ecologically relevant aspects of dinoflagellates, we sequenced the transcriptome of *Alexandrium tamarense*, an important HAB species and a cause of paralytic shellfish poisoning through the production of saxitoxin, using a 454-pyrosequencing shotgun approach. These data were assembled into the most comprehensive dataset yet for a dinoflaglleate, comprising >35,000 unique transcripts. We compared the gene content of *A. tamarense* to the genomes of sequenced marine diatoms, haptophytes and green algae to identify similarities and differences in the gene complement among these organisms. We also compared the *A. tamarense* transcriptome to the genomes of saxitoxin-producing cyanobacteria to identify dinoflagellates genes involved in toxin synthesis. Our results show that next-generation shotgun transcriptome sequencing is an effective alternative to whole genome sequencing for gene discovery."
(a) University of Arizona

P02029 Molecular mechanisms behind acclimatization to high light conditions in the marine diatom Phaeodactylum tricornutum
Nymark, Marianne (a) Valle, Kristin C (a) Brembu, Tore-presenter tore.brembu@bio.ntnu.no(a) Hancke, Kasper (a) Winge, Per (a) Andresen, Kjersti (a) Ostensen, Marianne (a) Johnsen, Geir (a) Bones, Atle M (a)
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"Photosynthetic organisms must be able to adapt their light harvesting systems to varying light conditions. Diatoms are responsible for 25% of total carbon fixing activity at a global scale and live in water with highly variable light qualities and intensities. Molecular mechanisms responsible for conditional adaptations/acclimatization in diatoms are largely unknown. We set out to investigate the mechanism of light acclimatization in Phaeodactylum tricornutum using global transcriptional profiling, fluorescence variable technique (PAM fluorometry) and metabolite profiling. Low light (35 µ;mol photons m-2 s-1) adapted cultures were subjected to high light conditions (500 µ;mol photons m-2 s-1). Molecular responses were studied at time points 0.5, 3, 6, 12, 24 and 48 h after transfer to high light. Global transcriptional profiling of cells exposed to high light intensities showed strong and consistent specific responses already after 30 minutes of exposure to high light. Hundreds of transcripts were differentially regulated, including genes encoding proteins of light harvesting complexes, ROS scavenging systems, chlorophyll binding proteins and enzymes involved in pigment biosynthesis. Fluorometric analyses and PAM show that photosynthetic capacity adapts to high light within a period of 12-24 h and metabolite profiling show that light protecting metabolites are rapidly synthesised over the first 12 h in high light. "
(a) Norwegian University of Science and Technology

SESSION P03 – ALGAL CORAL REEF ECOLOGY

P03001 – See PSA Bold Talk on Saturday, July 18

P03002 – See PSA Bold Talk on Saturday, July 18

P03003 Macroalgal communities of the papah-naumoku-kea marine national monument
Vroom, Peter (a) Braun, Cristi-presenter cristi.richards@noaa.gov(a)
"The Northwestern Hawaiian Islands (NWHI) are considered to be among the most pristine coral reef ecosystems remaining on the planet. These reefs naturally contain a high percent cover of algal functional groups with relatively low coral abundance and exhibit thriving fish communities dominated by top predators. Despite their highly protected status, these reefs are at risk from both direct and indirect anthropogenic sources. This study provides the first comprehensive data on percent coverage of algae, coral, and non-coral invertebrates at the species level and investigates spatial biodiversity patterns across the archipelago in order to document benthic communities before the onset of substantial environmental changes expected to occur in response to global warming and ocean acidification. Monitoring studies show that forereef habitats in atoll systems often contain high abundances of the green macroalga *Microdictyon setchellianum* and the brown macroalga *Lobophora variegata,* yet these organisms were found to be uncommon in forereefs of non-atoll systems. Species of the brown macroalgal genera *Padina, Sargassum,* and *Stypopodium* and the red macroalgal genus *Laurencia* are common in the 2 northernmost atolls of the island chain but are uncommon components of more southerly islands. Conversely, the scleractinian coral *Porites lobata* are common on forereefs at southern islands, but less common at northern islands. Data suggest that healthy subtropical reefs in the NWHI naturally contain high abundances of macroalgae, and these algal populations must be factored into future management plans."
(a) Joint Institute for Marine and Atmospheric Research, Research Corporation of the University of Hawaii, NOAA PIFSC Coral Reef Ecosystem Div.

P03004 The distribution of mesophotic macroalgae in Hawaii: a surprisingly diverse assemblage from the deep.
Spalding, Heather (a) Abbott, Isabella A. (a) Peyton, Kimberley A. (a) Smith, Celia M.-presenter limuwahine@gmail.com(a)
"The mesophotic (low-light) coral ecosystem in Hawaii ranges from ~50 to over 200 m. A conspicuous and yet to be described component of this ecosystem is the macroalgal flora. We used a combination of submersibles, remotely operated vehicles, and technical diving to survey mesophotic algae at 36 sites around the Main Hawaiian Islands (MHI). The deepest occurring alga was a filamentous chlorophyte (*Cladophora* sp.) at 212 meters. Expansive macroalgal meadows of siphonous green algae were found at multiple locations around Oahu (*Avrainvillea amadelpha*, *Udotea* sp.) and the Maui Nui Island complex (*Halimeda kanaloana*) to 90 meters. Surprisingly, these meadows were distinct to these specific islands. Numerous new records and species of macroalgae were discovered, suggesting the deepwater flora is unique from shallow water. Deep water algae previously described as endemic to the Northwestern Hawaiian Islands (e.g. *Kallymenia* spp., *Codium* spp.), were found in the MHI deep flora as well, showing some algal distributions to be fairly continuous across the Hawaiian Island chain. In contrast, other species (*Halymenia* spp., *Grateloupia* sp.) appeared unique to the MHI, or represented significant range extensions (e.g. *Caulerpa filicoides*, *Caulerpa mexicana*). Overall, the deep water flora appears to be abundant and biogeographically diverse with a combination of temperate, subtropical, and tropical affinities. The current study contributes greatly to our understanding of marine biodiversity and biogeography in the Pacific, and has significant implications regarding the unique nature of mesophotic coral ecosystems in Hawaii. "
(a) Botany, University of Hawaii

P03005 - **See PSA Plenary 3 on Tuesday, July 21 – Coral Reef Ecology**

P03006 - **See PSA Plenary 3 on Tuesday, July 21 – Coral Reef Ecology**

P03007 - **See PSA Plenary 3 on Tuesday, July 21 – Coral Reef Ecology**

P03008 "A Harmful Algal Bloom (*Prorocentrum gillespii*), Ciguatera Fish Poisoning and nutrients from Rarotonga, Cook Islands."
Skinner, Mark P-presenter mark_skinner59@yahoo.com.au(a,c) Lewis, Richard (b) Johnstone, Ron (c) Tuaine, Turua (d) Shaw, Glen (e)
"Ecological ciguatera fish poisoning (CFP) studies in the past have largely ignored the potential for benthic microalgae from the sediment to contribute to toxins in the associated environment, largely concentrating on the macroalgae present and associated micro flora and ignoring this other niche. Ciguatera field studies have also concentrated on the dinoflagellate genus *Gambierdiscus*, well known to be the producer of ciguatoxin precursors but ignoring the potential of toxins from other dinoflagellate genera *Prorocentrum and Ostreopsis* (except for studies on Caribbean *Ostreopsis*) to be causative of CFP. This study concentrates on a harmful algal bloom (HAB) of the genus *Prorocentrum*, on the sediment of a tropical high island fringing reef lagoon in the southern central Pacific Ocean which was described as a new species by SEM analysis. The field site, Muri lagoon was chosen, as the island of Rarotonga, has a very high occurrence of CFP. Sampling took place from November 2002 to September 2003 for microalgal abundance (*Prorocentrum gillespii* highest monthly count was 13,700 cells/g sand) and water samples were taken for nutrient analysis from the lagoon and catchment streams. Fish were collected from the actual lagoon site for toxin analysis both at the start and at the end of sampling. We surmise that the nutrients (over the critical limits for healthy coral reefs) are most likely responsible for the bloom of microalgae present and those toxins from this bloom could now be responsible for ongoing cases of CFP and also be impacting upon the biodiversity of the lagoon biota."
(a) Entox, National Research Centre for Environmental Toxicology, University of Queensland, Australia (b) Institute of Molecular Biology, University of Queensland. (c) Centre of Marine Studies, University of Queensland (d) Ministry of Marine Resources, Cook Islands (e) School of Public Health, Griffith University

SESSION P04 – ALGAL ECOLOGY AND POPULATION BIOLOGY

P04001 – See PSA Talk on Tuesday, July 21 – Algal Ecology & Population Biology II

P04002 - See PSA Bold Talk on Saturday July 18

P04003 – See PSA Talk on Saturday, July 18 – Algal Ecology & Population Biology I

P04004 – See PSA Bold Talk on Saturday, July 18

P04005 – See PSA Talk on Monday, July 20 – Algal Ecology & Physiology

P04006 – See PSA Talk on Monday, July 20 – Algal Cellular & Molecular Biology

P04007 – See PSA Bold Talk on Saturday, July 18

P04008 – See PSA Talk on Monday, July 20 – Algal Phylogenetics & Taxonomy II

P04009 - See PSA Talk on Saturday, July 18 – Algal Ecology & Population Biology I

P04010 – See PSA Talk on Tuesday, July 21 – Algal Ecology & Pouplation Biology II

P04011 – See PSA Talk on Monday, July 20 – Algal Ecology & Physiology

P04012 – See PSA Bold Talk on Saturday, July 18

P04013 – See PSA Talk on Saturday, July 18 – Algal Ecology & Population Biology I

P04014 – See PSA Talk on Tuesday, July 21 – Algal Ecology & Population Biology II

P04015 – See PSA Bold Talk on Saturday, July 18

P04016 – See PSA Talk on Monday, July 20 – Applied Phycology II

P04017 – See PSA Talk on Saturday, July 18 – Algal Ecology & Population Biology I

P04018 – See PSA Talk on Saturday, July 18 – Algal Ecology & Population Biology I

P04019 – See PSA Talk on Monday, July 20 – Algal Phylogenetics & Taxonomy II

P04020 – See PSA Bold Talk on Saturday, July 18

P04021 – See PSA Talk on Monday, July 20 – Algal Phylogenetics & Taxonomy II

P04022 - See PSA Talk on Tuesday, July 21 – Algal Ecology & Population Biology II

P04023 The secret garden: Life within Antarctic macroalgae
Amsler, Margaret O.-presenter mamsler@uab.edu(a) Amsler, Charles D. (a) McClintock, James B. (a) Lopez-Bautista, Juan M. (b) Peters, Akira F. (c)
"Although small filamentous epiphytes are uncommon in subtidal habitats from the Western Antarctic Peninsula, algal endophytes are more common. Thirteen species of macroalgae were surveyed for presence of endophytes. The endophytic community was dominated by phaeophytes: 99 isolates, comprising 10 species. Sequence data and digital images for all endophytes are presented. Representatives of *Geminocarpus* dominated the endophytic flora (63 of 99 isolates): *G. geminatus* (42 of 63 isolates) was found in all but two macroalgae; *G. austrogeorgiae* (20 of 63 isolates) was found in ten host species; and one isolate of *Geminocarpus* may represent a new species. *Laminariocolax eckloniae* was the second genus in frequency (25 of 99) and found in ten of the macroalgae. Five isolates of *Ascoseirophila violodora* were recovered from four hosts. Single isolates of *Petalonia fascia* (both crust and blade forms), *Desmarestia* sp. and *Australofilum incommodum* were also recovered. A single isolate of the commonly known epiphyte *Elachista antarctica* was isolated from a previously unrecorded host. Finally, an isolate of *Ectocarpus siliculosus* represents the first record of this species in Antarctica. "
(a) University of Alabama at Birmingham (b) University of Alabama at Tuscaloosa (c) Bezhin Rosko

P04024 – See PSA Talk on Saturday, July 18 – Algal Ecology & population Biology I

P04025 Scalar fluxes through dense *Sargassum* canopies
Nishihara, Gregory N-presenter greg@nagasaki-u.ac.jp(a)
"It is widely believed that coastal development, pollution, and herbivory have caused the loss of *Sargassum* forests of the East China Sea. However, many of studies in this region have overlooked hydrodynamic phenomenon that the biology of these organisms. Bare dissolved oxygen sensors were used to directly measure the flux (*J*) of dissolved oxygen through the canopy of a *Sargassum piluliferum* bed in a laboratory flow-chamber. I took 10 Hz measurements of *J* without significant interference by the fronds. Flux within the canopy was about 86 % of that in an empty flow-chamber and was directly proportional to the velocity. Turbulent flux (*J'*) within the canopy was also estimated and a spectral analysis of *J'* revealed that the power of the spectrum increased with water velocity and was lower in the canopy than in the empty flow-chamber. These results suggest that the fronds serve to dampen the flow with the canopy and that these effects vary with velocity."
(a) Nagasaki University

P04026 "Biogeography of microbiotic soil crust in high vs. low recreational use areas within the Wonderland of Rocks of Joshua Tree National Park, California"
Pietrasiak, Nicole-presenter npiet001@ucr.edu(a) Johansen, Jeffrey R. (b) LaDoux, Tasha (a) Graham, Robert C. (a)
"Microbiotic crusts in two geomorphologic similar areas of the Wonderland of Rocks region of Joshua Tree National Park, California were studied with respect to visible crust cover and frequency, chlorophyll a, soil stability, and a suite of abiotic soil parameters. The area northeast of the Keys Ranch was heavily disturbed in the past, but has been protected from further disturbance for over 35 years. The area northeast of Barker Dam was inaccessible to cattle, and so has had a long relatively disturbance-free period of time in which crusts could develop, but recently has experienced trampling disturbance from hikers and rock climbers that increasingly visit the area. The Keys Ranch area showed clear signs of recovery in this study, having higher visual cover of cyanobacterial crusts than the Barker Dam area. However, the area northeast of Barker Dam had more lichen and moss crusts, a sign that the crusts in this area are more successionally mature. In addition, three geomorphological features were characterized in this study: pockets, slopes, and wash banks. These geomorphic parameters clearly impacted crust development in both areas, with wash banks showing best crust development and slopes showing poorest crust development. Lichens and mosses were best developed in the pocket areas, which can accumulate and retain moisture during and following precipitation events. "
(a) University of California, Riverside (b) John Carroll University

P04027 "From cryptomonads to *Dinophysis*, a labyrinthine story of multi-species interactions among protists"
Myung, Geumog (a) Kim, Hyung Seop (a) Kang, Yi Gu (a) Ha, Na (a) Park, Jong Woo (a) Rho, Jung Rae (a) Park, Myung Gil (b) Yih, Wonho -presenter ywonho@kunsan.ac.kr(a)
"Some cryptophyte species serve as plastid donor as well as prey for a mixotrophic marine ciliate, *Myrionecta rubra* [1, 2]. Even karyoklepty (retention of prey's nucleus by the grazer) of the ciliate was reported quite recently [2]. Sequestration of the secondhand plastid in *M. rubra* by another protistan genus *Dinophysis* has been suggested, to evoke on-going controversial discussions [3]. The only known live prey for culturing *Dinophysis* spp. is the mixotrophic marine ciliate *M. rubra* [4], which can in turn ingest cells of a certain type cryptomonads [5]. Cryptomonds themselves already represent a long story of plastid evolution and are housing remnant nucleus of its old photosynthetic symbionts and ejectosomes, the highly sophisticated defense machinery in protist. Crowded cryptomond cells ($>10^5$ ml^{-1}) when mixed with *M. rubra* could compete out their grazer ciliate in a culture bottle [6], and may continue to grow using the organic material of dead ciliate cells. The cryptomonads can even compete out *Dinophysis* population in a co-culture system where high density prey's prey of the dinoflagellate is postulated to physically attack the grazer's grazer [7]. Recent exploration on bacterivory [8] and specific bacterial association [9] in *M. rubra* adds on another layer to the complexity of the multi-species interactions among the three marine protists. 1. Yih et al. 2004 Aquat Microb Ecol 36, 165 2. Johnson et al. 2007 Nature 445, 426 3. Park et al. 2008 J Phycol 44, 1154 4. Park et al. 2006 Aquat Microb Ecol 45, 101 5. Park et al. 2007) Aquat Microb Ecol 48, 83 6. Hansen et al. 2006 Mar Biol Res 2,169 7. Ha Na et al. 2009 MS thesis, Kunsan National University, 112pp. 8. Myung et al. 2006 Aquat Microb Ecol 44, 175 9. Hwang et al. 2009 Int J Syst Evol Microbiol 59, 609 "
(a) Kunsan National University (b) Chonnam National University

P04028 Nitrogen to phosphorus ratios in the microalga *Chlamydomonas reinhardtii*: removing the confounding of nutrient proportions and concentrations
Evens, Terence-presenter terence.evens@ars.usda.gov(a) Niedz, Randy (a)
"Our understanding of the importance of nutrient ratios, primarily NO_3 (N) and PO_4 (P), in relation to microalgal growth and growth rate (primary *production* and *productivity*, respectively) can be traced directly to Redfield's seminal work when he quantified the elemental ratios of marine phytoplankton in relation to the prevailing stoichiometry of the world's oceans. Since then the 'Redfield ratio', an N:P of 16:1, has become the gold

standard for all microalgal nutritional stoichiometry, and is often used as a benchmark for differentiating N-limitation from P-limitation with N-limitation occurring at N:P < 16 and P-limitation occurring at N:P > 16. Deviations from the Redfield ratio are seen as indicative of any number of physiological and ecological processes, and have been subject to a great deal of experimentation and speculation. Numerous studies have questioned the universality of the Redfield ratio, but it is still the accepted benchmark. We have recently begun applying mixture-based experimental designs to the N:P question, which allow us to remove the confounding between proportionality and concentration. Our first foray examines the interplay between medium NO_3 and PO_4 ratios at total [N+P] of 10-1000 µ;M and cellular stoichiometry in the green algal *Chlamydomonas reinhardtii*. Results indicate that our understanding of how N and P, ratios and concentrations, affect growth rates is complex but quantifiable, and needs to be reexamined."
(a) USDA-Agricultural Research Service, U.S. Horticultural Research Lab

P04029 Does *Dinophysis caudata* (Dinophyceae) have permanent plastids?
Park, Myung Gil-presenter mpark@chonnam.ac.kr(a) Kim, Miran (a) Kim, Sunju (b) Yih, Wonho (c)
"The marine photosynthetic dinoflagellates Dinophysis Ehrenberg species are obligate mixotrophs that requires both light and ciliate prey Myrionecta rubra (= Mesodinium rubrum) for long-term survival. Despite rapid progress on the study of Dinophysis using the laboratory cultures, however, whether it has its own permanent plastids or kleptoplastids (i.e. stolen plastids from its ciliate prey) remains unresolved. We addressed this issue here, using the established culture of D. caudata strain DC-LOHABE01 and cross feeding/starvation experiments encompassing the prey M. rubra strain MR-MAL01 cultures grown on two different cryptophytes (strains CR-MAL01 and CR-MAL11). To follow the fate of prey plastids, psbA gene as a tracer was amplified from individually isolated D. caudata cells and the PCR products were digested with a restriction enzyme SfaNI. The restriction fragment length polymorphism (RFLP) pattern of the PCR products digested by SfaNI revealed that D. caudata continued to keep CR-MAL01-type plastid, whereas it lost CR-MAL11-type plastid with increasing time of starvation. Our result suggests that Dinophysis does not treat plastids taken up from different cryptophytes via its ciliate prey M. rubra the same. Alternatively, D. caudata may already have its own CR-MAL01 type permanent plastid, with two type plastids (CR-MAL01 and CR-MAL11) obtained from M. rubra being lost within 1 month. Our result raises the need to address the origin of plastids in newly isolated Dinophysis in order to resolve the issue of plastid permanence in the photosynthetic Dinophysis species in the future study."
(a) Department of Oceanography, Chonnam National University (b) Smithsonian Environmental Research Center (c) Department of Oceanography, Kunsan National University

P04030 Investigating the genetic record of atmospheric carbon dioxide
Young, Jodi N-presenter jodiy@earth.ox.ac.uk(a) Lee, Renee B.Y. (a) Kapralov, Maxim (b) Moolna, Adam (a) Filatov, Dmitry (b) Smith, J. A.C. (b) Rickaby, Rosalind (a)
"The reconstruction of past climates, on timescales of millions of years, relies on the analysis of chemical or isotopic proxies in preserved organic matter. Such indirect approaches depend upon empirical calibration in modern species without understanding biological processes which underpin the incorporation of the climate signal. In order to address this major gap in climate research, we decided to study the evolution of photosynthesis and carbon concentrating mechanisms (CCMs) in algae, a living geological record. This will enable us to decipher past, and most importantly, future pCO_2 trends. Algae are responsible for up to 50% of total carbon fixed through photosynthesis and many have extensive fossil records used for past climate reconstruction, however, it is poorly understood how they have adapted to changing climate over time. Adaptation in the photosynthetic enzyme, RuBisCO, was investigated using Phylogenetic Analysis of Maximum Likelihood (PAML) and this was compared with the emergence of CCMs, in particular carbonic anhydrase (CA). Strong adaptation of RuBisCO was detected on branches leading to major groups of algae which are consistent with the variation of specificity factors between algal groups. While RuBisCO adaptation occurs during time periods of major changes in CO_2 and O_2, this adaptation appeared to stop at the point of divergence within groups. We subsequently demonstrated that this pattern corresponds to the distribution of δ-CA, a candidate CCM. Algae have adapted strongly to decreasing pCO_2 over time which would have perpetuated falling levels. With the current rise in anthropogenic CO_2, it is crucial to understand the rate of adaptation in algae and whether this will assist in negating further environmental damage. "
(a) University of Oxford, Dept Earth Sciences (b) University of Oxford, Dept Plant Sciences

P04031 Exploring the potential impact of native plant riparian zones for nutrient amelioration of non-point source pollution by examining changes in aquatic algal communities.
Norwich, Alyson R-presenter alynorwich@gmail.com(a) Casamatta, Dale (a) Moon, Daniel (a) Rossi, Anthony (a) Smith, Kelly (a)
"As anthropogenic impacts on the environment increase, aquatic ecosystems are under significant threat of elevated eutrophication. Recent research has indicated that one mechanism for ameliorating non-point source pollution may be the employment of native riparian plant communities. To test the impact of these communities on the lotic algal community, we studied nine first order tributaries along the St. Johns River (Jacksonville, Florida) over a three year period (January 2006-December 2008). Five of the sites were experimental and had native plant gardens established, with the remaining four sites serving as controls. Sites were pulsed with nitrogen and phosphorus additions in order to determine efficacy of plant communities at nutrient retention and sequestration. Physical/chemical parameters (e.g., total nitrogen, phosphorus, pH, conductivity) of the soils and associated streams were measured monthly over a three year period. For instance, preliminary results found a significant ($p<0.05$) decrease in soil nitrate and phosphate levels from the top of the experimental plots to the bottom after fertilization. Concurrently, algae was collected by benthic sampling and analyzed for cell counts and biovolumes. Cyanobacteria and Bacillariophyta dominated nearly all samples in terms of cell counts and biomass, with Chlorophyte mats (e.g., *Ulothrix, Cladophora* and *Mougeotia*) occasionally being temporally abundant. Preliminary analyses indicated that the associated algal communities shifted to chlorophyte dominance with increased fertilizer application, but did not exhibit a shift normally associated with eutrophic conditions such as luxurious growth of cyanobacteria. The dominant cyanobacteria tended to be rather ubiquitous members of the genera *Leptolyngbya, Aphanocapsa* and *Aphanothece*, none of which are typically associated with eutrophication. Thus, it appears that native plant riparian zones may have a role in preventing non-point source pollution by reducing nutrient loading. "
(a) University of North Florida

P04032 "Biomass and seasonal succession of thraustochytrids (stramenopiles, Labyrinthulomycetes)"
Ueda, Mayumi (a) Nomura, Yuka (a) Kadoe, Tomohiro (a) Honda, Daiske-presenter dhonda@konan-u.ac.jp(a)
http://syst.bio.konan-u.ac.jp
"Thraustochytrids are colorless unicellular stramenopiles, which are classified in the class Labyrinthulomycetes. It was reported the biomass of thraustochytrids in coastal waters could reach 3-43% of the bacterial biomass. The thraustochytrid cell is quite larger than the typical bacterial cell, and is a similar size to bacteria feeders, such as flagellates. It means that the thraustochytrids are directly ingested by higher trophic-level microorganisms, such as ciliates and copepods, that is, they serves as a larger starter than bacteria in microbial food chains with fewer trophic links and less trophic-level losses. Therefore, it is suggested that thraustochytrids play an important ecological role in the initial stage of the microbial food

chain as the decomposer. However, there is poor information about the seasonal changing of species and genus composition and biomass. In this study, we have monitored the seasonal dynamics of thraustochytrids every two or three weeks in 2008. The abundance of thraustochytrids was estimated using a modified MPN technique. The obtained isolates were identified based on 18S rDNA tree and morphological characteristics. The seasonal change of species composition was observed. In spring, *Aurantiochytrium mangrovei* appeared as the major species, then *Schizochytrium aggregatum* and *Botryochytrium* sp. appeared sequentially until summer. Two peaks were observed as a change in the number of cells in May and July, both of which were mainly composed of *A. mangrovei*. It is suggested that *A. mangrovei* is one of the most important thraustochytrid species from the ecological aspects in the coastal temperate environment."
(a) Faculty of Science and Engineering, Konan University

P04033 Reproduction in *Porphyra umbilicalis* Kutzing: Insights from amplified fragment length polymorphisms(AFLPs)
Blouin, Nicolas A-presenter nicolas.blouin@umit.maine.edu(a) Brawley, Susan H (a)
http://www.umaine.edu/marine/people/directory.php/profile/nic_blouin
" *Porphyra umbilicalis* is described as being mostly sexual in Europe; however, collections made each month over multiple years (n > 4000 individuals), show that P. umbilicalis reproduces exclusively via neutral spores (asexually) on the Maine coast. This suggests that the population could be clonal and lack genetic diversity. *Porphyra umbilicalis* haplotype P.um.1 was isolated from a collection made on the Maine (USA) shore, and is the subject of a complete genome-sequencing project by the US Department of Energy. Characterization of the parent population in Maine of P.um.1 will aid future investigations by scientists working with this haplotype. To investigate genetic diversity, we used amplified fragment length polymorphisms (AFLPs) with individuals of *P. umbilicalis* from locations where asexual or sexual reproduction is known to occur. If individuals were clones of each other, then their AFLP profiles would be expected to be identical. One hundred *P. umbilicalis* individuals were collected from four locations, n=25 per site. The sites selected included three locations in the USA (two from Maine) and a fourth location in the UK (known sexual individuals). Similarity measures and Bayesian analysis show that individuals within sites are more closely related than individuals between sites in the northwestern Atlantic. Additionally, individuals from the UK are more diverse than all of the USA sites. Because AFLP analysis revealed genetic diversity within all sites, we conclude that USA populations are not a single clone. "
(a) University of Maine, School of Marine Sciences

P04034 Genetic and morphological variation in *Codium fragile* in the NW Atlantic
Benton, Christopher S (a) Pleticha, Lucy E (b) Klein, Anita S.-presenter anita.klein@unh.edu(b) Mathieson, Arthur C (b)
http://www.biolsci.unh.edu/faculty/klein/index.html
"*Codium fragile* (Suringar) Hariot is an invasive Asiatic green alga that colonized coastlines in the NW Atlantic in the past 50 years. The plant has a dichotomously branched cylindrical thallus composed of tangled filaments surrounded by a densely packed outer covering of swollen filaments (utricles) and terminated by a pointed tip, the mucron. The goals of this study are to investigate the morphological and genetic differences in NW Atlantic *C. fragile* populations to determine which subspecies are present (invasive or non-invasive) and whether there is genetic variation among populations. In 2008, we surveyed 19 sites from the Canadian Maritimes to Long Island Sound and compared these to prior surveys by Mathieson et al. and Garbary et al. The distributions of *C. fragile* populations appear to be in flux, with a decrease in Prince Edward Island and an increase in Nova Scotia. For each population collected, we measured utricle and mucron size, two morphological characters commonly used to differentiate subspecies. There is a two-fold difference in both utricle and mucron length between populations. Haplotypes of chloroplast *rps*3-*rpl*16 region were determined; all extant collections corresponded with the invasive taxon *C. fragile* subsp. *fragile*. Since previous investigators (Malinowski 1974, Kusakina 2004) have observed genetic variation among populations, we are examining more variable genetic loci (*tRNA*G-5S, *rbc*L intron) in order to track how the invasive alga has spread across the NW Atlantic."
(a) Molecular Cellular and Biomedical Sciences, U New Hampshire (b) Biological Sciences, U New Hampshire

P04035 Algae of the Oxycline: Cultivation Using Strategies to Alleviate Oxidative Stress
Morris, James J.-presenter jmorri40@utk.edu(a) Szul, Martin J. (a) Buchan, Alison (a) Dunlap, John (a) Keller, Martin (b) Wilhelm, Steven W. (a) Saxton, Matthew (a) Zinser, Erik R. (a)
"Recent work in our laboratory has revealed that the abundant open ocean cyanobacterium *Prochlorococcus* is remarkably susceptible to damage by reactive oxygen species (ROS). We have demonstrated that pre-treatment of culture media to remove ROS, either by adding ROS-resistant 'helper' microorganisms or by adding antioxidant enzymes such as catalase, dramatically improve the cultivability of these organisms, allowing growth from inocula as low as a single cell. We report here our attempts to use similar strategies to recover ROS-sensitive algae from natural populations. On a 2007 cruise, we enriched samples from a hypoxic area in Chesapeake Bay. Community rDNA analysis of these cultures using phyto-specific primers suggests that pre-treatment with 'helpers' or with catalase significantly increases the phylogenetic diversity of the enrichment and leads to an increased representation of picophytoplankton lineages (e.g., *Synechococcus*) at the expense of larger organisms (e.g., diatoms). We further present two novel picoplanktonic isolates obtained on this cruise, tentatively identified as *Nanochlorum* sp. VOL9 and Prochlorales sp. VOL18. The possibility that these organisms, like *Prochlorococcus*, have undergone reductive evolution leading to a dependence on other organisms to tolerate ROS, is discussed."
(a) University of Tennessee Knoxville (b) Oak Ridge National Laboratory

P04036 Growth of *Myrionecta rubra* on divergent strains of cryptomonads
Schvarcz, Christopher R.-presenter schvarcz@email.arizona.edu(a) Hackett, Jeremiah D. (a)
http://www.eebweb.arizona.edu/Faculty/Hackett/Home.html
"The marine ciliate *Myrionecta rubra* is a bloom-forming phytoplankter that in natural environments serves as prey for the harmful toxin-producing dinoflagellates *Dinophysis spp*. *M. rubra* is interesting because of its ability to retain and regulate the plastids acquired from its cryptophyte prey *Teleaulax amphioxeia* or *Geminigera cryophila*. Whereas *M. rubra* is able to grow when fed related cryptophytes, it is unclear if the ciliate is retaining the plastids of these species. Furthermore, preliminary observations suggest that growth of *M. rubra* on some of these strains is suboptimal and may represent cases where kleptoplastidy is inhibited or inefficient. This study investigates the phylogenetic range of possible cryptophyte kleptoplastids for *M. rubra* and the physiological constraints preventing such relationships with other cryptomonad strains. Results from this work will provide additional insights into the physiological mechanisms involved in kleptoplastidy, as well as inform the growth ecology controlling *M. rubra* blooms (and thus *Dinophysis spp*. blooms) in nature. "
(a) University of Arizona, Department of Ecology and Evolutionary Biology

P04037 Quantitative response of Epithemia and Rhopalodia (Bacillariophyta) containing nitrogen-fixing cyanobacterial endosymbionts to low nitrogen and potential use in stream bioassessment in Southern California

Stancheva, Rosalina (a) Fetscher, A. Elizabeth (b) Kociolek, J. Patrick (c) Laslandes, Berengere (c) Sheath, Robert G-presenter rsheath@csusm.edu(a)
"The symbiosis between freshwater Epithemia and Rhopalodia species and nitrogen-fixing unicellular coccoid cyanobacteria has been observed under low-nitrogen field conditions. Hence, these genera can be potential indicators of nitrogen-limited waters. In a survey of >100 coastal streams in Southern California in the summer and fall of 2007 and 2008, representatives of Epithemia and Rhopalodia were recorded. We conducted a morphometric study of the cyanobacterial endosymbionts from 11 populations of the three most common Epithemia species and Rhopalodia gibba. The numbers of endosymbionts per diatom cell ranged from 1-2 in E. sorex, 2-6 in E. adnata, 2-12 in E. turgida and 1-4 in R. gibba. Endosymbiont average diameter, mean biovolume, and total biovolume per diatom cell were significantly negatively correlated to nitrate levels for all taxa. The distribution of the common filamentous cyanobacteria bearing heterocysts was compared to that of the diatoms studied. Both nitrogen-fixing diatoms and common filamentous cyanobacteria bearing heterocysts demonstrated preferences for low nitrates (< 1.4 mg/L), low total nitrogen, (<1.8 mg/L) and low total phosphorus (< 0.25 mg/L). However, the two groups differed in their light preferences and did not fully overlap in distribution. This study showed that nitrogen-fixing species can be powerful tools in stream biomonitoring and sub-cellular analyses of diatoms with cyanobacterial endosymbionts may provide an additional layer of resolution in this process beyond that obtainable through traditional assemblage composition analysis alone. "
(a) California State University San Marcos (b) Southern California Coastal Water Research Project (c) University of Colorado Boulder

P04038 "Biofouling diatoms at a static immersion test site in the Indian River Lagoon, Florida"
Zargiel, Kelli A.-presenter kzargiel@fit.edu(a) Swain, Geoff W. (a)
"Diatoms are a major component of biofilms in the marine environment and are particularly difficult to control on ship hulls. When these films form on the surface of ship hulls, they increase hydrodynamic drag, which results in increased fuel consumption of up to 20%. This poster describes diatoms that were present in biofilms on both polyvinylchloride(PVC) and a silicone fouling release antifouling coating at a static immersion test site in the Indian River Lagoon, Florida. Biofilm samples were collected every week from April 2008 to October 2008. Diatoms were identified to the lowest possible taxon and species abundances were compared between the two surfaces. Differences in biofilms were seen among surfaces and diatom composition varied by season and several water quality parameters. This data gives insight into biofilm formation on antifouling coatings and provides valuable information for ecological and engineering solutions to fouling of marine surfaces. "
(a) Florida Institute of Technology

P04039 The complete chloroplast genome sequences of two brown tide agents: *Aureococcus anophagefferens* CCMP1984 and *Aureoumbra lagunensis* CCMP1507
Ong, Han Chuan (a,g) Wilhelm, Steven W (b) Gobler, Christopher J (c) Bullerjahn, George (d) Rocap, Gabrielle (e) Jacobs, Michael A (f) Cattolico, Rose Ann-presenter racat@u.washington.edu(a,e)
"Members of the Pelagophyceae form massive brown tides that have continually plagued the coastal regions of the Eastern seaboard and the Gulf of Mexico. To gain a better understanding of the photosynthetic competence that may be linked to their success in forming massive blooms, we have sequenced the chloroplast genomes of *Aureococcus anophagefferens* (89,599 bp) and *Aureoumbra lagunensis* (94,346 bp). The chloroplast genomes of these algae are significantly smaller than those of the six stramenopiles that presently have been sequenced. Size reduction is augmented by a minimal (*A. anophagefferens*) or lost (*A. lagunensis*) of the inverted repeat. The fact that 8 of 10 small repeats found in both genomes are associated with genes coding for photosynthesis or energy production argues that these elements may have a functional constraint. High genomic synteny, a multi-gene phylogenetic analysis, and a synapomorphic change in an attenuated *psbA* gene, confirm these heterokont algae are closely-related sisters. Retention of three light-independent chlorophyll biosynthesis genes in *A. lagunensis* chloroplast DNA, but their absence in both chloroplast and nuclear DNA of *A. anophagefferens*, implies ongoing intracellular gene transfer, and also espouses the persistence of a more ancient (i.e.,dark-adaptive) potential in *A. lagunensis*. The contribution of this gene profile modification to a sustained bloom of *A. lagunensis* (continuously for ~8 years) but not *A. anophagefferens* (a few months) under reduced light conditions, remains an intriguing question."
(a) Department of Biology, University of Washington (b) Department of Microbiology, The University of Tennessee (c) School of Marine and Atmospheric Sciences, Stony Brook University (d) Department of Biological Sciences, Bowling Green State University (e) School of Oceanography, University of Washington (f) Department of Medicine, University of Washington (g) Division of Science, Lyon College

P04040 "A rapid underwater study on the impacts of climate change on marine algal distributions along the Jeju Island, south coast of Korea"
Shin, Hyun-Woung-presenter hwshin@sch.ac.kr(a) Mohandoss, Sidharthan (a) Cho, Sung-Hwan (b) Lee, Hanseong (a) Ko, Ji-Woong (b) Lim, Chi-Young (b)
"The algal vegetation cover on the sea bottom offer food, habitat and spawning bed for various benthic invertebrates as well as fishes. The temperate oceanic weather condition in the volcanic Island, Jeju is influenced by the major fronts such as Yellow sea cold current, Kuroshio and Tsushima current, influx of Changjiang river water and Jeju warm current that flows clockwise along the Island. In the recent past, unprecedented climatic changes in these major fronts have massively altered the physicochemical variables, therefore a radical shift in the diversity and biomass of algal distributions have been recognized. An intensive underwater (SCUBA) investigation was carried out with line-transects (at 5, 10 & 15 m depths) using quadrats and also CPCe with GPS-photo Link for a canopy forming species in eight scantily studied coastal zones along the Jeju Island. These stations were found to be much influenced by changing climatic conditions and are indicative of responding marine algal communities. Conspicuous changes in the sea bottom and associated algae with decline in species diversity and biomass; and incidence of patchy distributions were seen. A maximum mean coverage of 45.63% m^{-2} and relative biomass of 42, 411.2 g w. wt. m^{-2} was estimated for *E. cava* from Woljeong (north coast). Biomass of this canopy forming kelp species was comparatively less in west and south coasts (< 3,655.9 g w. wt. m^{-2}). Relative algal coverage of 7.18% m^{-2} (Aewol, west coast) and 6.74% m^{-2} (Sincheon, east coast) were clearly indicated the substantial reduction in algal vegetation. Results on algal coverage, species diversity and biomass showed decisive information on algal distributions that were influenced by perturbed environmental changes that evolved during the recent climate changes."
(a) Department of Marine Biotechnology, Soonchunhyang University (b) Jeju Fisheries Research Institute, National Fisheries Research & Development Institute

SESSION P05 – ALGAL PHYLOGENETICS AND TAXONOMY

P05001 – See PSA Talk on Tuesday, July 21 – Algal Ecoology & Population Biology II

P05002 - See PSA Talk on Saturday, July 18 – Algal Phylogenetics & Taxonomy I

P05003 – See PSA Talk on Monday, July 20 – Algal Phylogenetics & Taxonomy III

P05004 – See PSA Talk on Monday, July 20 – Algal Phylogenetics & Taxonomy II

P05005 "Species delimitation and systematics of Hawaiian Amansieae (Ceramiales, Rhodophyta)"
Sherwood, Alison R.-presenter asherwoo@hawaii.edu(a) Kurihara, Akira (a) Conklin, Kimberly Y. (a)
"Seventy Hawaiian specimens of representatives of the tribe Amansieae morphologically corresponding to taxa attributed to *Amansia*, *Melanamansia* and *Osmundaria* were collected from the Hawaiian Islands or examined from the holdings of the Bishop Museum. Sequences of three short DNA markers were obtained from as many of these specimens as possible and analyzed using a DNA barcoding approach. The nuclear 18S rRNA gene was amplified and sequenced from representative specimens of major clusters from the previous analyses. A phylogeny of the 18S rRNA gene indicated that although species-level systematics of Hawaiian Amansieae are relatively straightforward, generic concepts will require some revision. Specimens morphologically corresponding to *O. obtusiloba* and *M. fimbrifolia* formed well-defined clusters based on short marker molecular comparisons. In contrast, specimens morphologically identified as *M. glomerata* belonged to several molecular clusters, which corresponded to collections from most of the Main Hawaiian Islands, Hawaii Island, the Northwestern Hawaiian Islands, and deep water collections, indicating that *M. glomerata* in Hawaii is likely a species complex. Correlations of anatomy and molecular analyses will be discussed, along with implications for the taxonomy of the Hawaiian Amansieae."
(a) Botany Department, University of Hawaii

P05006 - See PSA Talk on Saturday, July 18 – Algal Phylogenetics & Taxonomy I

P05007 – See PSA Talk on Tuesday, July 21 – Algal Phylogenetics & Taxonomy IV

P05008 – See PSA Talk on Monday, July 20 – Algal Phylogenetics & Taxonomy II

P05009 Species of *Codium* from Continental Chile
Gonzalez, Alejandra-presenter apgonzalez@uchile.cl(b) Santelices, Bernabe (a)
"Seven species of subgenus Tylecodium have been traditionally recorded for the coastline of Chile. Some species (e.g. *C. dimorphum* Svedelius and *C. difforme* Kutzing) exhibit two utricles morphology, while some others (e.g. *C. adhaerens* C. Agardh, *C. arabicum* Kutzing, *C. setchellii* Gardner, *C. spongiosum* Harvey, and *C. subantarcticum* P.C. Silva) exhibit one utricle morphology. No critical morphological evaluation has been performed with the species collected, and their great morphological and geographic overlap makes difficult their identification. Along central Chile the species of Codium are ecologically important, the adherent thallus may coalesce, forming a permanent belt at mid intertidal levels often overgrowing and excluding other seaweeds and invertebrates. Using samples from 70 localities along the Chilean coast, we characterized morphologically and genetically the species found among 20 to 55 LS. We used 16 morphological traits and three neutral markers (chloroplast rbcL, mitochondrial rLSU and chloroplast Trn-Gly gene). Results indicate the presence of three, geographically discontinuous species. *Codium dimorphum* restricted to Guaitecas Islands in south of Chile (43 LS), *C. subantarcticum* extended from 53 to 55 LS, and a yet undescribed species *Codium* sp., extended from 25 LS in the north to 40 LS in the south of Chilean coast. Supported by CONICYT AT-4040047 to AG and FONDECYT 1060474 to BS. "
(a) Pontificia Universidad Catolica de Chile (b) Universidad de Chile

P05010 – See PSA Talk on Monday, July 20 – Algal Phylogenetics & Taxonomy III

P05011 – See PSA Talk on Tuesday, July 21 – Algal Phylogenetics & Taxonomy IV

P05012 – See PSA Talk on Tuesday, July 21 – Algal Phylogenetics & Taxonomy IV

P05013 - See PSA Talk on Saturday, July 18 – Algal Phylogenetics & Taxonomy I

P05014 - See PSA Talk on Monday, July 20 – Algal Phylogenetics & Taxonomy III

P05015 - See PSA Talk on Saturaday, July 18 – Algal Phylogenetics & Taxonomy I

P05016 - See PSA Talk on Saturday, July 18 – Algal Phylogenetics & Taxonomy I

P05017 – See PSA Bold Talk on Saturday, July 18

P05018 – See PSA Bold Talk on Saturday, July 18

P05019 – See PSA Bold Talk on Saturday, July 18

P05020 Taxonomy and life cycle of the *Prorocentrum emarginatum* species complex
Morton, Steve-presenter steve.morton@noaa.gov(a) Symon, Elizabeth (a) Richlen, Mindy (b)
"Examination of cultures of small, benthic/epiphytic *Prorocentrum* species reveals a number of possible cryptic species all sharing characters of both *Prorocentrum emarginatum* and *P. fukuyoi*. The gross morphology of these cultures includes a relatively small size (28-31 µ;undefinedm L x 19-23 µ;undefinedm W), with a prominent apical spine, and displays a pronounced cuneiform indentation into the middle of the right valve. The surface of the theca is smooth with pores of two sizes, the larger pores being arranged in a radial pattern. Sequences of the LSU rDNA gene help to resolve the phylogenetic relationships between these and other *Prorocentrum* species. These cryptic species appear to be a sister group to *P. emarginatum* and to the recently described *P. fukuyoi*. Vegetative cysts of these species form once cultures become phosphate limited. The complete life cycle of motile cells and cyst-like structures is hypothesized. "
(a) National Oceanographic and Atmospheric Administration (b) Woods Hole Oceanographic Institution

P05021 – See PSA Bold Talk on Saturday, July 18

P05022 - See PSA Talk on Monday, July 20 – Algal Phylogenetics & Taxonomy III

P05023 – **See PSA Talk on Saturday, July 18 – Algal Phylogenetics & Taxonomy I**

P05024 – See PSA Talk on Tuesday, July 21 – Algal Phylogenetics & Taxonomy IV

P05025 – See PSA Talk on Tueday, July 21 – Algal Phylogenetics & Taxonomy IV

P05026 – See PSA Talk on Monday, July 20 – Algal Phylogenetics & Taxonomy II

P05027 – See PSA Talk on Monday, July 20 – Algal Phylogenetics & Taxonomy II

P05028 – See PSA Talk on Tuesday, July 21 – Algal Phylogenetics & Taxonomy IV

P05029 "Reassessment of the genus *Dasya* (Rhodophyta, Ceramiales) in Hawaii"
Conklin, Kimberly Y.-presenter kyikemot@hawaii.edu(a) Kurihara, Akira (a) Sherwood, Alison R. (a)
"The red algal genus, *Dasya* (Ceramiales), is estimated to include more than 80 recognized species, of which many have not been fully described and are lacking complete diagnostic morphological characterization. Molecular and anatomical phylogenetic studies of the family Dasyaceae indicated that *Dasya* is a paraphyletic genus, further highlighting the taxonomic confusion and identification difficulties surrounding this group. In this study, molecular tools were used to reassess *Dasya* in Hawaii. Three short DNA markers (the mitochondrial COI DNA barcode, and portions of the nuclear and plastidal LSU rRNA genes) were analyzed using a DNA barcoding approach for 45 Hawaiian specimens of *Dasya*. The nuclear SSU rRNA gene was also amplified and sequenced for representative specimens and analyzed with GenBank sequences from other Dasyoideae taxa. Seven clusters corresponding to the previously recorded six *Dasya* species in Hawaii were recovered as well as a single unique sample of a currently undescribed species. Specimens identified morphologically as *D. corymbifera* separated into two clusters, one of which may represent *D.* '*collinsiana*,' a species that was more recently reported as invalid in 1999. Based on these analyses, the Hawaiian flora contains eight species of *Dasya*, and taxonomic changes will be proposed to reflect this. The results of this study highlight the benefits of molecular tools and their assistance in further clarification of the species limits of red algae."
(a) University of Hawaii

P05030 Preliminary data on the phylogeny of the family Achnanthidiaceae (Bacillariophyta)
Potapova, Marina-presenter potapova@ansp.org(a)
"The goal of this study was to obtain initial information on the phylogeny of the diatom family Achnanthidiaceae using both morphological and molecular data. Achnanthidiaceae are predominantly freshwater diatoms characterized by small size and secondary loss of the raphe on one valve. They are extremely common and abundant in benthic habitats of inland and coastal waters, rarely planktonic, but one representative, *Pauliella taeniata* is a marine planktonic species. The current classification of the family based on the phenetic approach is highly unstable and confusing. Morphology alone does not provide sufficient characters to establish generic boundaries and to unravel evolutionary history of the group. In addition to a few already available SSU rRNA, LSU rRNA, and *rbc*L sequences for the genera *Planothidium, Pauliella*, and *Achnanthidium*, sequences for the same genes were generated for other representatives of the same genera and for the genera *Rossithidium*, and *Psammothidium*. The preliminary results of phylogenetic analyses of these data will be presented."
(a) The Academy of Natural Sciences

P05031 Euglenoid phylogeny based upon the evaluation of protein and ribosomal coding genes
Watza, Donovan-presenter watzadon@msu.edu(a) Kim, JongIm (b) Bennett, Mathew (a) Lowery, Caitlin (a) Triemer, Richard (a)
http://euglena.msu.edu/
"Over the past decade molecular phylogenies have been used to determine relationships among the major genera of photosynthetic euglenoids. However, the majority of these studies have relied on the use of nuclear or chloroplast ribosomal genes. The purpose of this study was to incorporate protein coding genes into euglenoid phylogenies and compare the results with those of ribosomal genes. The psbO gene, a nuclear encoded plastid targeting gene involved in oxygen evolution, was sequenced for over 50 euglenoid taxa representing *Euglena, Monomorphina, Cryptoglena, Colacium, Trachelomonas, Phacus, Discoplastis*, and *Lepocinclis* . The dataset for the psbO gene was analyzed separately and in combination with the SSU rDNA gene. The results confirm that the psbO and SSU rDNA sequences resulted in phylogenetic trees that were congruent with previous analyses based on ribosomal genes only. Furthermore, the rooted Bayesian phylogenetic tree supported previously identified SSU and partial LSU rDNA clade relationships among the photosynthetic euglenoids. "
(a) Michigan State University (b) Chungnam National University

P05032 A method for obtaining nuclear gene sequences from field samples
Bennett, Matthew S.-presenter benne124@plantbiology.msu.edu(a) Triemer, Richard E. (a)

http://euglena.msu.edu
"One of the biggest issues in the field of taxonomy today is the inability to acquire, grow and sequence new taxa. This problem is very evident in the study of photosynthetic euglenoids. Most of the unique taxa in culture collections have been sequenced and many other taxa of interest have been resistant to culturing, and thus, sequencing. In an effort to address this problem, we have developed a technique, which allows for the sequencing of nuclear genes from a small number of cells. Cells are isolated from field samples using a Pasteur pipet and transferred through several rinses of sterile media. The isolated cells are then collected, processed with a standard DNA extraction kit and then run through a Multiple Displacement Amplification (MDA). After this process, all samples had their genomic DNA amplified many fold to microgram quantities which were then available for PCR analysis. We have successfully used this procedure to amplify multiple nuclear genes from several taxa. "
(a) Michigan State University

P05033 Phylogeny of a family of Dinoflagellates (Dinophysiaceae) based on rDNA sequences from single cells and environmental samples
Delwiche, Charles F-presenter delwiche@umd.edu(a) Handy, Sara M (a) Bachvaroff, Tsvetan R (b) Kim, Sunju (b) Timme, Ruth (a) Coats, Wayne (b)
http://www.life.umd.edu/labs/delwiche
"The dinoflagellate family Dinophysiaceae are considered to be heterotrophic, although many acquire kleptoplastids or cyanobacterial symbionts. Although environmentally important, their phylogeny is relatively little known, particularly in comparison to photosynthetic species. We examined the phylogeny of several dinophysiacean dinoflagellates using samples collected from four Atlantic sites. Roughly 3.5 kb of nuclear sequence comprising the nuclear ribosomal genes SSU, 5.8S, LSU, plus their ITS 1 and 2 regions, were determined for 26 individual cells. These include representatives of at least four morphological genera, including two for which molecular data were previously unavailable (Ornithocercus and Histioneis). To complement data from these individual cells, clone libraries targeting the dinophysiacean ITS2 and 28S regions were constructed from bulk environmental DNA from each of three sites. Three distinct phylogenetic analyses were performed: using only sequences from morphologically identified cells, (28 taxa, 3.5 kb); incorporating the environmental data (136 OTUs, 2.1 kb); and sampling across dinoflagellates (56 taxa, 1.9 kb). All trees were congruent for sequences from morphologically identified taxa. The cyanobacteria-harboring genera Histioneis and Ornithocercus were monophyletic, and together formed a single clade, although their symbionts appear not to be monophyletic. Despite proposals to submerge Phalacroma in Dinophysis, the representatives of these genera formed two distinct clades. Both of these genera include strains reported as toxic. The analyses reported here shed new light on phylogeny in the Dinophysiaceae, and may be important in understanding the evolution of cyanobacterial symbioses and the diagnosis of harmful strains. "
(a) University of Maryland - College Park (b) Smithsonian Environmental Research Center

P05034 "Reconstructing euglenoid evolutionary relationships using three genes: nuclear ssu and lsu, and chloroplast 16s rdna sequences and the description of *euglenaria* gen. nov. (euglenophyta). "
Linton, Eric W.-presenter eric.linton@cmich.edu(a) Karnkowska-Ishikawa, Anna (c) Kim, Jong-Im (d) Shin, Woongghi (d) Bennett, Mathew (b) Kwiatowski, Jan (c,e) Zakryś, Bożena (c) Triemer, Richard E. (b)
"Using Maximum Likelihood and Bayesian analyses of three genes, nuclear SSU and LSU rDNA, and chloroplast SSU (16S), the relationships among 82 plastid-containing strains of euglenophytes were clarified. The resulting tree contained two major clades: the upper clade contained *Euglena, Trachelomonas, Strombomonas, Colacium, Monomorphina, Cryptoglena* and *Euglenaria*; the lower clade contained *Lepocinclis, Phacus* and *Discoplastis*. The majority of the members of the genus *Euglena* were contained in the crown clade, but seven members were outside of this clade. *Euglena limnophila* claded with, and was thus transferred to the genus *Phacus*. *Euglena proxima* was a single taxon at the base of the upper clade and is unassociated with any clade. Five members of *Euglena* claded together in the upper clade and were transferred into the newly erected genus *Euglenaria*. The monophyly of the remaining genera was supported by both analyses. Combining datasets resolved the relationships among ten genera of photosynthetic euglenoids."
(a) Central Michigan University, Department of Biology (b) Michigan State University, Department of Plant Biology (c) University of Warsaw, Department of Plant Systematics and Geography (d) Chungnam National University, Department of Biology (e) University of California, Department of Ecology and Evolutionary Biology

P05035 "Plastids of *Climaconeis* species (Bacillariophyceae: Berkeleyaceae), including *C. petersonii* n. sp., from Guam and Palau, Western Pacific"
Lobban, Christopher S-presenter clobban@guam.net(a) Ashworth, Matt (b) Theriot, Edward C (b)
http://test.protistcentral.org/Project/get/project_id/17
"Plastids of *Climaconeis* were originally characterized as H-shaped in girdle view with pyrenoids, by Mereschkowsky when he resurrected *Okedenia* at the turn of the last century. More recently the characterization as H-shaped has persisted in the genus and species descriptions, even though new species were observed only from preserved material, but descriptions now implicitly or explicitly indicate that the H-shape is seen in valve view. In collections of benthic marine diatoms from Guam and Palau we have encountered five species of *Climaconeis* Grunow, including taxa that appear to conform to *C. silvae* Prasad and *C. inflexa* (Breb.) E.J. Cox (curved species), *C. fasciculata* (Grun. ex Cleve) Cox and *C. coxii* G. Reid & D.M. Williams (straight species, the latter with craticular bars), plus a straight species without bars that does not match known species. We document the appearance of plastids in freshly collected material of all five and demonstrate that while the plastids are distinctive, they are not generally H-shaped and the supposedly prominent pyrenoids are not consistently evident. Plastids generally occur as pairs of flat plates with a pyrenoid visible in girdle view when present. In *C. inflexa* the pyrenoids appear to join the opposing plastids, resulting in the only species with the plastids conceivably H-shaped in valve view, but even there Mereschkowsky was referring to the H-shape of each plate (not confirmed in our study). The number of plastids reported in the literature has been confounded both by different concepts of what constitutes one H-shaped plastid and by counts based on plastid remains in pickled cells. We have observed as many as 36 pairs in the new species, *C. petersonii*, and very long-celled species are reported to have even more. "
(a) University of Guam (b) University of Texas at Austin

P05036 Comparative molecular and morphological phylogenetic analyses of taxa in Chaetocerotaceae (Bacillariophyta)
Kooistra, Wiebe HCF-presenter kooistra@szn.it(a) Sarno, Diana (a) Hernandez-Becerril, David H (b) Assmy, Philipp (c) Di Prisco, Carmen (a) Montresor, Marina (a)
http://www.szn.it
"The diatom family Chaetocerotaceae includes two exclusively phytoplanktonic genera: *Chaetoceros* and *Bacteriastrum*. Its hallmark feature is the presence of setae hollow, spine-like appendages protruding from the valves. We gathered morphological, ultrastructural, and sequence data from 83 strains belonging to 19 morphologically delineated species in *Chaetoceros*, one in *Bacteriastrum (B.* cf. *hyalinum*), and related outgroup taxa

(*Eucampia, Hemiaulus*). A molecular phylogeny inferred from their partial LSU rDNA uncovered cryptic diversity in *C. curvisetus, C. debilis* and *C. socialis*. We also inferred cladograms from morphological character states. Both trees resolved *Bacteriastrum* inside paraphyletic *Chaetoceros* and revealed monophyly for subgenus *Chaetoceros (Phaeoceros*). The topologies of the morphological and molecular trees conflicted in details, but mapping of morphology over the topologies revealed several character states being shared derived in both of the trees. In contrast, characters showing several acquisitions and reversals on the molecular tree revealed multiple changes on the morphological tree as well. The results allow sorting through hypotheses as to how setae were acquired."
(a) Stazione Zoologica Anton Dohrn (b) Instituto de Ciencias del Mar y Limnologia, Universidad Nacional Autonoma de Mexico (c) Alfred-Wegener-Institute for Polar and Marine Research

P05037 Aleutian Islands Survey Reveals 16 New Species of Benthic Marine Algae
Lindeberg, Mandy R. (b) Lindstrom, Sandra C.-presenter sandracl@interchange.ubc.ca(a)
"Benthic marine algae have been inadequately surveyed in the Aleutian Islands due to the remote and harsh nature of this archipelago, which spans over 1,800 km from the Alaska Peninsula to Attu Island. Surveys conducted during 2006 and 2007 by the Alaska Department of Environmental Conservation (ADEC) as part of the EPA Environmental Monitoring and Assessment Program (EMAP) and in collaboration with NOAA Fisheries have resulted in a major collection of benthic marine algae. Collections were made from 97 sites throughout 24 different islands of the archipelago, resulting in a catalogue of over 900 specimens. Preliminary results show 151 species have been identified, including at least 16 species new to science, 9 of which appear to be endemic to the Aleutian Islands, and 52 new distribution records. Many of the new species were discovered west of Samalga Pass, where the last of Alaska Coastal Current waters enter the Bering Sea; Samalga Pass had been previously identified as major geographic boundary for marine species. A highlight of the collection was the discovery of a kelp representing a new genus and species, which we have formally named *Aureophycus aleuticus* H. Kawai, Hanyuda, Lindeberg et S.C. Lindstrom. "
(a) University of British Columbia (b) NOAA Fisheries

P05038 A new single cell *Phaeocystis* that produces layered cell walls.
Andersen, Robert A.-presenter randersen@bigelow.org(a) Dashiell, Cory (b) Sommer, Kristi (b) Bailey, J. Craig (b)
"An unusual alga was isolated into culture from a sample collected in the Arabian Sea during November, 1995. Vegetative cells were 6-10 µ;m in diameter and 8-15 µ;m long, and a layered cell wall surrounded each cell. The cell wall was unusual and often layered. The wall was thickest near the base. Based upon cell division activities, one daughter cell appeared to be released at the apical end, perhaps by dissolving the thinner apical wall layer. The chloroplast was shaped similar to a crown, with a cup-like base and several long finger-like projections. Flagellate cells, presumably zoospores, were approximately spherical with a diameter of 5-7 µ;m. There were two emergent flagella and a short haptonema. The ultrastructure of the cell was typical for haptophytes. The chloroplast had an embedded pyrenoid that was divided into two sections, and at least one thylakoid entered the pyrenoid. The Golgi body had a typical haptophyte inflated cisternae, arranged perpendicular to the nucleus. The mitochondria had tubular cristae. Vegetative cells had two short flagella and a short haptonema that presumably extended to full length once the cell escaped the cell wall. Based upon 18S rRNA and rbcL gene sequences, this walled alga belongs firmly within the genus *Phaeocystis*. The original culture contained a labyrinthulid that possessed long, thin pseudopods. Infected *Phaeocystis* cells contained large spheroidal labyrinthuloid cells and numerous small vesicles were produced by the parasite where it pressed against the algal cytoplasm."
(a) Bigelow Laboratory for Ocean Sciences (b) Center for Marine Science Research, UNC-Wilmington

P05039 Biodiversity survey of the Hawaiian bangiophycean algae (Rhodophyta)
Kurihara, Akira-presenter akirak@hawaii.edu(a) Conklin, Kimberly Y. (a) Sherwood, Alsion R. (a)
"Recent molecular phylogenetic studies have revealed that the bangiophyceae are not monophyletic, and a new classification of six bangiophycean classes has been proposed. Nuclear SSU rRNA gene analyses continues to reveal new genera and species within these lineages. Over the past several years we have been exploring the bangiophycean algae from marine, freshwater, and terrestrial habitats in the main Hawaiian Islands as part of the larger Hawaiian Rhodophyta biodiversity project. Taxa have been isolated from the Bangiophyceae, Compsopogonophyceae, and Stylonematophyceae, and analyzed based on the nuclear SSU rRNA gene as well as shorter markers from the plastid LSU rRNA and *cox*1 genes. Our preliminary SSU rRNA gene analyses show that Hawaiian *Porphyra* (collected from Hawaii, Kauia, Maui, and Oahu) and *Bangia* (Maui) collections are closely related to *P. acanthophora* from Brazil and a *Bangia* sp. from New Zealand, respectively, which is in contrast to the previous application of the names of *P. vietnamensis* and *B. atropurpurea*. In the Stylonematophyceae, *Chroodactylon*, *Chroothece*, and *Stylonema* are represented. In the Compsopogonophyceae, *Compsopogon*, *Erythrocladia*, *Erythrotrichia*, and an *Erythrocladia*-like alga are represented. The last taxon displays a monostromatic crustose base, giving rise to a few simple, constricted filaments (pit connections not recognizable with LM observation), however, it is place sister to *Rhodochaete* and the Erythropeltidales in the nuclear SSU rRNA gene analysis. This bangiophyceaen biodiversity survey has thus far resulted in the discovery of one new record and potentially one new genus, and it is anticipated that more will be forthcoming in the final stages of the project."
(a) University of Hawaii

P05040 New Species of the diatom genus *Gomphonema* Ehrenberg (Bacillariophyceae) from Hawaii
Kociolek, John P-presenter Patrick.Kociolek@colorado.edu(a) Lowe, Rex L. (b) Sherwood, Alison R. (c)
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"A survey of nearly ninety aerophilous and freshwater collections from four islands (Kauai, Oahu, Maui and Hawaii) showed the presence of six new species of the genus *Gomphonema* Ehrenberg. Some of these species are new interpretations of taxa previously reported from the Hawaiian Islands, others are new discoveries. We present descriptions of these new taxa based on light and scanning electron microscopy. The diversity of this genus as a proxy for the freshwater diatom flora of the Hawaii is discussed, in the context of a new three-year, NSF-supported biotic survey of the freshwater algae of the Hawaiian Islands. Based on preliminary observations of this and other freshwater raphid diatom genera, it would appear that the Hawaiian Islands support an endemic flora of freshwater diatoms far greater than previous reports would indicate. "
(a) University of Colorado, Boulder, Museum of Natural History (b) Bowling Green State University, Bowling Green, OH, Department of Biological Sciences (c) University of Hawaii, Manao, HI, Department of Botany

P05041 "*Licmophora* (Bacillariophyta) species in coral reef habitats of Guam, Western Pacific"
Ngirairikl, Isumechraard K.-presenter isu_09@yahoo.com(a) Lobban, Christopher (a)
"Biodiversity of benthic marine diatoms in the tropical Pacific Islands is poorly known, mostly from occasional sampling. Diatoms have recently been shown to be important in the diet of certain damselfish that cultivate algal 'farms' in coral reef habitats. Species in the genus *Licmophora* are among the most common components of these communities. To date, there has been no study of tropical *Licmophora* species comparable to Honeywill's

(1998) account of the *Licmophoras* of Britain. Species in the genus are easily recognized by the shape of the cell and the formation of colonies attached by mucilage pads or branched stalks, although in the same habitats are some similar species of *Podocystis* and *Ardissonea*. Fine structural elements of *Licmophora* such as the number of slits in the foot pole multiscissura, the number and location of rimoportulae, head pole spines, and the characters of the areolae define many species. So far we have documented at least 15 species of *Licmophora* in Guam, including several apparently new to science, a total similar to the British flora but large compared to tropical records. The objective of this project is to document and identify all *Licmophora* species found on Guam using light microscopy to study live cells and acid cleaned material, supplemented by scanning electron microscopy. Known species positively identified to date include *L. flabellata*, *L. remulus*, *L. ehrenbergii*, *L. gracilis*, and *L. proboscidea*. Among the putative new species are (1) *ripple fan*, which forms tightly adherent fans of extremely long, fragile, spatulate cells; (2) *curved* which is reminiscent of *L. normaniana*; (3) *wavy*, which has a distinct wave in the narrow lower part of the valve; and (4) *deep-bloomer*, a large species with very large rimoportulae. "
(a) University of Guam- College of Natural and Applied Sciences

P05042 – See PSA Talk on Monday, July 20 – Algal Phylogenetics & Taxonomy III

P05043 "'Birds of a Feather': Molecular Phylogenetic Analyses and Novel Life History Interpretations Ally *Colaconema subimmersum* and *Halosacciocolax kjellmanii* with the Rhodophysemataceae (Palmariales, Rhodophyta)."
Clayden, Susan L.-presenter susan.clayden@unb.ca(a) Saunders, Gary W. (a)
"*Colaconema subimmersum*, currently but incongruously a member of the Colaconematales, and *Halosacciocolax kjellmanii*, tenuously allied with the Rhodophysemataceae, Palmariales, were assessed with respect to their molecular phylogenetic placement, and associated life history patterns. Anomalous features *C. subimmersum* displays relative to other Colaconematales include lack of monosporangia and a reportedly diphasic life history consisting of gametophytic and carpotetrasporophytic generations. *Halosacciocolax kjellmanii* is tentatively assigned to the Rhodophysemataceae based on vegetative structure and reproduction. Lund documented tetrasporangia and spermatangia for the type, but not carpogonia, and the life history remains unknown. We addressed the uncertainty surrounding these taxonomic placements by sequencing large subunit rDNA (LSU) and the mitochondrial cytochrome C oxidase subunit I (COI) in the context of broader representation for members of the Colaconematales, Palmariales, and related Acrochaetiales. *Colaconema subimmersum* resolved as sister to *Rhodophysema* spp. in separate and combined analyses of these gene regions, consistent with inclusion in the Rhodophysemataceae. Our interpretation of direct development of a tetrasporophyte, rather than carpotetrasporophyte, from the fertilized carpogonium indicates a life history that is intermediate between the *Rhodophysema* and *Palmaria* types in support of generic status for *C. subimmersum* within this family. *Halosacciocolax kjellmanii* resolved within the genus *Rhodophysema* and, despite its parasitic nature, we assign it to the genus *Rhodophysema*. Tetrasporangia, spermatangia, and carpogonia - previously unreported, on the same thallus, suggest a *Rhodophysema* type life history in support of the molecular data."
(a) University of New Brunswick

P05044 "Are you the real *Nitella flexilis* (L.) Ag., em.?"
Meyer, Heather M (a,b) Karol, Kenneth G-presenter kkarol@nybg.org(a)
"In 1965 R. D. Wood and K. Imahori published A Revision of the Characeae, a global monograph of the fresh water green algal family Characeae. In this monograph, a broad morphological species concept was used to reduce more than 500 named species to approximately eighty loosely defined species, each with numerous subspecies, varieties and forms. Currently, we are using molecular phylogenetic methods in combination with vegetative morphology and oospore membrane architecture to test and revise this classification. For this study we present findings for *Nitella flexilis*, which includes more than thirteen previously recognized species either as synonyms or subspecific taxa. Chloroplast sequence data (*rbcL* and *atpB*) reveal that *N. californica*, *N. capitata*, *N. mexicana*, *N. mirabilis*, *N. missouriensis* and *N. opaca* each form distinct clades separate from *N. flexilis sensu stricto*. Gross morphological characters and oospore membrane architecture are consistent with these findings. Taken together, the taxa examined here appear to warrant species status separate from, but closely related to, *N. flexilis*."
(a) The New York Botanical Garden, Cullman Systematics Program (b) Sarah Lawrence College, Department of Biology

P05045 "A molecular *rbc*L-based phylogeny of *Champia* (Rhodymeniales, Rhodophyta), including the description of a new monoecious species from the Carribbean "
Schmidt, William E-presenter wes4500@louisiana.edu(a) Fredericq, Suzanne (a) Norris, James N (b)
"The red algal genus *Champia* Desvaux (1809) is widely distributed with representatives in tropical to cold temperate waters. Whereas the taxonomy of the genus is well characterized based on a long history of developmental and morphological studies conducted on *Champia parvula* (C. Agardh) Harvey, and on the type *Champia lumbricalis* (L.) Desvaux from South Africa, species concepts and relationships within the genus are in need of critical investigation. This study investigates *Champia* samples collected from multiple locations throughout the Gulf of Mexico and the Caribbean Sea using both morphological and chloroplast-encoded *rbc*L sequence data. The results indicate that species diversity and range extensions are greater than were previously reported. A new monoecious species from Caribbean Panama and Belize is discussed in light of the prevalent dioecious condition reported for the other species of *Champia*."
(a) University of Louisiana at Lafayette (b) Smithsonian Institution

P05046 A preliminary multigene phylogeny for the Chrysophyte algae
Julius, Matthew M-presenter mljulius@stcloudstate.edu(a) Lindgren, Rachel (a) Stepanek, Joshua (a) Hoffer, Jeannette (a) Conroy, Kathryn (a) Lingle, Kristin (a)
"Unlike other heterokant algae, the monophylly of the synurophytes and chrysophytes has not been obvious to phycologists studying this collection of taxa. A collaborative effort to resolve the evolutionary relationships of heterkant algae is currently ongoing. A byproduct of this project is a focus on the systematic relationships of synurophyte and chrysophyte taxa. Ultimately, seven genes for 50+ taxa will be used to produce this phylogeny. This study represents a hallmark interval towards this goal. The results for three genes for this taxic collection are presented. Results from the investigation support Andersons separation of the synurophytes from the chrysophytes, but corroborate very few other aspects the existing taxonomic scheme. At best historical taxonomic categories represent paraphyletic transitions on the larger evolutionary tree. Where possible, morphological features have been entered into cladistic data matrices to evaluate the legitimacy of diagnostic features in distinguishing Family level and below taxonomic categories. Overall, these results suggest the need for additional development for a detailed morphological data set useful in reflecting homologous states within the chrysophyte lineages."
(a) St. Cloud State University

P05047 New insights in the systematics of the Dumontiaceae-complex (Rhodophyta)
Fredericq, Suzanne-presenter slf9209@louisiana.edu(a) Krayesky, David (a) Freshwater, Wilson (c) Lopez-Bautista, Juan M. (d) Cho, Tae Oh (e) Norris, James N. (f) Hommersand, Max H. (b)
http://youtube.com/nemastoma2
"Two newly reported genera for the Gulf of Mexico that are currently placed in the marine red algal family Peyssonneliaceae, *Polystrata* and *Metapeyssonnelia*, are instead nested inside the Rhizophyllidaceae of the Dumontiaceae-complex as inferred from chloroplast-encoded *rbc*L and nuclear LSU rDNA sequence analyses. The Rhizophyllidaceae is a newly reported family for the Gulf of Mexico, with six species occurring in the region. The basis for interpreting morphological evolution in the Dumontiaceae-complex will be illustrated and discussed within a phylogenetic framework."
(a) University of Louisiana at Lafayette (b) University of North Carolina at Chapel Hill (c) Center for Marine Science, UNCW (d) The University of Alabama (e) Chosun University (f) Smithsonian Institution

P05048 "Preliminary assessment of marcoalgal diversity in Bocas del Toro, Caribbean Panama"
Norris, James N-presenter norrisj@si.edu(a) Wysor, Brian (b) Freshwater, D. Wilson (c) Fredericq, Suzanne (d)
"A PRELIMINARY ASSESSMENT OF MACROALGAL DIVERSITY IN BOCAS DEL TORO, CARIBBEAN PANAMA >Brian Wysor, D. Wilson Freshwater, Suzanne Fredericq & James N. Norris > The Bocas del Toro province, Republic of Panama, hosts a complex of diverse habitats, from coral and sponge reefs, to seagrass meadows and mangrove cays that yield a species rich marine flora and fauna. Recent investigations have uncovered a biota, although similar to Caribbean Islands, may be more speciose than other, better-studied areas. Based on our preliminary recent collecting efforts, we estimate macroalgal species richness for Bocas del Toro region to be very high. The high diversity is not surprising given the short history of marine botanical investigations in the region. > Currently a conservative estimate indicates there are 151 red, 76 green and 32 brown algal species. Our initial studies of Bocas del Toro intertidal and subtidal algae have resulted in one published new species (Gavio & Fredericq 2003), many new distribution records for Panama, numerous tentative new species, and revealed that much of the marine floristic diversity for Caribbean Panama is represented by species throughout this unique region. φ"
(a) Smithsonian Institution (b) Roger Williams University (c) University North Carolina Wilmington (d) University Louisiana Lafayette

P05049 "New insights in the systematics of *Peyssonnelia* and the Peyssonneliaceae (Rhodophyta), with emphasis on taxa from the Gulf of Mexico and Panama"
Krayesky, David M-presenter dkrayesky@yahoo.com(a) Norris, James N (b) Paul, Gabrielson W (c) Gabriel, Daniela (a) Fredericq, Suzanne (a)
http://youtube.com/nemastoma2
"*Peyssonnelia* Decaisne comprises a worldwide group of non-calcified or calcified, crust-forming red algae of great ecological significance, with some species involved in the establishment of rhodoliths. Of the eight genera currently recognized in the family, *Peyssonnelia*, is widely viewed to contain the largest number of species. The number of distinct species of Peyssonneliaceae present in the Gulf of Mexico has increased from 6 to 21. Comparative morphology, chloroplast-encoded *rbc*L and nuclear LSU rDNA sequence data suggest that species of *Peyssonnelia* do not occur in the Gulf of Mexico, and that previously reported *Peyssonnelia* species for the region actually belong to other genera of the Peyssonneliales."
(a) University of Louisiana at Lafayette (b) Smithsonian Institution (c) University of North Carolina

P05050 Comparative genomics of photosynthetic Heterokonts
phillips, naomi-presenter phillipsn@arcadia.edu(a) Braun, Ed (b) Moustafa, Ahmed (d) Bhattacharya, Debashish (d) Kapraun, Don (c) calhoun, sam (a) coaxum, teresa (a)
"Heterokonts (also called stramenopiles) comprise a monophyletic assemblage united by a shared endosymbiotic history that either exhibit a biflagellate heterokont condition (have distinct anterior and posterior flagella) or have heterokont ancestors. The group contains an enormous amount of biodiversity, with millions of species that can be divided into approximately 14 major lineages. The majority of these lineages are small unicells or simple filaments, but there is one lineage (the brown algae; Phaeophyceae) characterized by large multicellular thalli rivaling green plants in size and complexity. Despite the diversity of the heterokonts only a small number of genome sequences are currently available, especially for the photosynthetic heterokonts. We are interested in expanding the current dataset as efficiently as possible, so we are using relatively inexpensive methods (454 pyrosequencing and EST acquisition) to illuminate relationships among heterokont lineages and examine changes to genomic content that occurred during the shift to complex morphological and life histories in the brown algae. We present data on genome sizes as well as ESTs and 454 data from various heterokont lineages with an emphasis on the brown algae and its closest sister groups. "
(a) Arcadia University (b) University of Florida (c) University of North Carolina (d) University of Iowa

P05051 Detecting marine aliens: applying herbarium collections to investigate the genera *Ulva* and *Codium*
Maggs, Christine A-presenter c.maggs@qub.ac.uk(a) Mineur, Frederic (a)
"We show how using herbarium specimens both for morphological and molecular investigations can elucidate invasions by cryptogenic aliens, cryptic species and other algae responsible for taxonomic nightmares. DNA has been amplified from herbarium specimens up to 150 years old, in various herbaria. In Codium fragile, different subspecies are native to particular areas of the world and there is a single invasive subspecies. In Ulva, several clades are seen to be invasive, with some haplotypes becoming globally distributed and forming green tides. Some invasive clades are also associated with hull fouling, perhaps explaining how these have become global in extent. Morphological characters are misleading for identification and there seems little alternative but to use molecular data to identify taxa, especially those constituting green tides. "
(a) School of Biological Sciences, Queen's University Belfast, Belast BT9 7BL, Northern Ireland

P05052 "Study of a cyanobacterium from thermal, saline waters with relatives from unexpected habitats"
Banerjee, Meenakshi-presenter meenakshi.banerjee@rediffmail.com(a) Castenholz, Richard (b)
"Thermophilic cyanobacteria (i.e. growth > 45 0 C) that also grow at and above seawater salinity (i.e.>30-33 g L-1 TDS) have rarely been reported and studied. In the present study, a cyanobacterium that constitute this rare type of extremophile were isolated from a submerged siliceous crust at 40-45 0 C in a geothermal lagoon of altered seawater in southwest Iceland. Iceland Clone 2e, a Leptolyngbya morphotype, that barely survived in freshwater medium at 23 0-50 0 C or in saline medium at 23 0 C. At 45 0 C-50 0 C, in medium ranging from 28 to 94 g L-1 TDS, it grew with a maximum rate of over 3 doublings 24 h-1 under continuous illumination. This rate decreased by about one third at 54 0 C, and death occurred at 58 0 C. A comparison of the partial 16S rDNA sequence with others in the database revealed two apparent relatives in culture (>99% similarity) from slightly saline Greenland hot springs (1.3-1.6 g L-1 TDS). Three other similar sequences (93-98% similarity) were from periodically dry, endolithic habitats in Yellowstone National Park. All six formed a distinct phylogenetic clade, suggesting their common ancestry. In a search for phenotypic

similarities, the two Greenland Leptolyngbya-like strains grew best in moderately saline medium (28 g L-1) at 45 0 C and 50 0 C, but neither tolerated 54 0 C. Two of the three-endolithic Leptolyngbya isolates, grew well in saline medium at 50 0 C, but, remarkably, not in freshwater medium at 50 0 C. "
(a) Department of Biosciences,Barkatullah University (b) Center for Ecology and Evolutionary Biology,University of Oregon, Eugene, Oregon

P05053 Unlocking the mysteries of plastid establishment through comparative genome analysis of two *Paulinella* species
Yoon, Hwan Su-presenter hsyoon@bigelow.org(a) Yang, Eun Chan (a) Andersen, Robert A (a) Reyes-Prieto, Adria (b) Bhattacharya, Debashish (b)
http://www.bigelow.org/
"The acquisition of photosynthesis by eukaryotes was a pivotal event in evolution because algae and plants now form the base of the food chain for life on Earth. Plastids are the machines of photosynthesis in algae and plants and have a cyanobacterial origin through a single primary endosymbiotic event. Our knowledge of plastid evolution in algae and plants is still limited because the event occurred more than a billion years ago. Remarkably, a second primary endosymbiotic event has recently been uncovered. *Paulinella chromatophora*, a thecate amoeba, has a plastid that is only a few million years old. The plastid(s) in *Paulinella* arose from a separate, independent primary endosymbiosis involving a cyanobacterium related to *Prochlorococcus* and *Synechococcus*. The closely related *P. ovalis* lacks a plastid but feeds actively on cyanobacteria. Using these two model organisms (*P. chromatophora* and *P. ovalis*), we are sequencing genomic DNA and generating a transcriptome database from *P. chromatophora* strain FK01 and *P. ovalis* using the most modern pyrosequencing methods and comparing the gene inventories between *P. chromatophora* and *P. ovalis*. Genome comparisons between the photoautotrophic *P. chromatophora* and the heterotrophic *P. ovalis* will allow us to generate a gene/genome catalogue before and after the primary endosymbiotic event. We expect to gain insights into the initial genomic innovations that allowed the establishment of a new organelle; i.e., the critical process that allowed a transition from heterotrophy (animal) to autotrophy (plant). These genomic data could also be valuable to the field of genetic engineering."
(a) Bigelow Laboratory for Ocean Sciences (b) University of Iowa

P05054 DNA barcoding reveals massive diversity of dinoflagellates in marine envrionments
Stern, Rowena F-presenter rstern71@yahoo.com(a) Horak, Ales (a) Andrew, Rose (a) Keeling, Patrick J (a)
"The species concept in a taxonomically and ecologically diverse group as the Dinoflagellates species varies widely and many are difficult to identify. As such, many species have eluded classification and the real levels of their diversity is uncertain. DNA barcoding uses a short DNA marker as a proxy for species identification and has been utilized successfully for many protist groups. For the first time, this study has made a comprehensive database of nearly 360 DNA barcodes from described species in eleven different algal culture collections using the DNA marker, cytochrome *c* oxidase I (*COI*). This marker could identify almost 230 strains including 67 with no species or, for *Symbiodinium*, no clade classification. *COI* did not work some genera, most notably *Alexandrium*. Intra and Inter specific variation were largely similar to those of Cytochome Oxidase *b* marker for many species, recently studied in dinoflagellates by Lin *et al.*,2009. This database of barcodes was used to compare with approximately 700 environmental barcodes from three environments,including single cell barcodes. This revealed a huge diversity of unknown taxa and also an explosion in small genera, particularly in those in *Karenia* and *Karlodinium*. We also found depth related separation of dinoflagellate species. These results highlight the extent of unknown dinoflagellate diversity in the environment and their lack of representation in culture collections. This has wider implication for estimating protist diversity and their ecological impact."
(a) University of British Columbia, Canada

P05055 – See PSA Talk on Monday, July 20 – Algal Phylogenetics & Taxonomy III

P05056 Molecular divergence between Northwestern Atlantic populations of *Colpomenia sinuosa*
Parente, Manuela I-presenter nelaparente@hotmail.com(a,b) Costa, Filipe (b) Saunders, Gary W (a)
"*Colpomenia sinuosa* (Mertens ex Roth) Derbes et Solier is characterized by a spherical saccate thallus, plurilocular sporangial in punctuate sori with a cuticle, and commonly four to six layers of medullary cells. This species is generally reported to be widely distributed in tropical to warm temperate seas throughout the world. Morphological and molecular examinations were performed using isolates from different regions including the Macaronesian archipelagos, the Azores, Madeira and the Canary Islands, as well as the central coast of mainland Portugal. Cytochrome c oxidase 1 sequences, which are reported to resolve species-level diversity among brown algae, displayed 1.23 % divergence (7 of 566 bp) between isolates from the Macaronesian archipelagos and mainland Portugal suggesting a period of genetic isolation and possibly speciation. However, the low divergence between these populations in COI necessitates confirmation with a second, preferably nuclear (assess introgression and hybridization), marker. We thus generated nuclear ribosomal internal transcribed spacer (ITS) data for some of our isolates. Our preliminary results will be presented. . "
(a) 1Centre for Environmental & Molecular Algal Research, Biology, University of New Brunswick (b) Departamento de Ciencias e Engenharia do Ambiente Instituto do Mar IMAR, Faculdade de Ciencias e Tecnologia, Universidade Nova de Lisboa

SESSION P06 – ALGAL PHYSIOLOGY AND BIOCHEMISTRY

P06001 – See PSA Bold Talk on Saturday, July 18

P06002 – See PSA Talk on Monday, July 20 – Algal Ecology & Physiology

P06003 Laboratory and field studies of photoadaptation in the invasive red macroalga *Gracilaria salicornia*
Spafford, David C-presenter dspaffor@gmail.com(a)
"Mounds of *Gracilaria salicornia* thalli from Waikiki and Kaneohe Bay, Oahu, Hawaii show distinct yellow, green and red regions that were characterized using the University of Hawaii Marine Botany Laboratory pulse amplitude modulated (Diving PAM) fluorometer (photo system II electron transport activity), dissolved oxygen electrodes (O_2 evolution during photosysthesis), in vivo absorption spectrophotometer (relative pigment signatures), and diode array spectrophotometer (absolute chlorophyll quantification). *G. salicornia* photosynthetic adaptations contribute to its apparent competitive advantage over native species. Laboratory results compared with field data collected with the Diving PAM using SCUBA over a 10 meter depth gradient support the Diving PAM accuracy and usefulness as a rapid survey method. Facing the challenges of global warming,

eutrofication, and sedimentation, Diving PAM may allow marine resource managers and scientists to quickly and efficiently estimate the photophysiological status of marine flora."
(a) Univ of Hawaii at Manoa Botany Department

P06004 – See PSA Talk on Monday, July 20 – Algal Ecology & Physiology

P06005 – See PSA Bold Talk on Saturday, July 18

P06006 – See PSA Talk on Monday, July 20 – Algal Ecology & Physiology

P06008 – See PSA Talk on Monday, July 20 – Algal Ecology & Physiology

P06009 A PsbP-like protein (PsbP2) is required for singlet oxygen signaling and high-light growth in the green alga *Chlamydomonas reinhardtii*
Brzezowski, Pawel (a) Ken, Wilson-presenter ken.wilson@usask.ca(a)
"Photosynthetic organisms are particularly susceptible to photooxidative stress, because they are dependent on light for energy generation and generate high levels of oxygen in the chloroplast. While photosynthetic eukaryotes exhibit altered nuclear gene expression in response to changes in the production of reactive oxygen species in the chloroplast, little is known how this signal is transmitted from the chloroplast to the nucleus. Using a strain of the green alga *Chlamydomonas reinhardtii* harboring a singlet oxygen sensitive promoter construct, we performed a secondary mutagenic screen and identified mutant cell lines that are unable to regulate nuclear gene expression in response to singlet oxygen production. One of the mutants was shown to contain an insertion in the first exon of the *PsbP2* gene. This gene is part of a small gene family in *C. reinhardtii* that is conserved in higher plant species. The founding member of the gene family *PsbP1* (*Oee2*) is required for the assembly of the oxygen evolving complex of photosystem II. In *Arabidopsis*, other members of this gene family are involved in the assembly of photosynthetic complexes. In addition to disrupting the singlet oxygen signaling system, deletion of the *PsbP2* gene makes the cells sensitive to growth under high light. The role of the PsbP2 protein in singlet oxygen signaling and protection of the photosynthetic apparatus will be discussed."
(a) Department of Biology, University of Saskatchewan

P06010 – See PSA Talk on Monday, July 20 – Algal Ecology & Physiology

P06011 – See PSA Talk on Monday, July 20 – Applied Phycology II

P06012 The Physiological Effect of Submarine Groundwater Discharge on the Hawaiian endemic edible alga *Gracilaria coronopifolia*
Amato, Daniel W-presenter dwamato@hawaii.edu(a)
"Submarine groundwater discharge (SGD) is a significant source of nutrients to many coastal environments. The difficulty of experimental manipulation *in situ* has resulted in little evidence for a causal relationship between SGD and coastal biological processes. In this study, we examined the physiological response of the endemic edible Hawaiian rhodophyte, *Gracilaria coronopifolia*, to varied levels of simulated SGD in a laboratory setting. Forty-eight thalli were grown in a unidirectional flow-though mesocosm at 25oC and 250 µ;M $m^{-2}s^{-1}$ PAR during two replicate trials. To simulate increasing levels of SGD, four treatments, ranging from high salinity / low nutrient to low salinity / high nutrient water, were established. Treatment levels were determined from empirical relationships among salinity, nitrate, and phosphate from known sites of SGD near Kona, Hawaii. After 16 days, the growth rate, apical tip development (Tip Score and Tip Index), and photosynthetic parameters ($rETR_{max}$,α, I_k) via Pulse Amplitude Modulated (PAM) fluorometry were determined. The 27‰ SGD treatment (26.60µ;M-N, 1.10 µ;M-P, 27‰ salinity) had higher mean values for all parameters measured. The mean growth rate in the 27‰ SGD treatment (3% day^{-1}) was 3-fold greater than controls (0.20 µ;M-N, 0.05 µ;M-P, 35‰ salinity). $rETR_{max}$, I_k), Tip Index and growth rate were significantly greater in the 27‰ SGD treatment compared to control conditions. The parameters Tip Score and Tip Index are an informative and novel method for quantifying new algal apical tip development. The results of this study indicate moderate levels of SGD input to the coastal environment may increase the growth rate, apical tip development, and photosynthetic performance of *G. coronopifolia* on otherwise oligotrophic Hawaiian reefs."
(a) University of Hawaii at Manoa

P06013 The Physiological Effects of Submarine Groundwater Discharge on the Hawaiian Endemic Edible Alga *Gracilaria coronopifolia*
Amato, Daniel W-presenter dwamato@hawaii.edu(a)
"Submarine groundwater discharge (SGD) is a significant source of nutrients to many coastal environments. The difficulty of experimental manipulation *in situ* has resulted in little evidence for a causal relationship between SGD and coastal biological processes. In this study, we examined the physiological response of the endemic edible Hawaiian rhodophyte, *Gracilaria coronopifolia*, to varied levels of simulated SGD in a laboratory setting. Forty-eight thalli were grown in a unidirectional flow-though mesocosm at 25°C and 250 µ;M $m^{-2}s^{-1}$ PAR during two replicate trials. To simulate increasing levels of SGD, four treatments, ranging from high salinity / low nutrient to low salinity / high nutrient water, were established. Treatment levels were determined from empirical relationships among salinity, nitrate, and phosphate from known sites of SGD near Kona, Hawaii. After 16 days, the growth rate, apical tip development (Tip Score and Tip Index), and photosynthetic parameters ($rETR_{max}$,α, I_k) via Pulse Amplitude Modulated (PAM) fluorometry were determined. The 27ppt SGD treatment (26.60µ;M-N, 1.10 µ;M-P, 27ppt salinity) had higher mean values for all parameters measured. The mean growth rate in the 27ppt SGD treatment (3% day^{-1}) was 3-fold greater than controls (0.20 µ;M-N, 0.05 µ;M-P, 35ppt salinity). $rETR_{max}$, I_k, Tip Index and growth rate were significantly greater in the 27ppt SGD treatment compared to control conditions. The parameters Tip Score and Tip Index are an informative and novel method for quantifying new algal apical tip development. The results of this study indicate moderate levels of SGD input to the coastal environment may increase the growth rate, apical tip development, and photosynthetic performance of *G. coronopifolia* on otherwise oligotrophic Hawaiian reefs."
(a) University of Hawaii at Manoa

P06014 Towards unraveling sub-proteomic changes of cyanobacterium *Synechocystis* sp. Strain PCC 6803 in response to high-pH stress
Huang, Fang-presenter fhuang@ibcas.ac.cn(a) Zhang, Lifang (a) Norling, Birgitta (b)

"Towards unraveling sub-proteomic changes of cyanobacterium Synechocystis sp. Strain PCC 6803 in response to high-pH stress Zhang Lifang[1], Norling Birgitta [2] and Huang Fang[1]* 1 Research Center for Photosynthesis, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China, 2 Department of Biochemistry and Biophysics, Stockholm University, SE-10691, Stockholm, Sweden Cyanobacteria are ancient and the only prokaryotic organisms carrying out oxygenic photosynthesis. During long evolution cyanobacteria have adapted to all photic habitats with a remarkable capacity to survive in adverse environments including high pH stress. The molecular adaptation mechanisms underlying in cyanobacterial cells, however, remain to be clarified. In this study, we investigated how *Synechocystis* sp. Strain PCC 6803, a model cyanobacterium, responds to high pH stress. 2D-gel profiles of soluble and membranes fractions isolated from control (pH 7.5) and high pH-treated (pH 11.0) cells were constructed and compared quantitatively based on Coomassie Brilliant Blue staining as well as DIGE analysis. In our preliminary study, we have identified 39 differentially expressed proteins from the plasma membranes of *Synechocystis* using MALDI-TOF MS and MALDI-TOF/TOF MS coupled with database search. Of those identified proteins, 25 were enhanced/induced and 14 reduced by high pH stress. Approximately one-third of the up-regulated proteins are substrate binding proteins of ABC transporters. Eight hypothetical proteins were for the first time found expressed and involved in pH homeostasis in cyanobacteria. The physiological significance of the high-pH stress proteins is to be discussed. e-mail address of presenting author: fhuang@ibcas.ac.cn "
(a) Institute of Botany, Chinese Academy of Sciences, P. R. China (b) Department of Biochemistry and Biophysics, Stockholm University, Sweden

P06015 Light response of Photosystem II photochemistry in *Isochrysis galbana*
Obata, Mitsuko -presenter mobata@soka.ac.jp(a) Yamamoto, Shuichi (a) Taguchi, Satoru (a)
"Steady-state chlorophyll *a* (Chl *a*) fluorescence parameters including the maximum quantum yield (Fv/Fm), the quantum yield (Fv'/Fm'), the photochemical quenching (qP), and the effective quantum yield (ΔF/Fm') were assessed to investigate light response of photosystem II (PSII) photochemistry using *Isochrysis galbana* cultures that had been acclimated at four different photon flux density (growth PFD) levels. These parameters were measured by pulse-amplitude-modulation (PAM) fluorometory. The value of Fv/Fm was determined under dark condition while Fv'/F m', qP, and ΔF/Fm' were obtained by illuminating subsamples at step-wise increasing intensity of actinic light up to 2038 µ;mol photons m-2 s-1. The Fv'/Fm' was relatively constant to changes in the actinic PFD regardless of growth PFD and their levels decreased from 0.74 to 0.56 with growth PFD. The value of Fv'/Fm' was determined by amount of functional PSII and non-photochemical quenching involving thermal dissipation at PSII antenna. Since the level of Fv'/Fm' was very similar to the value of Fv/Fm at all growth PFD, the reduction of Fv'/Fm' with increasing growth PFD was due to loss of functional PSII rather than non-photochemical quenching. In contrast to Fv'/Fm', curvilinear decreases of qP and ΔF/Fm' with actinic PFD was observed regardless of growth PFD. Since the ΔF/Fm' = qP χ Fv'/Fm', the decline in ΔF/Fm' with increasing actinic PFD was due to redox state of PSII reaction centers rather than loss of functional PSII. The results obtained in the present study suggested that the proportion of absorbed energy being used in PSII electron transport of *I. galbana* depend on the short-term change in incident PFD and may be determined by redox state of PSII reaction centers."
(a) Soka University

P06016 Absoptance and estimates of photosynthetic performance in marine macroalgae.
Beach, Kevin S-presenter kbeach@ut.edu(a) Borgeas, Heidi B (a) Meyer, Kevin (a,c) Smith, Celia (b)
"*In vivo* transmittance and reflectance spectra from 280 to 700 nm were obtained from fourteen species for macroalgae in order to quantitatively compare to what extent species from different phyla, of dissimilar functional forms and with various cell wall constituents, absorb incident irradiance. Absorptance of PAR ranged from 64 to 92%. Irradiance reflected from the surface of macroalgae accounted for as little as 7% and as much as 23% of incident PAR. Reflectance needs to be quantified for accurate estimates of absorptance, comparable estimates of quantum yield via oxygen evolution and electron transport rates via chlorophyll fluorescence. In order to test this, oxygen evolution and PAM fluorometry were both used to estimate photosynthetic performance of *Caulerpa prolifera*. We took into consideration the light interval for PAM rapid light curve (RLC), algal absorbtance, and normalization routines. It was determined that the highest correlations were found when parameters were normalized to total chlorophyll levels, compared with algal fresh weight, dry weight, and total pigment levels, and determined using a model that incorporates photoinhibition. Surprisingly, the default absorbtance setting of 0.84% yielded stronger results than actual absorbtance values calculated for every sample due to high absorbtance variance between samples that was not related to photosynthetic performance. Regression analyses determined that the strongest relationship between PAM and O2 evolution was for parameters of ETRmax (60s light interval) and Pmax respectively. All other nonlinear parameters and light intervals were found to demonstrate a high degree of underestimation by PAM. "
(a) University of Tampa (b) University of Hawaii (c) University of Maryland

P06017 PSII efficiency and NO3 uptake dynamics of dinoflagellate *Alexandrium tamarense*
Murata, Ai-presenter amurata@soka.ac.jp(a) Taguchi, Satoru (a)
"We examined maximum PSII quantum efficiency (Fv/Fm), effective PSII quantum efficiency (ΔFv/Fm'), nitrate (NO3) uptake rate of dinoflagellate *Alexandrium tamarense* under five different NO3 concentrations from 6µ;M-NO3 to 100µ;M-NO3 by semi-continuous experiment. The Fv/Fm, ΔFv/Fm' and cellular NO3 uptake rate under 6µ;M-NO3 was highest among all culturing condition, and decreased with increasing NO3 concentration. The significant positive relationship between Fv/Fm or ΔFv/Fm' and cellular NO3 uptake rate was obtained in the present study. This result suggested that NO3 uptake rate of *A. tamarense* might be controlled by PSII efficiency. In general, when they grow under different NO3 concentrations, they take up NO3 and reduce NO3 by using 10 electrons which is produced at PSII. If *A. tamarense* takes up more NO3, they should produce more electrons and utilize the electrons for the reduction from NO3 to NH4. At low NO3 concentration, *A. tamarense* could demand more nitrogen than the cells at high NO3 concentration to maintain their continuous growth. Therefore, the PSII efficiency of dinoflagellate *A. tamarense* should be high at low NO3 concentration. The present study suggests that the changes of NO3 concentration may determine the PSII efficiency which affects the NO3 uptake dynamics of dinoflagellate *A. tamarense*."
(a) Soka University

P06018 Potential photosynthetic efficiency of expelled zooxanthellae from jellyfish *Cassiopea* KB8
Fuchinoue, Yumi-presenter leo-yumi-811@hotmail.co.jp(a) Murata, Ai (a) Kurosawa, Norio (a) Taguchi, Satoru (a)
"Global warming is one of possible causes for release of zooxanthellae from host animals. Recovery of host animals may be dependent upon how animals successfully recruit free living zooxanthellae cells from ambient environments into their animal tissues. When free living zooxanthellae cells are engulfed into animal tissues, the relaxation from nutrient exhaustion may be occurred to the cells. It is still unknown how zooxanthellae response to this process. Zooxanthelle cells isolated from host animals: jellyfish *Cassiopea* KB8 (clade: A) were grown at 25Co , 35 PSU salinity, and 300µ;mol photons m-2 s-1 of cool fluorescent light on 12h light :12h dark cycle under 50µ;M NH4 to harvest enough cells for further experiments. Harvested cells were transferred to low nitrogen condition (less than 10µ;M) in triplicate for 10 days. To determine the relaxation to ammonium replete conditions, subsamples from days 1, 5, 10 were transferred to incubate further in 50µ;M NH4 condition for 3 days. The photochemical efficiency (maximum PS II efficiency: Fv/Fm, electron transport rate in PS II: ETR) were measured by a Water-PAM chlorophyll fluorometer (Heinz Walz GmbH)

during experiments. The value of Fv/Fm in zooxanthellae were approximately 0.50 under low NH4 condition for 1 week. When cells were transferred to NH4 relaxation condition, Fv/Fm were also remained constant at approximately 0.50. In addition, ETR was increased with actinic light, and the response of ETR to the light did not show any differences between low NH4 and relaxation conditions. Those results suggested that expelled zooxanthellae could be maintained potential photosynthesis efficiency at least 1 week under low NH4 environment."
(a) Soka University

P06019 Electron transport rate of three phytoplankton species under blue light conditions
Gorai, Takako-presenter gorai2030@yahoo.co.jp(a) Mitsuko , Obata (a) Ai, Murata (a) Satoru, Taguchi (a)
"Electron transport rate (ETR) of photosystem II (PSII) under blue light condition was investigated in Bacillariophyceae *Phaeodactylum tricomutum*, Haptophyceae *Isochrysis galbana*, and Chlorophyceae *Dunaliella salina* that had been acclimated at 60 µ;mol photons m-2 s-1 under blue (400-550 nm) and white (400-700 nm) light condition (as a control). The ETR were calculated by consideration of both effective quantum yield (ΔF/Fm') and chlorophyll *a* absorption coefficient under each light condition (*a**[400-550] and *a**[400-700]). The value of ΔF/Fm' was obtained by illuminating subsamples at 60 µ;mol photons m-2 s-1 using pulse-amplitude-modulation (PAM). The chlorophyll *a* absorption coefficient under blue (*a**[400-550]) and white (*a**[400-700]) light condition were calculated by absorption spectral and chlorophyll *a* concentration. The values of ΔF/Fm' in blue light culture were 0.8-0.9 times lower than that in white light culture in *I.galbana* and *D. salina*, while significantly difference of ΔF/Fm' between blue and white light culture was not observed in *P. tricomutum*. The *a**(400-550) was 2 times higher than *a**(400-700) in three phytoplankton species. The ETR under blue light condition was 1.7-2.0 higher than that under white light condition. These results suggest that the high ETR obtained under blue light condition was due to high efficiency of light absorption. Phytoplankton may be able to photosynthesize more efficiently under blue light condition."
(a) Soka University

P06020 Response of photosynthetic characteristics in PSII and nutrient availability during dark and light conditions in diatom *Thalassiosira weissflogii*
Kaytayama, Tomoyo-presenter tomoponchiki@yahoo.co.jp(a) Murata, Ai (a) Yamamoto, Shuichi (a) Taguchi, Satoru (a)
"The photosynthetic characteristics in photosystem II (PSII) and nutrients availability in diatom *Thalasiosira weissflogii* were examined at dark and light conditions in the present study. Cells were stored in the dark for periods from 1 to 14 days. Samples from days 3, 8, and 14 were returned to light and monitored during a period of 1-120 hours. The photosynthetic characteristics, such as maximum quantum yields (Fv/Fm) in the dark and effective quantum yields (ΔF/F'm) at actinic light of 300 µ;mol photons m-2 s-1 were measured by pulse-amplitude-modulation (PAM). Nutrient concentrations such as silicic acid, nitrate, and phosphate were measured on nutrient auto-analyzer. During the dark storage period, the value of Fv/Fm increased gradually from 0.43 to 0.59 while ΔF/F'm remained virtually constant at 0.15. When returned to light, Fv/Fm and ΔF/F'm increased to 0.62 and to 0.25 regardless of dark storage period and then decreased to 0.41 and 0.058 after 48 hours. Nutrient concentrations remained relatively constant during the dark storage period, and then decreased when the cultures were returned to the light conditions. Especially, silicic acid concentration was depleted after 48 hours. The decreases of Fv/Fm, ΔF/F'm and silicic acid after 48 hours suggested that silicic acid availability may be associated with quantum yields of PSII of diatom *T. weissflogii*"
(a) Soka University

P06021 The brown algal pathogen *Eurychasma dicksonii*: a model to study the evolution of pathogenicity among oomycetes
Strittmatter, Martina (a) Gachon, Claire-presenter cmmg@sams.ac.uk(a) Kuepper, Frithjof (a)
http://www.sams.ac.uk
"Brown algae make up over 70% of the biomass on cold and temperate rocky seashores. The intracellular pathogen *Eurychasma dicksonii* is the most widespread eukaryotic pathogen of marine brown algae, with the broadest host range described so far for marine pathogens. It occurs worldwide in cold and temperate waters. The first-ever genome project on a seaweed (Ectocarpus siliculosus) is an unprecedented opportunity to develop a model interaction with Eurychasma dicksonii, and to study it using state-of-the art molecular approaches. This model has the strength of being both of environmental and fundamental relevance, enabling issues to be addressed ranging from the impact of diseases in marine ecosystems to the evolution of pathogenicity among protists. We have developed a comprehensive laboratory toolbox on this model, and contributed to a detailed description of Eurychasma infection cycle (1). Our results demonstrate, for the first time in brown algae, the existence of a genetically-determined immunity, most probably mediated by programmed cell death and conserved across the whole phylum. We have developed a Real-Time PCR assay that reliably quantifies Eurychasma infection in brown algae and found that various clonal Ectocarpus strains show differential susceptibility towards the oomycete pathogen. This assay is also applicable for the detection of the pathogen in natural brown algal populations (2). Due to its phylogenetic position at the basis of the oomycete lineage, we are currently using Eurychasma dickosnii as a model organism to study the evolution of pathogenicity among oomycetes and chromealveolates. (1) Sekimoto S, et al. (2008). Protist 159: 299-318. (2) Gachon CMM, et al. (2009). Appl Env Microbiol 75: 322-328."
(a) Scottish Association for Marine Science

P06022 The Spectral Dependence in Scattering Properties of Dinoflagellates *Prorocentrum* Species
Motokawa, Shozo-presenter regular.triangle@gmail.com(a) Dairiki, Chieko (a) Murata, Ai (a) Taguchi, Satoru (a)
"Phytoplankton are main primary producers in the ocean and exert an influence on the light scattering of seawater. Incident light into the seawater continue to be scattered still absorbed or available for photosynthesis of phytoplankton. The photosynthesis of phytoplankton depends on the wavelength of light. Cell size is the predominant factors influencing the scattering properties of phytoplankton. In the previous works, there are a few research examined large cell, e.g. dinoflagellates. Understanding the spectral properties of scattering of large cells is prerequisite to develop the bio-optical modeling primary production of water column, particularly in the coastal waters. The spectral dependence in the scattering properties of Dinoflagellates *Prorocentrum micans* (cell volume: 8.44E+03 µ;m3) and *P. minimum* (cell volume: 1.07E+03 µ;m3) under the different nitrate and light conditions was examined. The scattering coefficient (*b*[λ]) at nine wavelengths was calculated as the difference between attenuation and absorption coefficient by using an absorption and attenuation meter (ac-9). With decreasing wavelength, *b*(λ) of *P. micans* and *P. minimum* increased. Scattering coefficient at 555nm was selected as reference because of low absorption. The slope value derived from power approximation analysis of the *b*(λ) normalized to *b*(555) (*b*[λ]/b[555]) versus wavelength was different among each condition. Steeper slope was always observed in large-cell *P. micans* under the similar conditions. These observations indicate that *b*(λ)/*b*(555) of large cells could be more promising variables estimated from wavelengths to extend our understanding."
(a) Soka University

P06023 "Expression pattern of cold stress responsive genes under high concentration of carbondioxide in a freshwater green alga, *Spirogyra varians*"
Han, Jong Won-presenter jwhan@kongju.ac.kr(a) Yoon, Minchul (b) Kim, Gwang Hoon (a)
"Two novel stress-responsive genes were isolated by 2-dimensional gel electrophoresis (2DE) and differentially expressed gene method (DEG) from *Spirogyra varians*. These genes were characterized as an early light inducible protein (ELIP) and a chloroplast targeted transmembrane protein. The full-length cDNA sequences of the genes were obtained using PCR methods with cDNA library. The alga was subjected to various stress conditions such as high-light (1200 µ;mol photon m-2s-1), UV-A (350 nm), Blue-light (470 nm) and low-temperature (below 4 degree) and the expression level of two genes was analyzed. When S. varians was exposed to UV-A, the transcripts of two genes appeared within 3 hours and accumulated in a quantity which was more than 5 times higher in comparison with those in normal light (50 µ;mol photon m-2s-1). Different concentrations of CO2 (1%, 3%, 5%, 10%) were passed into the liquid medium under UV-A stress condition and the corresponding accumulations of stress responsive genes were analyzed using real time PCR and northern blot analysis. The transcription of stress responsive genes decreased in amount or disappeared within 30 minute after the treatment of high concentration of CO2 (>5%). These results implied that the elevated CO2 level may disturb the expression of stress responsive genes in freshwater algae which may alter the adaptability of the alga to changing environment."
(a) Department of Biology, Kongju National University, Kongju, Korea (b) Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup, Korea

P06024 "Differential expression of cytoskeletal genes in *Zygnema cruciatum* (Chlorophyta, Zygnematales) upon cold acclimation"
Kim, Gwang Hoon-presenter ghkim@kongju.ac.kr(a) Han, Jong Won (a) Yoon, Minchul (b)
"The distribution of cytoskeletons changed dynamically at each stage of the cell division in a psychrophilic alga *Zygnema cruciatum*. The actin filaments accumulated at the centre of the nucleus and associated with chromosomes from interphase to telophase. During cytokinesis a ring shaped actin filaments appeared at the cleavage furrow. The microtubules were arranged spirally beneath the protoplasma membrane during the interphase but they were observed only at the nuclear region from prophase to telophase. At the prophase a massive accumulation of microtubules occurred at the nuclear region and it developed into mitotic spindle during the metaphase. During the cold acclimation of *Z. cruciatum* the distribution of microtubules changed. As known, most tubulin dimers cannot polymerize into microtubules at temperatures below 4 degree but the cells of *Z. cruciatum* could divide and grow at this temperature. This ability may require molecular adaptation of the tubulin gene to low temperature. The gene had a typical modified site of amino acid sequence in this psychrophilic species. Northern blot analysis showed that higher transcription of α-tubulin gene occurred at 4 degree than at 20 degree. Interestingly, the transcription level of the β-tubulin and actin gene of *Z. cruciatum* was not affected by the temperature conditions."
(a) Department of Biology, Kongju National University, Kongju, 314-701, Korea (b) Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup, 580-185, Korea

P06025 Copper uptake kinetics of the marine diatoms *Thalassiosira oceanica* and *Thalassiosira pseudonana*
Guo, Jian-presenter jguo@eos.ubc.ca(a) Maria, Maldonado T. (a) Thomas, Ruth J. (b) Katie, Gagnon (c)
"The copper (Cu) uptake kinetics of the marine diatoms *Thalassiosira oceanica* and *Thalassiosira pseudonana* grown under sufficient and limiting Fe and/or Cu conditions followed classical Michaelis-Menten type kinetics. Interestingly, biphasic uptake kinetics as a function of Cu concentrations were observed, suggesting the presence of a high- and a low-affinity Cu transport system. The two Cu transport systems were controlled differently by Fe and/or Cu limitation. The half saturation constants (Km) of the high-affinity transport system were 48.3 nM and 188.4 nM in *T. oceanica* and *T. pseudonana*, respectively; the Km of low-affinity system is ~ 2410 nM in *T. oceanica*."
(a) Department of Earth and Ocean Sciences, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada (b) Tri-University Meson Facility (TRIUMF), Life Sciences Division, 4004 Wesbrook Mall, Vancouver, British Columbia (c) Medical Physics, University of Alberta

SESSION P07 – AGAL SPECIES CONCEPT IN MOLECULAR ECOLOGY

P07001 – See PSA Talk on Monday, July 20 – Algal Cellular & Molecular Biology

P07002 – See PSA Talk on Monday, July 20 – Algal Cellular & Molecular Biology

P07003 – See PSA Talk on Monday, July 20 – Algal Cellular & Molecular Biology

P07004 - **See PSA Plenary 2 on Monday, July 20 – Algal Species Concept in Molecular Ecology**

P07005 - **See PSA Plenary 2 on Monday, July 20 - Algal Species Concept in Molecular Ecology**

P07006 - **See PSA Plenary 2 on Monday, July 20 - Algal Species Concept in Molecular Ecology**

SESSION P08 – ABIOTIC STRESS

P08001 Identification of genes involved in salt tolerance of the mangrove plant *Bruguiera gymnorhiza*.
Tada, Yuichi-presenter tadayui@bs.teu.ac.jp(a) Miyama, Masashi (a,c) Ezawa, Shota (b)
http://www.teu.ac.jp/tada/Mangrove%20project1.html
"To identify key genes in the regulation of salt tolerance in the mangrove plant *Bruguiera gymnorhiza*, we performed three experiments. One is the gene expression profiling under salt-stress (500mM NaCl) using a microarray, followed by functional analysis of the salt-responsive genes. Expression vectors for selected salt responsive genes were constructed and transformed in *A. tumefaciens*, and then screened for salt tolerance. *A. tumefaciens* transformed with genes for lipid transfer and ankyrin repeat proteins showed enhanced salt tolerance. Transgenic *Arabidopsis* plants expressing these three genes also exhibited increased tolerance to NaCl. Second is the comprehensive functional screening of the *Agrobacterium* libraries expressing the mangrove cDNAs. Screening of the libraries on medium supplemented with 300mM NaCl identified 44 putative salt tolerance genes, including *Bg70* and *cyc02* homologue. Transgenic *Arabidopsis* plants expressing these two genes exhibited increased tolerance to NaCl. These results

demonstrate that *Agrobacterium* functional screening is an effective method to pre-screen genes involved in abiotic stress tolerance. Third is the proteome analysis of the mangrove plant. Comparative two-dimensional electrophoresis revealed that two, three and one proteins were differentially expressed in the main root, lateral root and leaf, respectively, in response to salt stress. Among these, three proteins were identified by internal peptide sequence analysis: fructose-1,6-bisphosphate aldolase and a novel protein in the main root and osmotin in the lateral root. Amounts of these proteins were not correlated to those of the respective mRNAs. The novel salt-responsive protein may provide insight into the salt-tolerance mechanism of the mangrove plant."
(a) School of Bioscience and Biotechnology, Tokyo University of Technology (b) Graduate School of Bionics, Tokyo University of Technology (c) Present address; Research Center of Advanced Bionics, Advanced Industrial Science and Technology

P08002 Cluster analysis of leaf quantitative traits in wheat lines with rigid pubescence
Aytasheva, Zaure G.-presenter zaure.aitasheva@kaznu.kz(a) Rysbekova , Ayman B. (b) Polimbetova, Fatima A. (b) Abdelsalam, Nader R. (a,c)
"Leaf rigid pubescence is regarded as one of principal features enabling resistance to a range of abiotic and biotic stressors. By Two Way ANOVA-guided cluster analysis based on a Euclidian similarity matrix applied to different morphometric properties, three conditioned types of leaf quantitative traits have been proposed. Starting from initial stages of ontogenesis, the area of the nucleus is referred to as *the major quantitative trait*. This primary trait has been shown to unify the set of rigidly pubescent lines already at the stage of seedlings. Length of rigid trichomes is attributed to *the flexible (or 'backward') quantitative traits* which appear to be less significant at early stages of ontogenesis to become critical by the stage of anthesis throughout waxy ripeness. Trichome density is ascribed to *the secondary quantitative traits* indicating the united cluster of rigid pubescence carriers only at the final stages beginning from waxy ripeness. In the latter case, such a belated unification of the cluster has been observed both for adaxial, and abaxial blade surfaces. Hence, unique properties of wheat rigid pubescence are defined by the area, as anticipatedly a functional state of trichomal nucleus rather early, at the stage of seedlings. So various developmental plots of rigid trichome functioning under stress conditions may be 'launched' namely at this stage."
(a) al-Farabi Kazakh National University (b) Institute of Plant Biology and Biotechnology (c) University of Alexandria

P08003 CBF/Cor/ABA and Freeze Survival of Winter Wheat
Sutton, Fedora-presenter fedora.sutton@sdstate.edu(a)
"Comparitive transcriptome analyses were performed with the Affymetrix Wheat chip and RNA isolated from control and 4 wk cold-acclimated crown tissue of two Hard Red Winter Wheat (HRWW) lines that differ in field freeze survival. These lines were generated by azide mutagenesis of the cultivar Winoka and designated FR (75% field survival) and FS (30% field survival). Contributions from the vrn allele that would occur with spring wheat were elimated because both lines were derived from the same winter cultivar. We have determined that the Cbf genes differentially regulated between FR and FS lines included genes from the central (Cbf12, 14), distal (Cbf3) and unlinked (Cbf6, 19) clusters on the long arm of chromosome 5. Additionally Cbf5 was identified as differentially regulated between FR and FS. However, it is not located on the long arm of chromosome 5. The differential Cbf gene expression levels observed for Cbf3, 5, 6, 12 14 and 19 did not correlate with gene expression patterns for any of the Cor genes examined. Thus we have assumed that at 4 wk cold acclimation these Cbf genes were not involved in regulation of this group of Cor genes. There were no significant differences between the cold-acclimated transcript accumulation between FR and FS lines. And there was also no difference in expression patterns between the group of ABA-dependent Cor genes (Wrab17, Wrab18, Wrab19 and Wcor825) and the ABA-independent genes (Wcor14A, Wcor14B, and Wlt10). This lack of ABA involvement in distinguishing between FR and FS lines at the level of gene expression is of great interest since it suggests that the role of ABA in confering freeze resistance may be in the control of other processes unrelated to Cor gene expression. "
(a) South Dakota State University

P08004 A genomic and proteomic study of Aluminum toxicity in tomato
zhou, suping-presenter zsuping@tnstate.edu(a,a) Sauve, Roger (a,a) Thannhauser, Theodore W (b,b)
"Growth inhibition in acid soils due to Al stress affects crop production worldwide. To understand mechanisms in sensitive crops that are affected by Al stress, a proteomic analysis of primary tomato roots tissue grown in Al amended and non amended liquid cultures was done. DIGE-SDS- MALDI-TOF-TOF analysis of these tissues resulted in the identification of 49 proteins that were differentially accumulated. The induction of dehydroascorbate reductase and glutathione reductase, catalase 2, and quercetin 3-O-methyltransferase could enhance the antioxidant activity in Al treated roots. Induced enzyme proteins associated with detoxification were mitochondrial aldehyde dehydrogenase, catechol oxidase, quinone reductase, and lactoylglutathione lyase while germin-like (oxalate oxidase) proteins, the malate dehydrogenase, wali7 and heavy-metal associated domain-containing proteins were suppressed. VHA-ATP that encodes for the catalytic subunit A of the vacuolar ATP synthase was induced and two ATPase subunit 1 isoforms were suppressed. Two enzymes associated with amino acid metabolism were affected; the NADPH-dependent flavin reductase was repressed while the isovaleryl-CoA dehydrogenase (IVD) was induced. A de novo fatty acid biosynthetic enzyme, beta-hydroxyacyl-ACP dehydratase, was reduced while the GDSL-motif lipase hydrolase family protein was induced. SAMS, quercetin 3-O-methyltransferase and AdoHcyase were also induced by Al stress. A parallel transcript profiling using Tom1 cDNA microarray confirmed some of the genes were regulated in similar patter at transcriptional level."
(a) School of Agricultural and Consumer Sciences, Tennessee State University (b) USDA-ARS, 102A R. W. Holley Center for Agriculture & Health, Cornell University, Ithaca, NY 14853

P08005 The Effect of Low Temperature and *BNCBF* Overexpression on Splicing-Related Proteins in *Brassica napus*
Savitch, Leonid V-presenter leonid.savitch@agr.gc.ca(a) Sprott, Dave (a) Ponomareva, Ekaterina (a) Golovkin, Maxim (b) Singh, Jas (a)
"The molecular response to cold stress involves the transcriptional modulation of stress related genes. The combined global transcriptome and proteome analyses of wild type and transgenic *B. napus* overexpressing *BnCBF5* and *BnCBF17* when undergoing cold stress and cold acclimation, revealed the involvement of post-transcriptional and translational mechanisms. Representation of a large number of proteins encoded by the genes related to transcription/translation was significantly increased (27% to 42%) in response to both low temperature exposure and *BNCBF* OE. Moreover, low temperature had a prominent effect on the quantitative (~ 26) representation of proteins related to pre-mRNA splicing and RNA stabilization. Transcriptional analyses of SR proteins and splicing-related LAMMER-type protein kinases revealed that *BnCBF* OE, cold stress and cold acclimation affected negatively the expression of *SRp30*, *AFC1*, *AFC2*, *SRPK1b*, *SRPK2b* while regulated positively the expression of *SRp34b*, *RSZ22*, *RSZp22a*, *RSZ32*, *SCL30a*, *SCL30*, *RSp41*, *RSp40*, *SR45*, *AFC3*, *SRPK1a*. Cold stress and cold acclimation also affected negatively the expression of *RSZ21*, *RSZ33*, *SC35*, *SCL33*, *RSp31*, *SR45a*. Proteomic analysis further confirmed that cold stress/acclimation and *BnCBF* OE, have helped to increases the amount and phosphorylation status of the following splicing-related proteins SR45, RSp41 and RSp40. Direct correlation of the expression levels of *SRPK1a* under these conditions with phosphorylation status of SR45, RSp41 and RSp40 suggests that these SR proteins might be

the targets of Clk/Sty Protein Kinases. To our knowledge, this is the first examination of the possible effects of *CBF/Dreb1* OE on the splicing-related gene activity and SR proteins accumulation in plants. "
(a) Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre, Ottawa, ON, Canada, K1A 0C6 (b) Biotechnology Foundation Laboratories, Thomas Jefferson University, Philadelphia, US/PA 19107

P08006 Allantoate amidohydrolase expression is independent of drought tolerance in soybean
Charlson, Dirk-presenter CharlsonD@gmail.com(b,a) Korth, Kenneth (c,a) Purcell, Larry (b,a)
"Nitrogen fixation in soybean (*Glycine max* L. [Merr.]) is decreased by drought, thereby resulting in reduced yields. During drought stress, shoot ureide concentrations increase in drought-sensitive genotypes, whereas they remain unaffected in drought-tolerant genotypes. The lack of increased shoot ureide concentrations during water-deficit stress appears to alleviate reduction in N2 fixation. Allantoate amidohydrolase (AAH) is a key enzyme involved in ureide breakdown in shoots. We hypothesized that *AAH* gene expression determines shoot ureide concentrations and differential sensitivities of N2-fixation response to water-deficit stress. Our objective was to examine *AAH* transcript expression for both a drought-sensitive (cv. Williams) and tolerant (cv. Jackson) genotype subjected to treatments of well-watered and water-deficit stress. In addition to *AAH* expression and shoot ureide concentrations, gene expression was examined for *DREB2*, which is expressed in response to water-deficit stress. As expected, shoot ureide concentrations were greater in Williams relative to Jackson, and *DREB2* expression was detected only during water-deficit stress for both genotypes, which indicated that the plants were experiencing water-deficit stress. However, contrary to our hypothesis, expression of *AAH* transcripts was similar among water-deficit treatments and between the two genotypes. These results indicated that reduced *AAH* expression is not likely associated with decreased ureide catabolism observed in drought-sensitive genotypes, such as Williams. Our results may indicate that further study at the post-translational level is warranted to dissect the potential role of *AAH* in drought tolerance. "
(a) University of Arkansas (b) Department of Crop, Soil, and Environmental Science (c) Department of Plant Pathology

P08007 Genetic engineering to improve drought tolerance in maize
Nuccio, Michael (a) Chen, Xi (a) Clarke, Joseph (a) Cates, Eddie (a) Duncan, Kateri-presenter kateri.duncan@syngenta.com(a)
"Water availability is the single most important factor limiting agricultural productivity. Although its precise function is not known, the trehalose pathway is essential in plants. It's been shown to influence sugar and starch metabolism. The position of the trehalose pathway in carbon metabolism suggests that it may regulate activated sugar pools. If so, then drought-inducible disruption of the maize trehalose pathway could redirect activated sugars toward sucrose and starch, enabling plants to increase production in water-stressed environments. To test this we identified a fragment of the maize homolog to the Arabidopsis T6PP-A gene encoding trehalose-6-phosphate phosphatase. This was used to construct a gene-specific RNAi knockout cassette driven by the drought-inducible Rab17 promoter. The construct was stably transformed into maize and, hybrid material was generated to examine construct performance in greenhouse and field experiments. The data show the construct functions to improve kernel set in water-limited environments and does not affect yield in well-irrigated environments."
(a) Syngenta Biotechnology

P08008 Improving drought tolerance in crops
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"Drought stress is one of the major factors that limit crop production worldwide. In order to keep crop production in pace with growing population, more food must be produced under stressful conditions including drought stress. To achieve such a goal, we must develop drought tolerant crops. One way to create drought tolerant crops is to introduce foreign genes into crops that can improve stress tolerance. For examples, overexpression of the Arabidopsis gene *AVP1* that encodes vacuolar membrane-bound pyrophosphatase (a proton pump) increases drought- and salt-tolerance in transgenic Arabidopsis and tomato, and overexpression of *IPT* that encodes an isopentyltransferase could significantly increase drought tolerance in tobacco plants. To test if these two genes can be used for improving other crops in the field, we introduced these two genes into cotton, peanut, and sweet potato. Our preliminary data with transgenic cotton plants that overexpress *AVP1* indicate that overexpression of *AVP1* can indeed improve drought- and salt-tolerance in cotton. The biomass of *AVP1*-expressing cotton plants is significantly higher than that of wild-type plants under both water-deficit and salt conditions. The fiber yield of *AVP1*-expressing cotton plants is also significantly higher than that of wild-type cotton under both water-deficit and salt conditions. More importantly, the *AVP1*-expressing cotton plants produced 25-30% more fiber under dry-land field conditions, suggesting that *AVP1* is a very promising gene that can be used to improve crop production in water limited areas."
(a) Texas Tech University (b) University of California (c) Arizona State University (d) USDA-ARS Cropping Systems Research Lab

P08009 "SHI2, A DEAD Box RNA Helicase Is Important for Abscisic Acid and Cold Response"
Wang, Bang S.-presenter wangbangshing@yahoo.com(a) Jiang, Jiafu (a) Shi, Huazhong (a)
"DEAD (Asp-Glu-Ala-Asp) box RNA helicases belong to the largest family of helicase proteins involved in gene expression from transcription to translation. Little is known about the precise functions of DEAD box RNA helicases in plant gene regulation and stress response. Using a forward genetic screening, a number of mutants, designated as *shiny* (*shi* for short), showing higher expression of luciferase reporter gene driven by a stress-inducible promoter native to the sulfotransferase gene *AtSOT12* were identified. Here we present the characterization and positional cloning of *shi2*. *shi2* mutant is more sensitive to ABA in seed germination and displays more inhibited growth at low temperature than wild type. Under salt stress, the expression level of both native *AtSOT12* and luciferase reporter gene is significantly higher in *shi2* mutant when compared with wild type, which suggests that SHI2 functions to repress salt induced gene expression. Map-based cloning and genetic complementation established that *SHI2* encodes a nuclear localized DEAD box RNA helicase protein. Promoter-GUS fusion analysis indicated that *SHI2* is expressed throughout the whole plant. Using yeast two-hybrid screening, a putative transcription factor was identified to interact with SHI2, which suggests that SHI2 may regulate gene expression through a chromatin-based process. "
(a) Department of Chemistry and Biochemistry, Texas Tech University

P08010 "A new insight into the role of chloroplast protein synthesis elongation factor, EF-Tu"
Ristic, Zoran-presenter zristic@ksu.edu(a) Fu, Jianming (b) Momcilovic, Ivana (b) Clemente, Thomas E. (c) Nersesian, Natalya (c) Trick, Harold N. (d)
"Chloroplast protein synthesis elongation factor, EF-Tu, is a highly conserved nuclear-encoded protein (45 kD) that plays a role in protein synthesis by promoting the GTP-dependent binding of aminoacyl-tRNA to the A site of the ribosome. Some evidence suggests that the role of chloroplast EF-Tu may not be limited to protein synthesis, as this protein seems to play a role in plant response to heat stress. EF-Tu is upregulated under supra-optimal temperatures, and in maize and wheat heat-induced accumulation of EF-Tu correlates positively with the ability of plants to tolerate heat

stress. In addition, chloroplast EF-Tu displays chaperone activity, as it protects heat-labile proteins from thermal aggregation and inactivation (Rao et al., 2004, Eur. J. Biochem. 271, 3684-3692). We hypothesized that increasing the expression of EF-Tu may lead to improved heat tolerance. To test this hypothesis we transformed wheat using a maize gene coding for EF-Tu. When compared to non-transgenic counterparts, transgenic wheat plants expressing maize EF-Tu displayed reduced thermal aggregation of leaf proteins, reduced injury to photosynthetic membranes, enhanced rate of CO2 fixation and higher yield following exposure to heat stress. The results support the concept that EF-Tu ameliorates negative effects of heat stress and improves heat tolerance by acting as a molecular chaperone. This is the first demonstration of ectopic expression of EF-Tu in plants leading to enhanced photosynthesis and protection against heat-induced injury following heat exposure. This is also the first demonstration that the improvement of heat tolerance is possible in a species with a complex genomic structure, hexaploid wheat. "
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P08011 A homolog of human ski-interacting protein in rice positively regulates cell viability and stress tolerance
Xiong, Lizhong-presenter lizhongx@mail.hzau.edu.cn(a) Hou, Xin (a) Xie, Kabin (a) Yao, Jialing (a)
"Abiotic stresses are major limiting factors for growth, development and productivity of crop plants. Here we report on OsSKIPa, a rice homolog of human SKIP (Ski-interacting protein) that can complement the lethal defect of the knock-out mutant of SKIP homolog in yeast and positively modulate cell viability and stress tolerance of rice. Suppression of *OsSKIPa* in rice resulted in growth arrest and reduced cell viability. The expression *OsSKIPa* is induced by various abiotic stresses and phytohormone treatments. Transgenic rice overexpressing *OsSKIPa* exhibited significantly improved growth performance in the medium containing stress agents (abscisic acid, salt, or mannitol) and drought resistance at both the seedling and reproductive stage. The *OsSKIPa*-overexpressing rice showed significantly increased ROS-scavenging ability and transcript levels of many stress-related genes, including *SNAC1* and rice homologs of CBF2, PP2C, and RD22 under drought stress conditions. More than 30 OsSKIPa-interacting proteins were identified, but most of these proteins have no matches with the reported SKIP-interacting proteins in animals and yeast. These data together suggest that OsSKIPa has evolved a novel function in positive modulation of stress resistance through transcriptional regulation of diverse stress-related genes in rice."
(a) Huazhong Agricultural University

P08012 Heat stress reduces the pH component of the proton motive force in light-adapted intact tobacco leaves
Zhang, Ru-presenter rzhang4@wisc.edu(a) Cruz, Jeffrey A. (b) Kramer, David M. (b) Magallanes-Lundback, Maria E. (c) Dellapenna, Dean (c) Sharkey, Thomas D. (a,c)
"Light triggers photosynthetic electron transport and proton translocation along and across thylakoid membrane, producing the pH gradient (ΔpH) and the electrical potential (Δψ). These are components of the transthylakoid proton motive force (*pmf*) driving ATP synthesis. The Δψ causes a shift in carotenoid absorbance bands (peak at 518 nm) called the electrochromic shift (ECS). The ECS can be used to estimate the magnitude of both the Δψ and ΔpH in vivo. By following the ECS for 25 s after turning off the light, we measured the two components of *pmf* within light-adapted, intact tobacco leaves before, during and after moderate heat treatment at 40oC. The ECS signal was deconvoluted by subtracting effects of zeaxanthin formation (peak at 505 nm) and chloroplast swelling/shrinking or movement (peak at 535 nm) from the signal measured at 520 nm. The data showed that *pmf* became smaller at high temperature, with reduced ΔpH while Δψ slightly increased, indicating that heat stress alters the partitioning of transthylakoid *pmf*. Elevated temperature accelerated ECS decay kinetics likely reflecting temperature-induced increases in proton conductance and movement of counter-ions. Simultaneous measurement of PSII chlorophyll fluorescence showed that the energy-dependent quenching (qE) was reduced by heat. However, the reduction of qE was less than that of ΔpH, indicating that qE is more sensitive to ΔpH at high temperature. Zeaxanthin did not increase at high temperature in light-adapted leaves but it was higher than that would be predicted at the reduced ΔpH found at high temperature. "
(a) Department of Botany, University Of Wisconsin-Madison (b) Institute of Biological Chemistry, Washington State University (c) Department of Biochemistry&Molecular Biology, Michigan State University

P08013 Protein phosphorylation regulates the transport activity of the wheat TaALMT1 aluminum tolerance protein
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"We studied the role of protein phosphorylation in modulating transport properties of the wheat aluminum tolerance protein and malate transporter, TaALMT1. Preincubation of *Xenopus laevis* oocytes expressing TaALMT1 with protein kinase and phosphatase inhibitors (K252a, staurosporine, okadaic acid, cyclosporine A) inactivated TaALMT1-mediated inward currents (malate efflux). In contrast, the protein kinase C (PKC) activator, PMA, enhanced TaALMT1-mediated currents, suggesting that TaALMT1 transport is regulated by PKC-mediated phosphorylation. Five truncated proteins were generated by sequential deletion of putative PKC phosphorylation sites in the C terminal tail. Interestingly, all sequential deletions abolished transport activity until the entire C-terminal tail was removed; then the basal malate efflux that was not enhanced by Al was recovered. This result suggests that that the N-terminal half of the protein can form a functional pore that allows malate release without Al-activation, and Al-binding residues are then located in the C-terminal domain. Finally, we performed site-directed mutagenesis on eight predicted phosphorylation sites in the entire sequence (S56A, S183A, S324A, S337A, S351-352A, S384A, T323A and Y184F), and evaluated their effect on TaALMT1 transport in oocytes. The results showed that the transport properties of all mutants except S384A were not altered relative to those observed in the wildtype TaALMT1. However, S384A completely abolished the activity of the transporter, indicating that S384 is an essential residue regulating the transport activity of the TaALMT1. Overall, our findings suggest that direct phosphorylation of TaALMT1 at residue S384 plays a crucial role in regulating the activity of the transporter."
(a) Robert W. Holley Center for Agriculture and Health, USDA-ARS, Tower Road, Cornell University, Ithaca, NY, 14853-2901

P08014 "Overexpression of *OsNUC1*, a novel salt-stress responsive gene, increases growth and salt tolerance in transgenic Arabidopsis"
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"Rice *nucleolin* gene, *OsNUC1*, was found to be differentially expressed in salt-sensitive and salt-resistant rice lines during salt stress condition. The expression was found in various organs of rice, leaves, flowers, seeds, and roots. During salt stress condition up to 9 days, the salt-resistant lines showed the higher level of gene expression. Moreover, the unique feature of rice nucleolin-like protein is the existence of two RNA recognition motifs (RRM) and Glycine- and Arginine-rich (GAR) repeat segments at the central and carboxylic domains. To determine the role of central and carboxyl domains of *OsNUC1*, the partial *OsNUC1* cDNA containing two RRM motifs and GAR was constitutively expressed under *35SCaMV* promoter, and was salt-inducible expressed under *rd29a* promoter in *Arabidopsis thaliana* L. In normal condition, the transgenic plants with the overexpression of partial *OsNUC1* showed the higher growth rate than the controls. They are also more resistant to salt stress. We propose that the central and carboxyl domains of *OsNUC1* play important roles on plant growth, and also contribute to salt-stress tolerance character in plants. This work defines a new

role of *nucleolin* in salt-tolerant ability and growth promotion."
(a) Dept. of Botany, Faculty of Science, Chulalongkorn University (b) Dept. of Biochemistry, Faculty of Science, Chulalongkorn University (c) College of Life Sciences, Peking University (d) National Plant Gene Research Center (Beijing)

P08015 Progress in phenotyping dehydration avoidance traits for drought resistance improvement in rice
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"Improvement of drought resistance and water productivity in rice requires the genetic dissection of the processes underlying plant response to soil drying, water-use efficiency, and dehydration avoidance during the most critical stages of stress development. Use of the Fraction of Transpirable Soil Water (FTSW) as a soil moisture parameter and stress co-variable allows the integration of multi-location field trials with controlled-environment experiments. It also facilitates analysis of gene expression profiles related to specific phenotypic traits under drought. Combining the FTSW dry-down method with non-destructive imaging techniques for plant growth and development measurement, and dissection of yield components, allows high-throughput, field-based, and precise drought phenotyping. These methods were used for large-scale evaluation of rice germplasm collections, breeding lines, and mutant and transgenic lines. Genetic sources of drought resistance have been identified and several QTLs have been characterized, with a focus on dehydration avoidance during reproductive-stage water deficit. Genetically diverse rice germplasm panels were phenotyped for allele mining and the application of an ideotype approach targeting root water extraction for maintaining plant growth and yield components under drought. Similarly, root traits were analyzed in sets of NILs differing in major QTLs for yield under drought. Traits related to deep rooting were found to be positively correlated to water uptake, dehydration avoidance and root growth rate at depth during progressive soil drying. This paper reviews the recent progress in understanding the physiological processes and traits involved in dehydration avoidance and growth regulation under water deficit in rice."
(a) International Rice Research Institute, Philippines (b) Barwale Foundation, Hyderabad, India (c) Tamil Nadu Agricultural University, Coimbatore, India

P08016 Induction of tomato galactinol synthase (SlGolS2) in response to heat stress
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"Recent studies indicated that raffinose-related oligosaccharides are synthesized in response to various stresses, such as heat, salt stress and drought, in higher plants and that the oligosaccharides play important roles in protection of proteins and biomembranes as osmolytes and antioxidants. The raffinose-related oligosaccharides are synthesized by galactinol synthase (GolS) with UDP-galactose and myo-inositol. In Arabidopsis, GolSs are expressed in seed and induced under drought, salt stress and chilling. Based on partial sequences of GolS homologs in MiBASE (Kazusa DNA Institute), we isolated a full length cDNA encoding a galactinol synthase in tomato (Solanum lycopersicum L. cv. Micro-Tom), designated SlGolS2. The predicted protein of SlGolS2 has high similarlity to AtGolS1 (75% in aminoacid identity) rather than LeGolS1 (61%). SlGolS2 was transiently induced in response to salt, heat and chilling stresses. Heat stress induced H2O2 production in tomato leaf under light. It was examined whether oxidative stress functions as a signal molecule to trigger induction of SlGolS in tomato under heat stress. H2O2 treatment enhanced heat tolernce of tomato plant, and SlGolS2 and oxidative stress-specific transcription factor HsfA2 were induced in response to H2O2. Furthermore, expression of full length SlGolS2 significantly enhanced salt stress tolerance of E. coli in the presence of myo-inositol. These results suggest that induction of SlGolS2 and synthesis of osmolyte, galactinol, are involved in aquired heat tolerance of tomato and that oxidative stress and SlHsfA2 are implicated in gene regulation of SlGolS2 in response to heat stress. Involvement of phytohormones on SlGolS2 expression in response to salt stress and chilling will be discussed."
(a) Faculty of Agriculture, Kyushu University (b) Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University (c) School of Agriculture, Kyushu University

P08017 High light acclimation processes are altered in *Arabidopsis thaliana* L. (Heynh.) lacking chloroplast protease SPPA
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"SPPA1 is a plastid-localized protease. In this study we examined T-DNA insertion mutants of the *SPPA1* gene in *Arabidopsis thaliana* (At1g73990). Under moderate, non-stressful conditions, mutation of SPPA1 had no effect on growth and development of plants. It also did not affect quantum efficiency of photosynthesis as measured by dark-adapted *Fv*/*Fm* and light-adapted ΦPSII. Chloroplasts from sppA mutants were indistinguishable from wild type. However, loss of SPPA appears to affect photoprotective mechanisms during high light acclimation: mutant plants maintained a higher level of non-photochemical quenching of Photosystem II chlorophyll (NPQ) than wild type, while wild type plants accumulated more anthocyanin than the mutants. Quantum efficiency of Photosystem II was the same in all genotypes grown under low light, but was higher in wild type than mutants during high light acclimation. Further, the mutants retained the stress-related Early Light Inducible Protein (ELIP) longer than wild type leaves during the early recovery period after acute high light plus cold treatment. These results suggest that SPPA1 may function during high light acclimation in the plastid, but is non-essential for growth and development under non-stress conditions. "
(a) Smith College, Biological Sciences

P08018 Two MAP kinases preferentially expressed in guard cells positively regulate ROS-mediated ABA signaling
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"Abscisic acid (ABA) plays an essential role in protection of plants from environmental stresses such as drought, salt, and cold. Reactive oxygen species (ROS) have been suggested to function in guard cell ABA signaling. To further genetically dissect guard cell ABA-ROS signaling, we identified two MAPK genes, GCMPK3 and GCMPK4, that are preferentially and highly expressed in guard cells. To provide direct genetic evidence, RNAi-based gene silencing plant lines were generated in which both genes are simultaneously silenced. In parallel, Arabidopsis single and double mutants carrying deleterious point mutations in these genes were identified. ABA-induced stomatal closure was strongly impaired in two independent RNAi lines in which both GCMPK3 and GCMPK4 transcripts were significantly silenced. Consistent with this result, gcmpk3-1/4-1 double mutants showed an enhanced transpirational water loss and ABA- and H2O2-insensitive response in stomatal movement assays, whereas mutants carrying a mutation in one of these genes did not show any altered phenotype, indicating functional redundancy in these genes. A GCMPK4-YFP fusion construct rescued the gcmpk3-1/4-1 double mutant phenotype in ABA-induced stomatal movements, demonstrating that the mutations in these genes caused the phenotype. GCMPK4 protein is localized in the cytosol and the nucleus, and ABA enhances the protein kinase activity of GCMPK4. Together, these results provide genetic evidence that GCMPK3 and GCMPK4 function downstream of ROS to positively regulate guard cell ABA signaling."
(a) University Of Maryland (b) University of Sassari (c) University of British Columbia (d) CNRS-CEA-Universite Aix-Marseille II

P08019 The role of cell wall pectin in aluminum resistance
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"Among the Al exculsion mechanisms, Al-induced organic acids secretion have been well documented in quite some Al resistant plant species. However, it still could not explain all the difference herein observed in some Al resistant plant species. In our studies, we investigated the role of cell wall pectin in aluminum resistance in some plant species with or without organic acids secretion under Al stress. First of all, most of Al was found to be bound to cell wall, while partial remove of Al by citrate washing could significantly help the plant to recove better from Al toxicity, indicating the role of cell wall in expressing Al toxicity. The amount of Al absorbed to cell walls of wheat was decreased by pectinase treatment. While oxalate efflux could not explain genotypic Al differences between Al-resistant and Al-sensitive buckwheat cultivars, we found that cell wall pectin were substantially higher in the Al-sensitive cultivars. We further found that cell wall pectin properties showed some correlations with root tip Al content and different Al resistance in two rice cultivars. Immunolocalization of pectins showed a higher proportion of demethylated pectins in Al-sensitive rice cultivar, indicating a higher proportion of free pectic acid residues, which could satisfactory explain the higher Al content in the cell walls of Al-sensitive rice root tips. All these results indicate that cell wall pectin may play an important role in plant Al resistance"
(a) College of Life Science, Zhejiang University

P08020 ABA response element binding proteins are convergence points for metabolic and stress signalling pathways involving SNF1-related protein kinases
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http://www.rothamsted.bbsrc.ac.uk/
"Interest in links between stress and metabolic signalling began when several mutations that affected the sugar response in Arabidopsis were found to be ABA-related and protein kinases related to the metabolic regulator, SnRK1, were placed in stress signalling pathways. SnRK1 is activated in response to high cellular sucrose / low cellular glucose and regulates carbon metabolism through the modulation of enzyme activity and gene expression. Plants contain two large and diverse subfamilies of related protein kinases, SnRK2 and SnRK3 that are involved in signalling pathways that regulate responses to drought, cold, salt and osmotic stress. ABA-response element binding proteins (AREBPs) are a family of bZIP transcription factors that are expressed in response to different stresses. SnRK2s have been shown previously to phosphorylate AREBPs and we have found that both SnRK1 and SnRK3 will also phosphorylate them. Peptides based on two highly-conserved target sites were phosphorylated by purified SnRK1 and by calcium-dependent and-independent activities present in crude Arabidopsis extracts. Most of the calcium-independent activity could be precipitated out of the extracts by anti-SnRK1 antisera. It is possible that the calcium-dependent activity was SnRK3, making AREBPs convergence points for signalling by all three SnRK subfamilies. We hypothesise that SnRK2 and SnRK3 arose initially by gene duplication of SnRK1, and then diverged rapidly during plant evolution to fulfil new roles that enabled plants to develop networks that link stress and ABA signalling with metabolic signalling. The research is being transferred to wheat, in which we have identified AREBP transcripts in seedlings subjected to several stress treatments. "
(a) Plant Science, Rothamsted Research, Harpenden, Herts UK

P08021 A Hybrid type Histidine Kinase from *Oryza sativa* L. cv IR64 is regulated by multiple abiotic stresses
Karan, Ratna-presenter karan.ratna@gmail.com(a) SinglaPareek, Sneh L (b) Pareek, Ashwani (a)
"Abiotic stresses severely affect the growth and development of crop plants. Plants have distinct signaling systems to recognize and respond to these stresses. Prokaryotes and some eukaryotes use two-component signaling systems to sense and respond to changes in different environmental conditions. A typical two-component system consists of a sensor histidine kinase and a regulatory protein referred to as a response regulator. Little is known about the involvement of osmosensor genes in the osmotic adjustments of monocotyledonous plants such as rice. Rice is a very important cereal crop and also a major food source for more than one-third of the worlds population. In the present study, we have characterized the putative osmosensor OSHK3b from the crop plant rice. We have cloned OSHK3b from salinity sensitive rice variety IR64, encodes for 95.89 kD protein, containing a chase domain followed by a transmembrane domain and a receiver domain which shows this gene is structurally similar to hybrid type histidine-kinase. Expression of OSHK3b ORF functionally complemented the temperature sensitive yeast mutant for SLN1. Site directed mutagenesis of conserved Histidine and Aspartate residue of OSHK3b failed to complement the SLN1 mutant.This analysis revealed the catalytically conserved role for OSHK3b and SLN1. qRT-PCR analysis of OSHK3b transcripts under multiple abiotic stress condition showed differential accumulation in rice cv IR64, in response to different durations of stress. Western analysis also confirmed that OSHK3b protein shows differential accumulation under a range of abiotic stress conditions. OSHK3b protein is localized in plasma membrane. Upstream DNA sequence of OSHK3b has been found to be inducible by abiotic stresses as revealed by reporter gene expression. "
(a) Jawaharlal Nehru University, New Delhi, India (b) International Centre for Genetic Engineering and Biotechnology, New Delhi, India

P08022 Glycerophosphodiester phosphodiesterases play an important role in phosphate recycling and phosphate sensing in white lupin
Cheng, Lingyun-presenter cheng111@umn.edu(a,b) Liu, Junqi (b) Miller, Susan S (c) Bucciarelli, Bruna (c) Zinn, Kelly E (d) Zhang, Fusuo (a) Allan,, Deborah A (e) Shen, Jianbo (a) Vance, Carroll P (b,c)
"White lupin (*Lupinus albus* L.), a well adapted species to phosphate (Pi) impoverished soils, develops short, densely clustered lateral roots (cluster/proteoid roots) to increase Pi uptake. Here, we report two white lupin glycerophosphodiester phosphodiesterase (*GPX-PDE*) genes which share strong homology with bacteria, mammalian and plant *GPX-PDE*. However, phylogenetic analysis showed that these two genes belonged to different clades, suggesting functional diversity. RNA blot and qRT-PCR analysis showed that both *LaGPX-PDE* genes are highly upregulated in mature Pi-deficient cluster roots. A *LaGPX-PDE1* promoter::GUS reporter construct showed a greatest activity in root hair, phloem and epidermal cells of the P-deficient cluster roots. Mutation of PHR1 binding site in the promoter of *LaGPX-PDE1* abolished the response to P stress. Resupply of 1 mM Pi or phosphonate (Phi) directly to P-starvation roots resulted in a rapid decrease in both *LaGPX-PDE* transcripts. Although *LaGPX-PDE* transcripts were highly abundant in Pi-stressed cluster roots, protein was low to non-detectable. One of the *LaGPX-PDE* genes annotates as both a GPX-PDE and a PHO85 like kinase, and may be bifunctional. Taken together our results indicate that Pi signaling can be localized as well as systemic in white lupin."
(a) Department of Plant Nutrition, Laboratory of Plant-Soil Interaction, China Agricultural University (b) Department of Agronomy and Plant Genetics, University of Minnesota (c) USDA-ARS, Plant Science Research (d) Department of Biochemistry and Molecular Biology, University of Nevada (e) Department of Soil, Water and Climate, University of Minnesota

P08023 Ectopic expression of coffee HD-ZIP *CAHB12* confers drought tolerance to Arabidopsis thaliana
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http://www.biologia.ufrj.br/~lgmv/MAF/

"Homeobox genes present a conserved 61-amino acid motif (homeodomain/HD) and are found in all eukaryotic organisms, generally regulating cellular development and differentiation. Recent studies have shown that homeobox genes of the HD-Zip family are also involved in transcriptional regulation of stress responses in plants. HD-Zip proteins have the HD associated to a leucine zipper and are exclusively found in plants. In our previous work, we identified two contigs named *CAHB12* and *CAHB1* (*Coffee Arabica Homeobox12 and 1*) in **Genoma Cafe** Sequencing Project Consortium database combining phylogenetic methods and *in silico* analysis of gene expression. To gain futher information in the *CAHB12* and *CAHB1* gene function, a comprehensive expression analysis were carried on in coffe plants. qPCR experiments showed that *CAHB1* and *CAHB12* are induced in leaves and roots of *C. arabica* submitted to drought. In addition *CAHB1* shows a conspicuous expression along this organ, while *CAHB12* is preferentially expressed in lateral roots, and in upper part of the main root. Spatial analysis of gene expression by *in situ* hybridization experiments revealed that *CAHB1* and *CAHB12* are expressed in root phloem. cDNAs of both genes were cloned in binary vectors, and those vectors were used for transformation of *Arabidopis* plants. Transgenic *Arabidopis* ectopically expressing *CAHB12* presented a higher tolerance level to drought stress during the transition from the reproductive to frutification phases. *35S:CAHB12* seedlings are also more tolerant to salt stress. Our data strongly suggest that at least one homeobox gene of HD zip I family in Coffee has a crucial role in the tolerance to drought stress. These results indicate that *CAHB12* can be a key gene in coffee response to drought stress."
(a) Federal University of Rio de Janeiro (b) Embrapa Recursos Geneticos e Biotecnologia (c) Institute of Molecular Physiology and Biotechnology of Plants, University of Bonn (d) Facultad de Bioquimica y Ciencias Biologicas, Universidad Nacional del Litoral

P08024 "Overexpression of AtABCG36 improves drought, osmotic, and salt stress resistance in Arabidopsis"
Jun-Young, Jin-presenter jjan@postech.ac.kr(a) Do-Young , Kim (a,c) Enrico, Martinoia (a,b) Youngsook, Lee (a)
"Drought and salt are major abiotic stresses that adversely affect crop productivity. Thus identification of factors that confer resistance to these stresses would pave a way to increasing agricultural productivity. When grown on soil in greenhouse longer than 5 weeks, transgenic Arabidopsis plants that over-express AtABCG36/AtPDR8 produced higher shoot biomass and less chlorotic leaves than the wild type. We investigated whether the improved growth of AtABCG36-overexpressing plants was due to their improved resistance to abiotic stresses, and found that AtABCG36-over-expressing plants were more resistant to drought, salt and osmotic stress and grew to higher shoot fresh weight than the wild type. In contrast, T-DNA insertional knockout lines were more sensitive to drought stress than the wild type and were reduced in shoot fresh weight. To understand the mechanism of enhanced salt and drought resistance of the AtABCG36-over-expressing plants, we measured sodium contents, and found that AtABCG36-over-expressing plants were lower in sodium content than the wild type. Our data suggest that AtABCG36 contributes to drought and salt resistance in Arabidopsis by a mechanism that includes reduction of sodium content in plant."
(a) Global Research Laboratory, Division of Molecular Life Sciences, POSTECH (b) Institute of Plant Biology, University of Zurich (c) Department of Plant Biology, Carnegie Institution of Washington

P08025 Application of infra-red thermal imaging for the screening of osmotic stress tolerance in cereals
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"Excessive salt concentration in soils (>10 dS.m&−1) is a limiting factor to cereal production in Australia. Munns et al. (2006) suggested that improvement of crop performance in saline soils could be achieved by improving tolerance to osmotic stress. Genotypic variation in tolerance to osmotic stress has been previously assessed by measuring stomatal conductance of plants grown in salt, relative to non-salt controls (James et al. 2008). As leaf temperature differences due to variation in transpiration rates can be visualised by infra-red thermal imaging, it is likely that this technology could be used to assess osmotic stress tolerance in cereal crops. The aims of this study were two-fold: 1) to develop an accurate, high through-put screening protocol for osmotic stress tolerance using IR thermography, and 2) to validate the new screening protocol on a collection of durum wheats previously characterised for osmotic stress tolerance (James et al. 2008). An IR camera (SC660, FLIR) detecting long wavelength infra-red radiation was used to capture leaf temperature images. The temperature difference between treated and non-treated plant was in the range of 2degK. Simultaneous measurements of leaf conductance were recorded using an AP4 steady-state porometer (Delta-T Devices). A high through-put, automated image analysis protocol for the capture, identification and analysis of thermal images of wheat and barley was developed in Matlab to compute average whole-plant temperature, which was used as a surrogate for stomatal conductance. A complementary, non-destructive growth analysis was undertaken using a Scanalyser (Lemnatec). Relationship between stomatal conductance and change in leaf temperature for seedlings grown in a range of salt treatments is reported."
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P08026 "Varietal differences in rates of root growth and leaf photosynthesis, hydraulic conductivity and expression of aquaporins in barley seedlings under salt stress conditions "
Watanabe, Tamaki (a) Katsuhara, Maki (b) Yamaguchi, Makoto (a) Ookawa, Taiichiro (a) Takeda, Kazuyoshi (b) Hirasawa, Tadashi-presenter hirasawa@cc.tuat.ac.jp(a)
"We observed significant varietal differences in dry matter production under short and long term salt stress conditions among barley varieties. To investigate their mechanisms of salt tolerance, we selected OUE 812* as a variety that shows a small reduction in dry matter production and OUC 613* as a variety that shows a large reduction in dry matter production. We then compared the rates of root growth and leaf photosynthesis, water transport properties and gene expression of barley plasma-membrane type (HvPIP) aquaporins of roots in the seedlings under salt stress conditions. Plants were grown with one-half strength Hoagland's solution in an environment-controlled chamber. When the first leaves were expanding, plants were transferred to a solution with 100 mM NaCl and were grown for 24 and 48 hours, referred to here as the 24 h NaCl treatment and 48 h NaCl treatment, respectively. The rates of root growth and photosynthesis decreased in both types of plants with NaCl treatment but the decreases were smaller for OUE 812 than OUC 613. The difference in the reduction in the rates was remarkable for the plants in the 48 h NaCl treatment. The hydraulic conductivity remained high in OUE 812 compared to OUC 613 for plants in the 24 h NaCl treatment. Changes in the accumulation of aquaporin mRNA were not evident in either type of plant for the 24 h NaCl treatment. However, the accumulation tended to decrease in OUC 613 and, in contrast, to increase in OUE 812 in the 48 h NaCl treatment. Root growth and the function and expression of aquaporin genes in response to the salt stress might be responsible for the varietal difference in the reduction in leaf photosynthetic rate in the NaCl treatment. *Accession number at the Barley Germplasm Center of Okayama University. "
(a) Graduate School of Agriculture, Tokyo University of Agriculture and Technology (b) Research Institute for Bioresources, Okayama University

P08027 Cytokinin-mediated mechanisms involved in the enhanced drought tolerance of transgenic plants expressing isopentenyltransferase.
Rivero, Rosa M (a) Walia, Harkamal ` (b) Blumwald, Eduardo-presenter eblumwald@ucdavis.edu(a)
"Water scarcity makes drought the single biggest challenge for present and future agricultural production. To begin to address this critical issue we

recently proposed an experimental approach involving delayed senescence in plants under water-limiting conditions. Using tobacco as a model crop, we introduced cytokinin biosynthesis isopentenyltransferase transgene driven by stress- and maturation inducible promoter (PSARK::IPT) and tested for drought tolerance. The transgenic plants displayed remarkable ability to delay senescence and ameliorate drought caused yield losses. We have combined transcriptomic, physiological, and biochemical approaches for mechanistic elucidation of the PSARK::IPT action in the drought-tolerant plants. Array-based analysis indicated major expression level difference between the tolerant and wildtype plants for genes involved in several photosynthesis processes localized to the chloroplasts. Some of the key genes of PSII, PSI and, ATPase complexes were strongly repressed in the wild-type stressed plants but were unaffected or marginally repressed in stressed PSARK::IPT plants. Transcript abundance of genes associated with oxidative stress tolerance and photo-protection were significantly higher in PSARK::IPT compared to wild-type plants. At protein level, photosynthesis genes such as D1, Cyt b6, and, □$B&A□(B-ATPase were strongly repressed in stressed wildtype plants only. In vivo comparisons using chlorophyll fluorescence were also consistent and indicated that during stress PSARK::IPT plants maintained PSII and NPQ efficiencies comparable to unstressed plants. By integrating several data we will propose hormone-mediated mechanism(s) which enable the PSARK::IPT plants to delay senescence and display striking drought tolerance."
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P08028 Cytokinin-dependent photorespiration and the protection of photosynthesis during water deficit
Rivero, Rosa M (a) Shulaev, Vladimir (b) Blumwald, Eduardo-presenter eblumwald@ucdavis.edu(a)
"We investigated the effects of the expression of IPT (isopentenyltransferase) and cytokinin production on several aspects of photosynthesis in transgenic tobacco plants grown under optimal or restricted (30% of the optimal) watering regimes. There were no significant differences in stomatal conductance between leaves from wild-type and transgenic plants expressing the IPT gene under the control of the SARK promoter grown under optimal or restricted watering. On the other hand, there was a significant reduction in the maximum rate of electron transport as well as the use of triose phosphates only in the wild-type plants during growth under restricted watering, indicating a biochemical control of photosynthesis during the growth under water deficit. The transgenic plants displayed an increase in catalase inside peroxisomes, a physical association between chloroplasts, peroxisomes and mitochondria and an increase in the CO2-compensation point, indicating the cytokinin-mediated occurrence of photorespiration in the transgenic plants. The contribution of photorespiration to the tolerance of the transgenic plants to water deficit was also supported by the increase in transcripts coding for enzymes involved in the conversion of glycolate to RuBP. Moreover, the increase in transcripts was further enhanced in the transgenic plants grown under restricted watering conditions, indicating a cytokinin-induced increase in photorespiration and the contribution of photorespiration to protecting photosynthetic processes and playing a beneficial role during water stress. Our results indicate the possibility of generating transgenic plants with increased water use efficiency and increased tolerance to water deficit."
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P08029 The DDB1a interacting proteins CSA and DDB2 are critical factors for UV-B tolerance in *Arabidopsis thaliana*
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"Genotoxic stress imposed by UV-irradiation or chemical treatment is a permanent threat potentially affecting the genomic integrity of all live forms, but especially that of sessile organisms like plants. Consequently, several DNA repair mechanisms have evolved, one of which is the nucleotide excision repair (NER). NER comprises two separate pathways, the global genomic repair (GGR) and the pathway of transcription-coupled repair (TCR). Critical for recognition of UV-induced DNA damages to initiate GGR and TCR dependent repair processes are the proteins DDB2 (Damaged DNA Binding 2), and Cockayne Syndrome A (CSA), respectively. Both DDB2 and CSA assemble with an ubiquitin E3 ligase that contains the cullin CUL4 and the substrate adaptor DDB1. This assembly leads to the ubiquitination and subsequent degradation of DDB2, CSA and additional proteins via the 26S proteasome. Although GGR and TCR have been intensively described in mammalian cells, only poor knowledge is present for plants. Here, we report that loss of the CSA and DDB2 orthologs from *Arabidopsis thaliana* leads to an increased sensitivity of affected plants to UV stress. We provide evidence that these plants exhibit an impaired NER-dependent repair of UV induced thymidine dimers. Additionally we demonstrate that CSA assembles into a CUL4-DDB1a complex and that it is degraded by the 26S proteasome in a CUL4 and UV-B dependent manner. Furthermore we describe tissue specific expression patterns of *Arabidopsis* CSA and DDB2 using promoter:GUS constructs and RT-PCR analysis, and investigate subcellular localization of the two proteins. In summary, this work describes for the first time functions of *Arabidopsis* CSA and underscores the significance of CUL4-based E3 ligases, DDB2, and CSA for DNA repair in higher plants. "
(a) Angewandte Genetik, Freie Universitaet Berlin (b) School of Biological Sciences, Washington State University

P08030 A calcium sensor calcineurin B-like gene is involved in the regulation of cold tolerance via calcium signalling in *Arabidopsis thaliana*
Song, Chun-Peng-presenter songcp@henu.edu.cn(a) Guozeng, Zhang (a) Xiaohua, Hao (a) Ling, Bai (a)
"Low temperature is a common abiotic stress that leads to economic losses in agriculture. The earliest reported events in plant responses to low temperature are a transient increase in the concentration of cytosolic free calcium. Here, we report the function of a calcium sensor, enhanced-cold tolerance 1 (*eht1*) in response to low temperature in plants. We measured cold-induced elevation of cytosolic free calcium concentration ([Ca2+]cyt) by using aequorin-expressed Arabidopsis plants. The *eht1* mutant plants have shown more tolerance to freezing than wild-type plants under both cold-acclimating and non-acclimating conditions. Cold-triggered cytosolic free calcium responses in *eht1* mutant plants were higher than those in control, but the calcium signature adjacent to the vacuolar membrane rose to similar levels. Pharmacological research indicated the main involvement of extracellular calcium in this response. In the cbl9 mutants, transcript levels of *RD29A*, *KIN1* and *COR15A* were higher than that in the wild-type. As a Ca2+ sensor in the membrane EHT1 participates in the cold signal transduction and acts to negatively control the expression of the COR genes through calcium signaling."
(a) Henan University

P08031 Role of *GIGANTEA* gene in the regulation of salt stress signaling in Arabidopsis
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"Plants depend on environmental cue to control the flowering. Many physiological studies of the floral transition led to the identification of the several environmental signals such as day length, temperature, water supply, nutrient states and so on. Recent molecular genetic studies revealed mechanisms of these inductions of flowering. In Arabidopsis, four pathways were identified: the vernalization, gibberellin (GAs), photoperiodic and autonomous pathways. Besides these four genetic pathways, plants could flower under the intensive abiotic stress conditions, such as nutrient starvation, disease, salinity and so on. The molecular mechanisms of stress flowering pathway have not been studied. To understand whether the stress signaling could regulate flowering, Arabidopsis wild type plants had been treated salt stress (0 – 250 mM NaCl) and were examined flowering phenotypes under long day conditions. Stress treated plants exhibited early flowering phenotype. This result suggested that stress signaling could integrate the flowering pathways. We also treated salt stress to late flowering mutant, *gigantea* (*gi-3*), to detect whether the stress pathway interact

with known photoperiodic pathway or novel flowering pathways. *gi-3* plants showed hypersensitive phenotype to salt than wild type with 200mM and 250mM NaCl treatments. In *gi-3* mutant, the mRNA expression levels of *RD29A* was higher than that of wild type and the mRNA expression levels of *CBF1*, *CBF2*, and *CBF3* were lower than that of wild type. These results suggested that *GIGANTEA* could have dual functions for photoperiodic flowering pathway and stress signaling pathway."
(a) Iwate University

P08032 Effect of high temperature on pollen tube growth and energetics in the cotton pistil
Snider, John-presenter jsnider@uark.edu(a) Oosterhuis, Derrick (a) Skulman, Briggs (a) Kawakami, Eduardo (a) Storch, Diana (a)
"Successful pollen tube growth and fertilization of the ovule is a prerequisite for the development of seeds and yield development in cotton. As pollen tube growth has a high energy requirement relative to vegetative tissues, any abiotic stress negatively affecting the availability of energy reserves in the pistil should negatively impact fertilization and yield. To test the effects of heat stress on source leaf activity, pistil energy reserves and in vivo pollen tube growth, cotton plants were maintained at optimal day/night temperature regimes (30/20C) or exposed to heat stress (38/20C) conditions. Pollen tubes were observed in ovules 24 h after anthesis via UV microscopy, and pollen performance was expressed as the ratio of fertilized ovules to total ovules per ovary. At flowering, measurements included pistil soluble carbohydrates and ATP levels and subtending leaf photosynthesis, stomatal conductance, photochemical efficiency, chlorophyll content, and ATP levels. Heat stressed pistils had significantly lower pollen tube to ovule ratios, decreased soluble carbohydrate contents, and lower ATP levels relative to the control. Subtending leaf photosynthesis, photochemical efficiency, and chlorophyll content decreased under heat stress, whereas stomatal conductance increased and ATP levels remained unchanged. We propose that the major limitations to subtending leaf photosynthesis under heat stress are reduced quantum efficiency and enhanced cyclic electron flow, which maintains ATP content but decreases CO2 fixation in source leaves. Because ATP and carbohydrate levels in heat stressed pistils declined concomitantly with pollen performance, we also conclude that the energy requirements of growing pollen tubes can not be sufficiently met under heat stress. "
(a) University of Arkansas

P08033 Changes in -omics profilles in *Arabidopsis thaliana* L. exposed to explosives: serching for genes involved in their degradation
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http://www.vurv.cz
"Plants are subjectedto many stresses. Some of them are caused by polluted environment. Some gebnotypes have better ability to withstand such condition and they may be used for phytoremediation e.g. removal of polutants from the eco-systems. Up to now established phytoremediation-based systems appear to adequately and efficiently remove them from various environment at a relatively low costs. However, such successes have been achieved against a background of still limited knowledge of the mechanisms involved, so that a more systematic investigation concerning the selection of plants and optimisation of remediation processes is required. To achieve better knowledge on the mechanism how the plants cope with environmental pollution we *Arabidopsis thaliana* as a model plant and investigated changes in transcritome, proteome and metabolome after exposition to an explosive (tri-nitro-toluen) that polutes some ecosystems. Using Affymterix chips we were able to identify clusters of up/down regulated genes, with 2D electrophoresis we identified corresponding proteins and monitor changes in the metabolome. Together with investigation of physiological parametr we accquired more complex picture how the plant may overcome such stress. Genes were identified that may be potentially used for engineering of plants fitting for remediation of such xenobiotics. The research was supported by the Czech ministry of Education, Youth and Sports 2B06187"
(a) Crop Research Institute (b) Institute of Experimental Botany, Czech Academy of Science

P08034 A Novel MYBS3-dependent Pathway Confers Cold Tolerance in Rice
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"Rice seedlings are particularly sensitive to chilling in early spring in temperate and subtropical zones and in high elevation areas. Improvement of chilling tolerance in rice may significantly increase rice production. MYBS3 is a single DNA-binding repeat (1R) MYB transcription factor and previously known to mediate sugar signaling in rice. In the present study, we showed that MYBS3 also plays a critical role in cold adaptation in rice. Gain- and loss-of-function analyses indicate that MYBS3 is sufficient and necessary for conferring cold tolerance in rice. Transgenic rice constitutively overexpressing MYBS3 tolerated chilling temperature for at least 1 week, and plants were only slightly shorter than the wild type and exhibited no yield penalty in greenhouse and field growth conditions. Transcription profiling of transgenic rice overexpressing MYBS3 led to identification of two sets of genes up- and down-regulated by MYBS3. Surprisingly, MYBS3 negatively regulated the well-known CBF/DREB1-dependent cold signaling pathway in rice. DREB1 responded quickly and transiently while MYBS3 responded slowly to cold stress, which suggests distinct pathways may act coordinately in the fine tuning of gene expression necessary for cold adaptation in rice. Promoter activities of both MYBS3 and DREB1 were up-regulated and their mRNA half-lives were prolonged under cold stress, indicating a general regulatory mechanism might regulate the expression of these genes under the cold stress condition. Our studies thus reveal a hitherto undiscovered novel pathway in which cold and sugar signalings crosstalk and control cold adaptation in rice."
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P08035 The role of AIR12 as a putative extracellular regulator of developmental and abiotic stress responses in *Arabidopsis thaliana*
Gibson, Shawn W.-presenter shawn.gibson@usask.ca(a) Taylor, Janet L. (b,a) Todd, Chris D. (a)
"The development of plants challenged by environmental stress alters plant architecture through several pathways including modified hormonal responses and reactive oxygen species (ROS) production. Auxin, a phytohormone associated with every aspect of development, and abscisic acid (ABA), a phytohormone involved in abiotic stress responses use ROS. These ROS are used as secondary messengers to activate transcription of abiotic stress genes, and in developmental responses. To understand the mechanisms involved in the abiotic stress response, and how the response intersects with auxin, ABA, and ROS, we focused on *AUXIN INDUCED IN ROOTS 12 (AIR12)* in *Arabidopsis thaliana*. BLAST queries indicated high identity of *AIR12* to the putative abiotic stress response gene *COPPER INDUCED IN LEAVES 1 (CIL1)* in *Brassica carinata* suggesting a link between auxin and ROS production resulting from abiotic stress. *air12* T-DNA knockout plants display decreased bud inhibition resulting in the over-production of axillary leaves during vegetative growth and increased bolt production during reproductive growth. *air12* plants also show a 50 % reduction in lateral root length, and H2O2 concentration. Growth in the presence of H2O2 is able to restore lateral root length to wild type amounts. Preliminary analysis of *A. thaliana air12* promoter trap lines detected transcript in the apex of elongating lateral roots. Subcellular localization using *35S:GFP-*

AIR12 translational fusions confirms that AIR12 localizes to the plasma membrane and is secreted into the apoplast. We demonstrate that *air12* plants are susceptible to salt stress, but not osmostic stress and are ABA insensitive suggesting AIR12 is an extracellular protein acting downstream of abiotic stress recognition."
(a) University of Saskatchewan (b) Plant Biotechnology Institute

P08036 Functional characterization of members of stress associated proteins family for their role in tolerance to multiple abiotic stresses
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"Exposure to heavy metals as well as abiotic stresses such as drought, salt, and nutrient deficiency adversely affect the growth and productivity of crop plants and are serious threats to agriculture. Previously, plants have been engineered by overexpressing genes that provide tolerance to a single abiotic stress. However, the knowledge about the genes/gene networks that provide tolerance to multiple stresses is lacking. Recently, members of the Stress-Associated Proteins (SAP) gene family have been suggested to play significant roles in multiple abiotic stress responses in plants, however, their exact functions are not known. These proteins have been shown to be induced in response to multiple environmental stresses. In the Arabidopsis and rice genomes a total of 14 and 18 genes, respectively, coding for SAPs have been identified. These proteins contain either A20 or AN1 or both A20/AN1 zinc finger domains and some contains extra Cys2-His2 motifs at the N- or C-terminus. We have isolated and cloned members of the SAP gene family both from rice and Arabidopsis. We overexpressed two Arabidopsis AtSAP genes (AtSAP13 with two AN1 and one extra C2H2 domain; AtSAPI0 with one A20 and AN1 domain each) and two rice OsSAP genes (OsSAP16 with two AN1 and one C2H2 domain and OsSAP18 with only one A20 domain). Overexpression of AtSAP13 in Arabidopsis provided strong tolerance to multiple stresses such as zinc, cadmium, arsenic, salt, and drought, whereas, AtSAP10 provided strong tolerance to zinc, nickel, manganese and heat. We hypothesize that these proteins may interact with other proteins via protein-protein interactions. Further characterization of these proteins for their role in providing tolerance to multiple abiotic stresses is in progress. "
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P08037 Relationship between expression patterns of protein phosphatase 2A genes and mutant phenotypes
Hrabak, Estelle M. (a) Thompson, Megan M.-presenter mmp32@unh.edu(a) Wang, Enhua (a)
"Protein Phosphatase 2A (PP2A) is important for regulating a variety of cellular processes such as auxin transport, microtubule organization, and ethylene, jasmonate and abscisic responses. PP2A is composed of three subunits: A (scaffolding), B (regulatory), and C (catalytic). In *Arabidopsis thaliana*, there are five isoforms of the C subunit. We have isolated mutants in the *c4* gene (At3g58500) which have a NaCl-induced root skewing phenotype. Based on this phenotype, we expected that the *C4* gene is expressed in roots. To test this, a translational fusion construct was created with the beta-glucuronidase (GUS) reporter gene. This construct contained a genomic fragment encompassing the promoter region and the entire coding sequence. A minimum of 15 transgenic lines were generated for each construct. We analyzed: i) overall *C4* gene expression patterns at major developmental stages in the life cycle and ii) relative expression levels by incubating the transgenic plants with GUS substrate for different periods of time. Results showed that *C4* genes were expressed at about the same level throughout the plant at different developmental stages. The highest levels of expression were in the root elongation zone and root vascular cylinder. No change in expression of the *C4* gene in roots was observed in the presence of NaCl. Expression if the PP2A *C4* gene in roots is consistent with the observed phenotype of the *c4* mutants. "
(a) University Of New Hampshire

P08038 Expression of a viral aquaglyceroporin gene (AQPV1) from chlorella virus MT325 in tobacco and its effect in mitigating drought
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"Plant growth and development depend on water availability, uptake from the soil, and transport and delivery from the roots to aboveground plant organs. Aquaporins (AQPs) are trans-membrane water channel proteins that are responsible for the majority of the plant symplastic water movements. This study aimed at analyzing the expression of aquaglyceroporin gene (AQPV1) from Chlorella virus MT325 in tobacco plants and investigating its role in plant response to drought. The AQPV1 gene was placed under control of the 35S CaMV promoter and introduced into tobacco by Agrobacterium-mediated transformation. Drought tolerance was evaluated by measuring gas exchange parameters, PSII maximum photochemical efficiency (Fv/Fm), plant water relations, and biomass related parameters in the control and transgenic tobacco plants. Under favorable growth condition, there was no significant difference between the transgenic and the control plants. However, under drought condition, the transgenic tobacco plants exhibited higher photosynthetic and stomatal conductance rates, less negative water potential, and lower osmotic potential than the control plants. Total leaves dry weight, above ground dry weight, and specific leaf area was moderately improved in transgenic plants compared to the controls. No significant difference was observed in Fv/Fm. Overall, transgenic plants performed better than the control plants under drought condition suggesting that AQPV1 might improve plant drought tolerance through its involvement in plant water status regulation."
(a) School of Natural Resources, University of Nebraska-Lincoln (b) Department of Horticulture and Agronomy, University of Nebraska-Lincoln (c) Department of Plant Pathology, University of Nebraska-Lincoln (d) Department of Biochemistry, University of Nebraska-Lincoln (e) Center for Plant Science Innovation, University of Nebraska-Lincoln

P08039 Enhancement of salinity tolerance by engineering a chloride-volatilizing enzyme into plants
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"Several organisms possess enzymes that can catalyze one-step methylation of Cl- ions to chloromethane gas using *S*-adenosyl-L-methionine as methyl donor. Presence of this enzyme in organisms that live in saline habitats has been interpreted as a mechanism for Cl- detoxification via its volatilization (Science 249: 160-162), but this possibility has never been experimentally tested. While searching for chloride-methylating enzymes in plants, we identified a thiol methyltransferase (TMT) in cabbage that, aside from its natural role in the methylation of thiol compounds produced upon glucosinolate hydrolysis, was also able to methylate Cl- ions with greater efficiency than any other similar enzyme reported (J Biol Chem 270: 9250-9257; Plant Cell Environ. 23: 165-174). We cloned the gene encoding this TMT (Plant Mol. Biol. 50: 511-521), and engineered it under the control of CaMV 35S promoter into tobacco, which otherwise lacks the ability to methylate Cl-. Transgenic tobacco plants acquired the ability to efficiently transform Cl- to chloromethane over extended periods, parallel with a dramatic enhancement in their salinity tolerance. Whereas both wild type and transgenic plants grew normally in 50 NaCl, transgenic plants grew significantly better at higher concentrations of the salt. The latter were able to complete their life cycle and produce viable seed at 200 mM NaCl, which was lethal to the wild-type plants. The results convincingly demonstrate that volatilization of Cl- is a detoxification event, which can contribute to the plant's ability to withstand salinity stress. This ability can, therefore, be used to engineer crop species with enhanced salt tolerance."

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P08040 UV resistance with somatic hybrid plant regeneration of *Bupleurum scorzonerifolium*

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"To investigate the UV resistance with somatic hybrid plant regeneration of *Bupleurum scorzonerifolium*, some physiological and biochemical indicators were compared between the protoplasts of fusion parents. SCGE showed that more DNA fragments appeared in *Arabidopsis thaliana* than in *B. scorzonerifolium* after different doses of UV treatment. The accumulation of ROS was enhanced with increase in the time of exposure of samples to UV-radiation. Activities of POD increased after UV irradiation in *B. scorzonerifolium*,but did not in *A. thaliana*. UV result in GR activities decreased obviously in protoplasts of *A. thaliana*, but only little decreased in that of *B. scorzonerifolium*. Flavonoid amount increased in an extent of UV dose in the two plants, but *B. scorzonerifolium* really had a higher basal and increased content than *A. thaliana*. Protoplasts of *B. scorzonerifolium* irradiated with UV (at an intensity of 380 μ;W/cm2 for 5 min) were fused by using the PEG method with the protoplasts of *A. thaliana*. Hybrid leaves and plants resembling *B. scorzonerifolium* were obtained with a high frequency. Analysis of chromosome size and number, RAPD and nuclear SSR of *A. thaliana* respectively confirmed their hybrid nature. The UV-tolerance of *B. scorzonerifolium* with the exclusion of *A. thaliana* chromosomes and hybrid plant regeneration was discussed. Key words: *Bupleurum scorzonerifolium* Willd; *Arabidopsis thaliana* (L.) Heynh; Physiological and biochemical indicators; Asymmetric somatic hybridization"

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P08041 A proteomic study of the response to salinity and drought stress in an introgression strain of bread wheat

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"The effect of drought and salinity stress on the seedlings of the somatic hybrid wheat cv. Shanrong No. 3 (SR3) and its parent bread wheat cv. Jinan 177 (JN177) was investigated using two dimensional gel electrophoresis and mass spectrometry. Out of a set of 93 (root) and 65 (leaf) differentially expressed proteins (DEPs), 34 (root) and six (leaf) DEPs were cultivar-specific. The remaining DEPs were salinity/drought stress responsive, but not cultivar-specific. Many of the DEPs were expressed under both drought and salinity stress. The amounts of stress responsive DEPs between SR3 and JN177 were almost equivalent, whereas only some of these DEPs were shared by two cultivars. Drought responsive DEPs were less common than salinity responsive ones, and most of the drought responsive DEPs also responded to salinity. A parallel transcriptomic analysis showed that the correlation between transcriptional and translational patterns of DEPs was poor. The enhanced drought/salinity tolerance of SR3 appears to be governed by a superior capacity for osmotic and ionic homeostasis, a more efficient removal of toxic byproducts, and ultimately a better potential for growth recovery. Key words: Bread wheat, Introgression strain SR3, Salinity and drought resistance, Proteome and transcriptome"

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P08042 Fluorescent parameters of wheat genotypes *Triticum aestivum* L. under water deficit

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"Two wheat (Triticum aestivum L.) genotypes differing in drought-resistance Azamatli-95 (drought-tolerant) and Giymatli-2/17 (drought-sensitive) were used. All genotypes were grown in field conditions and dehydration was imposed by withholding water supply. Potential quantum yield of photochemical reactions of PSII (Fv/Fm ratio) in chloroplasts from control (non-drought stressed) plants was 0.74 for Azamatli-95 and 0.81 for Giymatli-2/17, that is typical for normally grown plants. State of PSII in dehydration process was being significantly changed. Potential yield of photochemical reactions of PSII undergoes appreciable changes in comparison with control plants; the highest value of Fv/Fm was in Azamatli-95 (Fv/Fm=0.71) and the lowest one in Giymatli-2/17 (Fv/Fm=0.69). It is interesting to note, that chloroplasts from non- drought-tolerant genotype Giymatli-2/17 have higher value of photochemical efficiency of PSII under regular irrigation conditions of growing. However, low ratio of Fv/Fm again confirms that strong effect of drought is appeared in genotype Giymatli-2/17 (genotype Giymatli-2/17 is strongly affected by drought). Decreasing of a photochemical efficiency (Fv/Fm) under severe drought can be considered as a fact of damage of photosynthetic reaction centers. "

(a) Institute of Botany, Azerbaijan

P08043 Light irradiation declines the chilling-induced glutathione accumulation in mung bean

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"Application of a 8oC, 36 h-chilling in dark resulted in a 2-fold increase in the glutathione (GSH) levels in 7-d-old mung bean seedlings, compared to 25oC unchilled plants. However, this chilling-induced GSH accumulation was prohibited by white-light irradiation under the same chilling treatment. The inhibitory effect of light on the GSH accumulation was enhanced with the increasing of light intensity and reached the maximal level at 100 μ;mol m-2 s-1. Whereas this light-dependent inhibitory effect promptly declined when the white-light intensity was more than 100 μ;mol m-2 s-1. Noteworthily, electrolyte leakage analysis showed that the light-reduced GSH accumulation had no negative impact on the cold tolerance of mung bean seedlings. A combination of cold treatment (8oC, 36 h) and 100 μ;mol m-2 s-1 red (640-660 nm) or blue light irradiation (420-460 nm) further indicated that red light was the main contributor for GSH inhibition in mung bean seedlings. Taken together, these results strongly suggest that in addition to growth and development, red/far-red light receptor phytochrome is also involved in the development of cold tolerance in plants."

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P08044 Effect of salicylic acid on physiology and biochemistry properties in turfgrass under salt-stress

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"The salt stress is one of the main environmental factors that inhibit plant growth and 20% global cultivated lands are imperiled. It was found that salicylic acid (SA) could induce plants to produce salt resistance, but its mechanism is not yet clear. In this study, Red Fescue (*Festuca rubra* L.) and Ryegrass (*Lolium perenne* L.) were used to study the mechanism of SA increasing salt-resistance in turfgrasses, including the change of the cell membrane permeability, the contents of proline, soluble sugar, malondialdehyde(MDA), and the activities of superoxide dismutase(SOD), peroxidase(POD)and catalase(CAT) after the treatment of exogenous SA under the 1% salt stress. The results are that Spraying SA could increase the contents of proline and soluble sugar of both turfgrass, which have positive role for increasing the capacity of osmotic stress-resistant in turfgrasses, and is able to reduce the penetration rate of the electrolyte and improve the stability of the cell membrane of two kinds of lawn grasses, and also could protect the cell membrane system from salt-stress injury, and can reduce the MDA content and increase the enzyme activity of the

SOD, POD, CAT and many other antioxidant enzymes, and improve the antioxidant ability of the cell. It was showed that the best effective concentrations of salicylic acid is 200mg/L, spraying with different concentrations of salicylic acid on the grass lawn. Key words: salicylic acid; salt stress; turfgrass; Ryegrass(*Lolium perenne* L.); Red Fescue(*Festuca rubra* L.). "
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P08045 "A bZIP transcription factor, SlAREB1, confers tolerance to drought and salinity in tomato"
Casaretto, Jose A.-presenter jcasaretto@utalca.cl(a) Orellana, Sandra (a) Yanez, Monica L. (a) Espinoza, Analia (a) Ruiz-Lara, Simon (a)
"As part of their responses to environmental stresses, plants have developed mechanisms that involve expression of genes with roles in conferring different degrees of tolerance. Among these genes, transcription factors are key regulatory end points of signal transduction cascades. Towards a better understanding of how crops respond to abiotic stress such as drought and salinity, we have previously isolated two bZIP transcription factors from tomato (*Solanum lycopersicum*), *SlAREB1* and *SlAREB2*, that share high homology with the ABF/AREB group of plant transcription factors. Their expression is induced by the phytohormone abscisic and in response to dehydration and salt treatment, however, transcript levels of *SlAREB1* showed the most significant change in shoots and roots under both stress conditions. In order to evaluate its role in conferring stress tolerance, transgenic tomato plants over-expressing and down-regulating *SlAREB1* were generated. Physiological parameters measured in transgenic sense and antisense lines subjected to water withholding or treated with 300 mM NaCl, indicate that the degree of drought and salinity tolerance correlates with the level of *SlAREB1* expression in transgenic and wild type plants. These results along with expression analyses of stress-related genes in transgenic plants, suggest that this transcription factor is involved in mediating responses to abiotic stress in tomato"
(a) Universidad de Talca, Instituto de Biologia Vegetal y Biotecnologia

P08046 Functional analysis of AP2/ERF like-transcription factor in *Physcomitrella patens*
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"Tolerance of dehydration, not avoidance of stress, may be especially advantageous for crop improvement but first it is necessary to gain an understanding of the molecular, biochemical and physiological mechanisms that underlie this trait. We have chosen plants that are desiccation tolerant, in this case *Tortula ruralis*, to address this problem. In an effort to identify genes that are essential for the expression of this important trait, we undertook extensive EST sequence expression profiling of a cDNA library from rapidly dried and rehydrated *T. ruralis* gametophytes. Using polysomal RNA isolates from hydrated, dried and rehydrated gametophytes for probe construction we identified a large number of desiccation/rehydration responsive transcripts. One of the genes that is up-regulated in response to desiccation and rehydration is an AP2/ERF like-transcription factor. For functional assessment of this transcription factor we have constructed transgenic lines of *Physcomitrella patens* (a desiccation-sensitive moss) that over-express the *Tortula* AP2/ERF like-transcription factor under the control of both constitutive and inducible promoters. We have also developed a dehydration assay using *P. patens* protonemal filaments and gametophores, to accurately assess any alteration in the dehydration tolerance of the moss resulting from transgene expression. Our preliminary studies indicate that expression of the *Tortula* AP2/ERF factor does increase the dehydration tolerance of *P. patens*. The use of this strategy for functional analysis of desiccation responsive genes should shed much light on the cellular level processes that underlie dehydration tolerance in plants. "
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P08047 Root hairs: a causal mechanism for P-deficiency tolerance in rice?
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"Pup1 [Phosphorus (P) uptake 1] is a major quantitative trait locus (QTL) for P uptake capability in rice, with Pup1 near isogenic lines (NILs) acquiring 3-4 times the amount of P from P-fixing soil than the recurrent parent (cv. Nipponbare). Genotypes containing Pup1 sustain root growth under P deficiency; however, studies have shown that the increased P uptake (used to sustain root growth) was not due to excretion of acid phosphatases and organic acids by roots or due to associations with mychorriza, nor linked to Pi transporter activity. However, among genes with the highest fold change in roots of a Pup1 NIL (NIL6-4) in a recent microarray study were three xyloglucan endotransglycosylases/hydrolases (XHTs) and a NAD(P)H-dependent oxidoreductase, all putatively associated with root hair extension. In the present study, a series of growth chamber experiments (using agar) and a glasshouse experiment (using soil) were conducted to examine root hair growth and expression of the above mentioned genes in several Pup1 NILs. The results and their implications for P uptake and root growth in rice are discussed."
(a) Japan International Research Institute for Agricultural Science

P08048 Influences of High Temperature during Grain Filling Stage on Accumulation of Storage Proteins and Grain Quality in Rice (*Oryza sativa* L.)
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"High temperature (HT) can reduce the grain yield and quality of rice. Storage proteins are important for both the development and quality of rice grains, but effect of the HT on the accumulation of storage proteins is clear. Our study was to understand the effects of HT during filling stage on the expression of storage proteins and the quality of rice grains. Storage proteins were analyzed by 1D SDS-PAGE, and differential expressed gel bands were further identified by LC'MS'MS. Transcriptions of the genes for key proteins were also determined. Results showed that HT reduced the wight, amylase content, flour gel consistency of grains. HT increased accumulation of storage proteins at early filling stage, but decreased the accumulation of prolamines and globulins at maturation. Among storage proteins prolamine and globulin were most sensitive to HT. Proteins of cyclophilin 2, peroxireducxin, glyoxalase I, RAB24 and HSP16.9 were differentially enhanced by HT. At transcription level, HT enhanced the expressions of genes for glutelin, prolamine, globulin, and protein disulfide isomerase at early filling stage; but decreased that of these genes at later stage. HT also decreased the expressions of starch biosynthesis related genes GBSS and SSIIa, and HT increased the expression of stress responsive genes PRDX, RAB24, HSP16.9c, and GloI. A schematic model is proposed to depict the influence of HT on grain quality formation in rice."
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P08049 Role of temperature stress on chloroplast biogenesis and protein import in Pea (*Pisum sativum* L)
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"Temperature stress impairs transcription and translation of several enzymes involved Chl biosynthesis and chloroplast development resulting in substantial loss of plant productivity. Modulation of photosynthesis and chloroplast protein import, by low and high temperatures, was studied in pea plants grown at 250C and subsequently exposed to 70C or 400C up to 48h. The decline in Fv/Fm (variable /maximum fluorescence) in temperature-stressed plants was substantially restored when they were transferred to room temperature. The ATP-driven import of precursor of small subunit of

Rubisco (pRSS) into plastids was downregulated by 67% and 49% in heat-stressed and chill-stressed plants respectively. Reduction in binding of the pRSS to the chloroplast envelope membranes in heat-stressed plants could be due to the down regulation of Toc159 gene/protein expression. In addition, reduced protein import into chloroplast in heat-stressed plants was likely due to decreased gene/protein expression of certain components of TOC complex (Toc75), TIC complex (Tic20, Tic32, Tic55, Tic62), stromal Hsp93 and stromal processing peptidase. In chill-stressed plants the gene/protein expression of most of the components of protein import apparatus other than Tic110 and Tic40 were not affected suggesting the central role of Tic110 and Tic40 in inhibition of protein import at low temperature. Heating of intact chloroplasts at 350C inhibited protein import implying a low thermal stability of the protein import apparatus. Results demonstrate that in addition to decreased gene and protein expression, downregulation of photosynthesis in temperature-stressed plants is caused by reduced post-translational import of plastidic proteins required for the replacement of impaired proteins coded by nuclear genome."
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P08050 Functional analysis of the cold shock domain protein AtCSP4 in *Arabidopsis thaliana*
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"In response to cold, *E. coli* accumulates cold shock proteins (CSPs) that function as RNA chaperone to destabilize RNA secondary structure for the regulation of translation under low temperature. Plant cold shock domain (CSD) proteins contain a CSD that is highly conserved with bacterial CSPs. Wheat CSD protein (WCSP1) has been characterized and shown to have an RNA chaperone activity in our previous studies. CSD proteins or Y-box proteins in animals are known to have broad function related to growth and development, however function of CSD proteins in plants is poorly understood. Here, we performed functional characterization of one of the four *Arabidopsis* CSPs, AtCSP4. *AtCSP4* mRNA was detectable at ambient temperature but was up-regulated in response to cold. AtCSP4 complemented a low temperature sensitive phenotype of the *E. coli csp* mutant. AtCSP4 showed melting activity against double-stranded DNA as a model of RNA secondary structure. These result indicated that AtCSP4 have an RNA chaperone activity. AtCSP4-promoter::GUS transgenic plants revealed tissue specific expression of *AtCSP4* in shoot apex, vascular tissues, pollens and developing seeds. Subcellular localization of an AtCSP4::GFP fusion protein was observed in the nucleus and nucleolus of *Arabidopsis* leaf cells. 35S::AtCSP4 plant showed delayed seed germination on 150mM NaCl plates, whereas a knock-out mutant of AtCSP4 exhibited accelerated seed germination under the same condition. These results demonstrated that AtCSP4 negatively regulates seed germination under salt stress condition."
(a) National Agriculture Research Center for Hokkaido Region

P08051 Response to Aluminum in ecotypes of *Medicago truncatula*
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"Aluminum (Al) makes up approximately 7% of earth's crust. Most forms of Al are non-toxic; however, under acidic conditions, as often found in crop production areas, Al is solubilized into the soil solution where it rapidly inhibits root growth. To study mechanisms of aluminum tolerance in legumes, we assessed aluminum responses in eighty-two *Medicago truncatula* accessions collected from different geographic regions. Root growth measurement and root staining assays were used to define the sensitivity of each accession. Seedlings were grown in an agar medium, containing three levels of Al and root length was measured at 24, 48 and 72 h after exposure to Al. Relative root growth (RRG) differed among genotypes. Root viability and Al sensitivity was also tested by fluoroscein diacetate and hematoxylin staining. Sensitivity/tolerance to Al, as measured by RRG was correlated with results of the root staining assays. Based on these results, we selected 3 sensitive lines and 3 tolerant lines for further analyses. Crosses were performed between tolerant and sensitive lines to develop segregating populations for genetic mapping. Results from these ongoing studies will be presented. The identification of molecular mechanisms underlying Al responses could provide information to breed for improved Al tolerance in crop legume species and other."
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P08052 "Serpins in *Oryza*, *Arabidopsis* and *Chylamydomonas*"
Ersoy, Renan A.-presenter renanaliersoy@yahoo.com(a) Ahn, Joon W. (a) Roberts, Thomas H. (a) Atwell, Brian J. (a)
"Serpins are highly adaptable proteins, most of which irreversibly inhibit protein-degrading enzymes using a unique spring-loaded mechanism. Serpin genes are ubiquitous in the Plant Kingdom, but the biological functions of plant serpins remain unknown. We aim to elucidate their roles in three model organisms: *Oryza*, *Arabidopsis* and *Chylamydomonas*. We determined the basal expression levels of eight selected serpin genes in rice at different developmental stages, and in various organs and tissues. We investigated the effects of several abiotic stresses (e.g. cold, DNA damage) and other plant treatments on expression of the gene Os03g41419, which encodes the LRS serpin. We found that LRS serpin expression at the mRNA level was much higher than that of the other serpin genes in all tissues and was relatively unchanged by a range of plant stresses; i.e. it appears to behave as a housekeeping gene. Thus the LRS protein may have an important central function required by all cell types. We are attempting to silence the LRS gene using RNAi. We have amplified the full-length LRS serpin cDNA and are producing the recombinant protein and to study its properties. We have recently shown that two *Arabidopsis* serpin genes (At1g64030 and At2g14540) are involved in growth responses to alkylating DNA damage using a reverse genetics approach. We are conducting a microarray experiment using one of the knockout mutants from this study to identify other genes associated with this plant response to MMS. The *Chlamydomonas reinhardtii* genome contains a single serpin gene. We are currently producing RNAi transformants using fragments of the serpin cDNA corresponding to the N-terminal, C-terminal and 3UTR regions inserted into a Maa/X vector to provide clues to function. "
(a) Macquarie University, Faculty of Science

P08053 "Arabidopsis thaliana calcium-dependent lipid-binding protein: involvement in plant stress responses, DNA- and lipid-binding characteristics. "
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"Understanding the components that mediate environmental stress responses is essential to enhance future genetic engineering strategies for developing stress tolerant crops. Ca2+ is one of the most important messengers of plant signaling and is an ideal target for manipulating signals from variety of pathways. The study of functions of plant proteins containing Ca2+ binding component (C2-domain) and the mechanisms behind the role of proteins in Ca2+ signaling pathways will open new perspectives for crop genetic improvement. By using a yeast one-hybrid screen assay we identified an *Arabidopsis thaliana* calcium-dependent lipid-binding protein - a small protein with a C2-like domain. Here, we define the expression of this protein as well as its DNA-binding and lipid-binding characteristics. Transcripts of this gene can be detected in all tissues examined including leaf, stem, root and flower of *Arabidopsis thaliana*. By using a gel-shift mobility assay we found that this protein can bind the specific promoter region of the pentacyclic triterpene synthase gene, *ATPEN1*. Immunological characterization of this calcium-dependent lipid-binding protein revealed that it is present in the nucleus of *Arabidopsis* root tip cells. Lipid-binding characteristics were studied by using lipid strips (Echelon, Inc.). We found that *Arabidopsis* calcium-dependent lipid-binding protein was able to interact with sulfatide (3-sulfogalactosyl ceramide). Additionally, we discovered that a mutation in the gene of calcium-dependent lipid-binding protein apparently leads to increased tolerance to salt stress in *Arabidopsis* mutant

seedlings (knockout lines from Salk Institute Genomic Analysis Laboratory). Our hypothesis is that the protein of interest is involved in stress signaling. "
(a) Department of Applied Science, University of Arkansas at Little Rock (b) Department of Plant Biology, North Carolina State University (c) Kenan Institute, North Carolina State University

P08054 Functional characterization of *A.thaliana* Glyoxalase 2-1
Devanathan, Sriram-presenter sriramdevanathan@gmail.com(a) Makaroff, Christopher A (a)
"The glyoxalase system has been studied in a number of organisms. It has been proposed that detoxification of 2-oxoaldehydes is its primary function, although the exact role(s) of this system, which consists of two enzymes: glyoxalase I (lactoylglutathione lyase) and glyoxalase II (hydroxyacylglutathione hydrolase) is unknown. While glyoxalase I, has been well characterized in several systems, much less is known about glyoxalase II enzymes, which exist as multiple isozymes in plants. In *Arabidopsis thaliana*, four putative glyoxalase II isozymes have been identified. One of these, Glx 2-1 is lacking conserved substrate binding ligands and cannot bind or hydrolyze their known substrate SLG. This indicates that GLX2-1 is not a glyoxalase II enzyme and raises the question of the role of GLX2-1? Neither insertional deletion nor constitutive over expression of GLX2-1 in *A. thaliana* has an effect on plant growth and development. Analysis of publicly available microarray data and several analysis tools did provide some insight into the functional role of GLX2-1. In experiments designed to test predictions made using the bioinformatic approaches, we have shown that in the absence of *A.t* Glx2-1, plants are more susceptible to anoxic stress, and high levels of L - threonine. Therefore we propose that *A.t* Glx2-1 is involved in amino acid degradation (more specifically threonine degradation), which is a normal plant response under stress conditions."
(a) Department of Chemistry and Biochemistry, Miami University, Oxford, OHIO

P08055 The WRKY30 transcription factor: a node of convergence for abiotic and biotic signals in plant defence
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http://www.ibr.gov.ar/eng/index.html
"In plants, oxidative stress is one of the major causes of damage as a result of various environmental stresses. Oxidative stress arises from an imbalance in the generation and removal of reactive oxygen species (ROS). Despite the deleterious effects of ROS, recent studies indicate that ROS act as signalling molecules. We used the GeneChip technology in order to monitor gene expression of the entire genome of *A. thaliana* under oxidative stress generated by MV. *WRKY30* transcription factor was highly expressed under this MV treatment. Its expression profile was studied *in silico* using Genevestigator database and *in vivo* with the reporter gene GUS fused to the *WRKY30* promoter. *WRKY30* was clearly induced under biotic or abiotic oxidative stress conditions. The convergence of these stresses was also analysed by cross tolerance experiments carried out with Arabidopsis plants. Results showed that there are multiple stress perception and signalling pathways, some of which are specific, but others may cross-talk at various steps. WRKY30 could be a node of convergence for integrating biotic and abiotic signalling events, regulating downstream genes involved in the defence against ROS damage."
(a) Instituto de Biologia Molecular y Celular de Rosario (IBR-CONICET) (b) Facultad de Ciencias Bioquimicas y Farmaceuticas, Universidad Nacional de Rosario

P08056 Characterization of physiological function of Plasma Membrane Protein 3 in salt tolerance of rice and Arabidopsis plants
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"The regulation of Na+ ion uptake and accumulation is critical for plant salt tolerance. Although the function of Plasma Membrane Protein 3 (PMP3) is not fully understood in plants, it has been shown in yeast that it plays a role in salt tolerance via contributing to the restriction of Na+ uptake. To determine its role in plants we have investigated the role of PMP3 homologues in rice and *Arabidopsis*. We have found that: 1. Disruption of *RCI2A* (*PMP3* homolog in *Arabidopsis*) leads to over-accumulation of Na+ and increased salt sensitivity in *Arabidopsis* plants. 2. Over expression of *RCI2A* decreases Na+ uptake and mitigates salinity-induced damages in *Arabidopsis* plants. The increase of malondialdehyde and of H2O2 production caused by high salinity was greater in the shoots of wild type than in those of transgenic plants. 3. Nine expressed *OsPMP3* genes have been identified (homologous to yeast *PMP3* in rice plants) and the transcript of three *OsPMP3* genes was induced by high salinity. The mRNA of salt-inducible *OsPMP3* genes was detected in mesophyll cells of leaf blades and lateral root cap cells of roots. The over expression of *OsPMP3-3* in rice plants alleviated the suppression of shoot growth under NaCl treatment via reducing Na+ uptake into the shoots. Additionally *PMP3*-knockout yeast showed hypersensitivity not only to Na+ but also to various chemicals and was complemented by six *OsPMP3* genes. These results suggest that PMP3 contributes to maintaining ion homeostasis under saline conditions and that the abundant expression of *PMP3* homologues can improve salt tolerance in plants."
(a) Graduate School of Agricultural Sciences, Nagoya University

P08057 Identification of candidate genes for enhancing multiple abiotic stress tolerances based on comparative analysis of cold stress responses among several rapeseed varieties
Jeong, Yu Jeong (a) Choy, Yoon Hi (a) Joo, Hye Joon (a) Hwang, Ji Hye (a) Byun, Yoon Jeong (a) Lee, Dong Hee (a) Lee, June Seung-presenter jslee@istep.re.kr(a,b)
"To develop the cold stress tolerant rapeseed not showing the cultivation limit in Korean, we studied the cold sensitivity of some of Korean rapeseeds and analyzed their gene expression profile using Arabidopsis 1.6K cold specialized cDNA chip. According to the hierarchical clustering pattern among the rapessed 5 varities, it was revealed that the survival results of previous freezing test was well consistent with the gene expression pattern during cold stress. Gene sets were developed from cold network model for early cold stress response and a co-expression gene set groups including each small square form gene set combinations. A few gene set were selected from cross-talk map using expression data of Arabidopsis under 9 different environmental stresses. Among the selected gene sets, a few genes were identified. As a result, genes including GST and TRX showed the enhancement of cold tolerance. These genes can be considered to be an engineering target gene to apply to enhance multiple abiotic stress tolerances."
(a) ewha womans university (b) Korea Institute of S&T Evaluation and Planning (KISTEP)

P08058 "GLO1, a Genetic Locus Important for the Gene Regulation of AtERF3"
Shen, Yun-presenter yun.shen@ttu.edu(a) Guerrero, Noemi (a) Shi, Huazhong (a)
"Ethylene responsive element binding factors (ERFs) are part of a family of transcription factors that have only been found in higher plants. In *Arabidopsis*, one of the ERF genes, *AtERF3*, is moderately induced by salinity and drought stress. To identify the signaling components controlling *AtERF3* gene expression, the promoter of *AtERF3* was fused with a luciferase reporter gene (AtERF3P-LUC). Homologous lines of transgenic plants harboring the chimeric gene construct were generated. Seeds of one homologous line were subjected to EMS-mutagenesis. A number of mutants,

designated as *glowing* (*glo* for short), showing high expression of luciferase were identified from M2 seeds by using a high sensitive, low light CCD imaging system capable of monitoring the luciferase expression level. Here we present the characterization and positional cloning of *glo1*. *glo1* mutants are more sensitive to ABA and sodium in root growth and display morphological and developmental growth phenotypes when compared with wild type. Map-based cloning established that *GLO1* is located on the upper arm of the fifth chromosome of *Arabidopsis*. Genetic crosses indicated that *glo1* is not allelic to several other *glo* mutants being characterized and genetically mapped. Further study of the expression of the native *AtERF3* gene, the luciferase transgene and other abiotic stress-responsive genes in *glo1* mutant and molecular identification of GLO1 is in progress."
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P08059 A Na+/Ca2+ exchanger AtNCL in Arabidopsis and its function in regulating Calcium ion homeostasis during salt stress
Cui, Sujuan-presenter cuisujuan@263.net(a,b) Sun, Daye (a,b) Wang, Peng (a) Li, Zhaowei (a)
"Calcium ion (Ca2+) plays a crucial role in many important physiological processes. It is important to keep the Ca2+ homeostasis under stimulation. The plasma sodium/calcium exchanger (NCX) plays a crucial role in animal excitable cell in keeping Ca2+ homeostasis. In Arabidopsis genome, there was one putative NCX gene, named AtNCL (Arabidopsis Na+/Ca2+ exchanger Like gene), encoding protein with similar NCX structure by bioinformatics prediction and different from previously known calcium/hydrion exchangers (CAX). AtNCL protein was identified to localize in the cell plasma membrane and inner membrane, have Ca2+ binding property, and show Na+/Ca2+ exchange activity in cultured mammalia CHO-K1 heterologous expression cell. AtNCL was expressed broadly in Arabidopsis, and up regulated by abiotic stresses, such as NaCl, abscisic acid, heat shock and cold stress. atncl loss of function mutants were less sensitive to salt stress than wild type and AtNCL transgenic over-expression lines in seedling growth. There was more total Ca2+ in the whole seedlings of atncl mutants than wild type by atomic absorption spectroscopy. And cytosolic free Ca2+ and Ca2+ flux, detected by transgenic Aequorin and scanning ion-selective electrode, needed longer time to recover after NaCl stress in the atncl mutants root tip than that in wild type. All these data indicated that AtNCL may be a Na+/Ca2+ exchanger in Arabidopsis and participate in maintaining Ca2+ homeostasis. And AtNCL may represent a new type of Ca2+ transporter in higher plant."
(a) Institute Of Molecular Cell Biology in Hebei Normal University (b) Hebei Key Laboratory of Molecular and Cellular Biology

P08060 Overexpression of DnaK from a holotolerant cyanobacterium *Aphanothece halophytica* enhances growth rate as well as abiotic stress tolerance of poplar plants
Takabe, Tetsuko-presenter tptakabe@agr.nagoya-u.ac.jp(a) Kajita, Hiroshi (b) Takabe, Teruhiro (c) Hayashi, Takahisa (d) Mitsuya, Shiro (a)
"The DnaK/HSP70 family is a molecular chaperon that binds non-native status of other proteins, and concerns to various physiological processes in the bacterial, plant and animal cells. Previously, we showed that overexpression of DnaK from a halotolerant cyanobacterium *Aphanothece halophytica* (*ApDnaK*) enhances tolerance to abiotic stresses such as high temperature in tobacco and rice plants. Here we tested the transformation of poplar (*Populus alba*) with *ApDnaK* for enhancing the growth rate of transformed poplar plants. Under control growth conditions, transgenic poplar plants exhibited similar growth rates with the wild-type plants during young seedlings under low light intensity, whereas they showed faster growth, larger plant size, and higher cellulose contents when poplar plants were grown under high light intensity. Transgenic young poplar plants exhibited more rapid recovery from the stresses of high salinity, drought, low temperature, and high temperature compared with those of the wild type plants when poplar plants were grown under low light intensity. These results suggest that *ApDnaK* could be useful to enhance the growth rate as well as to increase the stress tolerance."
(a) Graduate School of Bioagricultural Sciences, Nagoya University (b) SCIVAX Co. (c) Reserach Institute, Meijo University (d) Wood Research Institute, Kyoto University

P08061 Functional characterization of peroxisomal and cytosolic betaine aldehyde dehydrogenases in barley
Fujiwara, Takashi-presenter madridista_taka@hotmail.com(a) Mitsuya, Shiro (a) Hattori, Tasuku (a) Takabe, Tetsuko (a)
" Betaine aldehyde dehydrogenase (BADH, EC 1.2.1.8) is an important enzyme that catalyzes the last step in the synthesis of glycine betaine, a compatible solute accumulated by many plants under various abiotic stresses. In barley (*Hordeum vulgare* L.), we reported previously the existence of two BADH genes (*BBD1* and *BBD2*) and their corresponding proteins, peroxisomal BADH (BBD1) and cytosolic BADH (BBD2). However, little information exists on the enzymatic properties of BBD1 and BBD2. In this report, we have investigated the enzymatic properties of BBD1 and BBD2. Enzymatic analyses indicated that the affinity of BBD2 for betaine aldehyde, a precursor of glycine betaine, was 1,000-fold higher than that of BBD1 with apparent *K*m of 18.9 µ;M and 19.9 mM, respectively. *V*max/*K*m with betaine aldehyde of BBD2 was about 2,000-fold higher than that of BBD1. In addition, both BBD1 and BBD2 prefer NAD+ to NADP+ as an electron acceptor, which is agreement with those reported for other plant BADHs, and the difference in the affinity for NAD+ was not observed. These findings strongly suggest that BBD2, cytosolic BADH, plays a main role in glycine betaine synthesis in barley plants. However, BBD1 catalyzed the oxidation of σ-aminoaldehydes such as 4-aminobutyraldehyde (AB-ald) and 3-aminopropionaldehyde (AP-ald) as efficiently as BBD2 did. We have also found that both BBDs oxidized 4-N-trimethylaminobutyraldehyde (TMAB-ald) and 3-N-trimethylaminopropionaldehyde (TMAP-ald). "
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P08062 Improved tolerance to drought and salt in transgenic poplar producing glycinebetaine
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"Genes involved in biosynthetic pathway of glycinebetaine from *Escherichia coli* were modified to be expressed in plants. Transgenic poplars containing both chimeric genes (*p35S-betA* and *p35S-betB*) were developed via two rounds of transformation using *nos-nptII* and *nos-hpt* gene as selectable markers. Seven double-transgenic lines were tested for their drought tolerance using leaf disks on callus inducing medium containing different levels of PEG. The transgenic plants grew significantly better than did nontransgenic plants. Stem segments with a shoot apex were also cultured on root inducing medium containing different levels of NaCl to evaluate their tolerance against salt stress. At 125 mM NaCl, more transgenic plants survived than did nontransgenic plants. Among them, one clone (*betA*2+*betB*2) survived 100% at 125 mM NaCl. Compared to nontransgenic controls, the transgenic plants exposed to 100mM NaCl for 16 days developed far less necrosis symptoms. The transgenic plants also had higher rate of photosynthesis than did nontransgenic control under NaCl stress. Taken together, the transgenic plants attained enhanced salt and drought tolerance by the expression of *betA* and *betB* genes."
(a) Biotechnology Division, Korea Forest Research Institute

P08063 Suppression of cell division and elongation in coleoptiles of rice *reduced adh activity* (*rad*) mutant.
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"Under limited oxygen conditions, such as in submergence, plants synthesize ATP by glycolysis. Glycolysis requires NAD+, which is generated by

alcohol dehydrogenase (ADH). Rice can survive under submergence by elongation of the coleoptile. In the *reduced adh activity* (*rad*) mutant of rice, which has a reduced amount of ADH1 protein as a result of point mutation in the *Adh1* gene, coleoptile elongation is repressed. To understand what other genes are affected by the loss of ADH1 in the metabolism of submerged coleoptiles, we isolated coleoptiles from rad and wild-type (WT) embryos using laser microdissection (LM). RNA extracted from these cells was analyzed with a rice 22K oligo-DNA microarray. We identified 994 genes that were up-regulated or down-regulated in *rad* compared to the WT (p-value<0.01). The expressions of several cell elongation related genes were decreased in *rad*. This was expected, because cell elongation has been thought to be the main cause of coleoptile elongation. However, intriguingly, the expressions of cell division related genes were also repressed in *rad*, suggesting that cell division also has a role in coleoptile elongation. Transcript abundance of some cell elongation- related genes and cell division-related genes as measured by RT-PCR confirmed that, under submergence, these genes were up-regulated in the WT and down-regulated in *rad*. Furthermore, DNA synthesis, an indicator of cell division, was assayed by BrdU incorporation. Using an anti-BrdU antibody, BrdU signals were detected in the WT but not in rad, confirming a lack of cell division in *rad*. These results indicate that cell division was also activated in rice coleoptile elongation in the WT and was suppressed in *rad*. "
(a) University of Tokyo (b) National Institute of Agrobiological Sciences (c) Iwate Biotechnology Research Center

P08064 Calmodulin binding associated with cell death suppression activity of Arabidopsis Bax Inhibitor-1
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"Bax inhibitor-1 (BI-1) is a highly conserved cell-death suppressor protein that resides in the endoplasmic reticulum (ER) membranes of a range of organisms. BI-1 is important in the response of organisms to abiotic and biotic stresses. The *atbi1-1* plant has T-DNA inserted into the Arabidopsis BI-1 protein C-terminal region, which contains potential coiled-coil structures and is essential for inhibiting both Bax-induced lethality in yeast and oxidative stress-induced cell death in plant cells. We found that C-terminal 14 amino acids of AtBI-1 were capable of binding to calmodulin molecule, a mediator of calcium signaling. Furthermore, the mutant BI-1 protein (AtBI-CM) produced in *E. coli* could no longer bind to calmodulin. A promoter-reporter assay demonstrated compartmented expression of BI-1 during HR, introduced by the inoculation of *Pseudomonas syringae* possessing the *avrRTP2* gene, Pst(*avrRPT2*). In addition, both BI-1 knockdown plants and *atbi1-1* showed increased sensitivity to Pst(*avrRPT2*)-induced cell death. The results indicated that the loss of calmodulin binding reduces the cell-death suppressor activity of BI-1 in planta. "
(a) Saitama University (b) University of Tokyo (c) Iwate Biotechnology Research Center

P08065 Subtractive hybridation for water stress gene expression studies in chili pepper (Capsicum annuum L.)
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"Chili pepper is one of the most important horticultural crops in Mexico and worldwide. This crop is usually cultivated under irrigation due to its sensitivity to drought. In this work a subtractive cDNA library was established as an approach to study genes that are differentially expressed under water deficit conditions. Seeds of chili pepper (C. annuum L.) cv. Tampiqueno 74 (Serrano type) were germinated at 27 oC and 16 h light/8 h dark. Seedlings were individualized in pots and when they were 2 months old, five groups of ten plants were subjected to water deficit by water withholding (0, 3, 6, 9, 12, 15 and 18 d). Control plants (4 out of each group) were watered every 3 days. Plants (one from each group) were collected at the indicated times and divided into roots, stems and leaves. Plant tissues were frozen in liquid nitrogen and stored at -74 oC. Total RNA was extracted from pooled root, stem and leaf tissues of treated or control plants and the mRNA was isolated. Subtractive library was generated with the PCR-Select cDNA Subtraction Kit (Clontech) using the treated plants as tester and the control plants as driver. cDNA clones were sequenced at the 5 prime end, processed (http://mazorka.ira.cinvestav.mx:8080/), and 2,147 filtered sequences with an average size of 515.6 bp and a 42.6 average quality value were obtained. cDNA expressed sequences were analyzed by Gene Ontology and the highest frequency was associated to chloroplast (131) and nucleus (59), related to hydrolase (96) and transferase (80) activities, and more frequently associated to protein metabolism (183), response to abiotic or biotic stimulus (136) and response to stress (114). Thionin, metallothionein, Pin II type proteinase inhibitor were among the cDNAs with higher expected hit values."
(a) Cinvestav-Unidad Irapuato

P08066 Copper induces a calcium response involving calmodulin and protein kinases during gene expression for antioxidant proteins in *Ulva compressa* (Chlorophyta)
Moenne, Alejandra-presenter alejandra.moenne@usach.cl(a) Cabello, Susana (a)
"We studied the biochemical and molecular mechanisms involved in copper tolerance in the marine macroalga *Ulva compressa* (Chlorophyta) by analyzing a copper-induced calcium-dependent calcium release response involving calmodulin and protein kinases during the activation of genes coding for ascorbate peroxidase (AP) and metallothionein (MET). Intracellular calcium was detected by confocal microscopy and fluorophor Fluo3-AM in tissue incubated without copper and with 10 µ;M copper. Other treatments included the use of EDTA, the calcium channel inhibitors niphendipine and verapamil, the calmodulin inhibitors W-7 and fluphenazine, the protein kinase inhibitors staurosporine and cheleritrine and the inhibitor of intracellular calcium receptor ryanodine. All inhibitors decreased the release of intracellular calcium. In addition, the level of AP and MET transcripts was analyzed by real-time PCR in the alga cultivated without copper, with 10 µ;M copper for 3 days and preincubated with niphendipine, W-7, staurosporine and ryanodine. The level of AP and MET transcripts decreased with niphendipine, W-7, staurosporine and ryanodine. MET with ryanodine did not follow the same pattern and, in this case, a new PCR-amplified MET transcript was detected, probably corresponding to a novel MET gene. These results indicate that copper triggers a calcium-dependent calcium release response involving calmodulin and protein kinases in the activation of antioxidant protein gene expression. Fondecyt-Chile 1085041 to A.M."
(a) Departamento de Biologia, Facultad de Quimica y Biologia, Universidad de Santiago de Chile

P08067 Functional analysis of a novel heat shock protein isolated from soybean and *Arabidopsis*
Chung, Eunsook (a) So, Hyun-A -presenter love2284@yahoo.co.kr(a) Cho, Chang-Woo (a) Choi, Hong-Kyu (a) Kim, Kyoung-Mee (a) Kwack, Yeon-Joo (a) Kim, Kyoung-Sook (a) Lee, Jai-Heon (a)
"We have isolated wound-inducible genes from soybean using suppression subtractive hybridization (SSH) method and were able to obtain the full-length clone of W23 gene encoding DnaJ-like protein. The full-length cDNA of W23 is 689 bp with an open reading frame (ORF) consisting of 163 amino acid (aa). Genomic southern blot confirmed that soybean genome has two copies of W23 gene. Northern blot analysis showed that the RNA expression of W23 gene is specifically induced by heat, NaCl, wounding and drought stresses. It was demonstrated that W23-GFP was targeted to the nucleus in tobacco cell. Bacterial cells expressing W23, or DnaJ protein were shown to be more resistant to heat shock than the control cells with LacZ. Arabidopsis AtDnaJ gene DnaJ-like protein homologous to W23 was further characterized for the functional analysis of DnaJ-like protein. It was shown that AtDnaJ RNA expression is induced by heat shock stress and AtDnaJ-GFP was targeted to the nucleus of protoplasts. The AtDnaJ promoter (1 kb) was isolated and fused to the GUS reporter gene to investigate gene regulation of AtDnaJ specific to heat shock stress or to developmental organ in the transgenic lines. RNAi construct was employed to generate AtDnaJ knock-out plants for the study of the function of AtDnaJ. Molecular

function of AtDnaJ will be further identified in response to heat shock and also developmental stages in Arabidopsis. "
(a) Dong-A University

P08068 Improved Drought Tolerance and Increased Yield in Rice by Overexpressing *AtHDG11*
Xiang, Chengbin-presenter xiangcb@ustc.edu.cn(a) Chen, Xi (a) Wang, Yu-Ping (b) Wang, Shi-Mei (c) Wu, Yue-Jin (d) Zhu, Qi-Sheng (c) Li, Shi-Gui (b)
"The Arabidopsis *HDG11*, a homeodomain-START transcription factor, was previously demonstrated to confer improved drought tolerance and increased biomass when overexpressed in the Arabidopsis mutant *edt1* and transgenic tobacco (Yu et al., 2008). Here we demonstrate that overexpressing *AtHDG11* in rice, one of the most important cereals in the world, also confers similar phenotypes to those observed in the *edt1* and transgenic tobacco, which include improved drought tolerance and root system, reduced stomatal density, increased photosynthesis and water use efficiency. Most importantly, through three consecutive year's field trials from 2006 to 2009 transgenic rice showed consistent and significant yield increase under both normal and drought stressed conditions, demonstrating the capability of *AtHDG11* simultaneously enhancing drought tolerance and increasing yield. Results will be presented and discussed. [Yu et al. (2008). Activated Expression of an Arabidopsis HD-START Protein Confers Drought Tolerance with Improved Root System and Reduced Stomatal Density. Plant Cell 20: 1134-1151]."
(a) School of Life Sciences, University of Science and Technology of China (b) Rice Research Institute, Sichuan Agricultural University, Chengdu 611130, China (c) Rice Research Institute, Academy of Agricultural Science of Anhui Province, Hefei 230031, China. (d) Key Laboratory of Ion Beam Bioengineering, Institute of Plasma Physics, Chinese Academy of Sciences, Hefei, Anhui 230031, China.

P08069 Production of soybean transgenic lines with various functions using highly efficient soybean transformation system
Kim, Mi-Jin-presenter cutin-kmj@hanmail.net(a) Kim, Hye-Jeong (a) Jeon, Eun-Hee (a) Pak, Jung-Hun (a) Lee, Dong-Hee (b) Chung, Young-Soo (a)
"Korean soybean variety Kwangan was transformed with several genes using highly efficient soybean transformation system. The genes are RNAi construction of soybean mosaic virus coat protein gene (SMV-CN-2 RNAi), leaf senescence delaying gene (ORE7), salt stress resistance gene (AtSIZ). Three genes were transformed into Kwangan and kept growing all the transgenic soybeans to harvest seeds. Most transgenic soybean lines produced T1 seeds and their T1 plants are investigated for gene introduction and their expression. PCR, RT-PCR, southern blot were carried out using those putative transgenic plants. Soybean mosaic virus test has been performed to select genuine transgenic soybeans among SMV-RNAi transformants with viral resistance. For the two other genes, Ore7 and AtSIZ, we prepared a set of seeds to investigate target trait expression such as senescence delay and salt stress resistance. "
(a) Dong-A University (b) Venture Bldg 306 Pohang Techno Park

P08070 "Overexpression of ubiquitin conjugating enzyme, *OgUBC*, derived from wild rice (*Oryza grandiglumis*) gave resistance against UV-B radiation"
Kim, Mi-Jin (a) Jeon, Eun-Hee-presenter ccomadakgy@hanmail.net(a) Pak, Jung-Hun (a) Kim, Hye-Jeong (a) Lee, Hye-Young (a) Jeung, Ji Ung (b) Chung, Young-Soo (a)
"One of the wild rice species *Oryza grandiglumis* is tetraploid (2n=48, CCDD). It has been known to own resistance against sheath blight, rice blast and bacterial leaf blight. *OgUBC* contains 447 nucleotides and 148 amino acids. The protein has 95.4% sequence homology to *Oryza sativa* ubiquitin conjugating enzyme (UBC). The *OgUBC* gene induced by wounding, fungal elicitor, jasmonic acid (JA) and salicylic acid (SA), protein phosphatase inhibitors cantharidin (CN) and endotall (EN) as well as UV-B. To identify *in vivo* function of *OgUBC* gene, the gene was transformed into *Arabidopsis thaliana*. After the *OgUBC* transgenic plants were exposed to UV-B for 4 hours and for further observation exposed to UV-B for 2 hours, the degree of damage was divided into 3 levels. The survival rate of *OgUBC* transgenic plants under UV exposure greatly increased and damage level of *OgUBC* transgenic plants was lower compared to the wild type. After initial tolerance screening on whole plants, root-bending assay carried out. The root growth was less inhibited by UV-B exposure in *OgUBC* transgenic plants compared to the wild type. To investigate the effect of *OgUBC* on cell damage by UV-B radiation, ion leakage was measured after exposed to UV-B for 30, 60, 90 min. UV-B damaged more on wild type plants than *OgUBC* transgenic plants and led to more severe breakage in cell membrane. Our results suggest that *OgUBC* gene may involve in tolerant response of plant against UV irradiation. "
(a) Dong-A University (b) National Institute of Crop Science

P08071 Novel wild rice class IV chitinase (*OgChitIV* and *OgChitIVa*) mRNA expression is modulated by signaling components and enhanced fungal resistance in Arabidopsis
Kim, Mi-Jin (a) Pak, Jung-Hun -presenter 19970820@hanmail.net(a) Jeon, Eun-Hee (a) Kim, Hye-Jeong (a) Lee, Hye-Young (a) Chung, Young-Soo (a)
"*Oryza grandiglumis* Chitinase IV (*OgChitIV*) and Chitinase IVa (*OgChitIVa*) cDNA encoding a class IV chitinase were cloned from wild rice. The deduced amino acid sequence shows 55 to 95% identity to chitinases from other plants. *OgChitIV* cDNA contains an open reading frame of 690 nucleotides encoding 229 amino acid residues with a predicted isoelectric point (pI) and molecular weight (Mw) of pI/Mw: 8.79/25278.49. *OgChitIVa* cDNA contains an open reading frame of 867 nucleotides encoding 288 amino acid residues with a predicted of pI/Mw: 8.48/30423.85. Deduced amino acid sequences of *OgChitIVa* include the signal peptide and chitin-binding domain in the N-terminal domain and conserved catalytic domain. *OgChitIV* and *OgChitIVa* showed significant similarity at the amino acid level with related monocotyledonous rice and maize chitinase, but low similarity with dicotyledoneous chitinase. Southern blot analysis showed that *OgChitIV*a genes are present as two copies in the wild rice genome. It was shown that RNA expression of *OgChitIV* and *OgChitIVa* was induced by defense/stress signaling chemicals, such as jasmonic acid, salicylic acid, and ethephon or cantharidin and endothall or wounding, and yeast extract. It was demonstrated that overexpression of *OgChitIV* and *OgChitIVa* in Arabidopsis resulted in mild resistance against the fungal pathogen, *Botrytis cinerea*, by lowering disease rate and necrosis size. Here, we suggest that a novel *OgChitIV* and *OgChitIVa* gene may play a role in signal transduction process in defense response against *B. cinerea* in plants."
(a) Dong-A University

P08072 Identification of novel RNA masking system during deacclimation of *Arabidopsis* plants
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"Overwintering plants are capable of exhibiting high levels of freezing tolerance, which is acquired through cold acclimation. Exposure to non-freezing low temperature increases freezing tolerance of plants. In contrast, the acquired freezing tolerance is rapidly reduced in deacclimation step. In deacclimation step, plants resume growth after sensing warm temperature. It is important to clarify the mechanism of deacclimation step for understanding plant growth and development. However the detailed mechanism of deacclimation is not fully understood. In order to understand the

molecular mechanism of deacclimation step, we focus on the RNA masking system, which is an RNA regulation mechanism in translational step involved in RNA stability. RNA masking regulates protein expression by repressing translation and the translation is started by environmental changes or developmental signals. To understand the RNA masking system, we performed comparative analysis between transcriptome and proteome to identify the target mRNAs of RNA masking. According to these analyses, we identified several candidates of target mRNAs whose proteins were specifically increased in deacclimation step. These target mRNAs encoded enzymes involved in primary metabolism providing energy to resume plant growth. The initial response system in deacclimation step of plants will be discussed. "
(a) RIKEN Plant Science Center (b) Iwate University, Cryobiofrontier Research Center (c) Yokohama City University, Kihara Institute for Biological Research

P08073 Rice OsDEG10 encoding a small RNA-binding protein is involved in abiotic stress signaling
Park, Hee-Yeon-presenter phy820511@hanmail.net(a) Kang, In Soon (a) Han, Ji-Sung (a) Lee, Choon-Hwan (a) An, Gynheung (b) Moon, Yong-Hwan (a)
"Excessive light can be harmful to photosynthetic apparatus since it causes photoinhibition and photooxidation, and plants often encounter hypoxic or anoxic environments when they become submerged by heavy rain or an ensuing flood. In this study, *Oryza sativa Differentially Expressed Genes* (*OsDEGs*) from rice under photooxidation and anoxia conditions were isolated using DD-PCR. Among them, *OsDEG10* is predicted to encode a small RNA-binding protein (RBP) and the transcript levels of *OsDEG10* strongly increased under most of abiotic stress treatments such as high light, anoxia, NaCl, ABA, MV and cold. However, the transcript levels of two rice OsDEG10 homologs were not changed under those treatments. *OsDEG10* RNAi transgenic plants were more sensitive to high light and cold stresses compared to wild-type plants. Our results suggest that OsDEG10 is a small RBP involved in the response to various abiotic stresses."
(a) Pusan National University (b) Pohang University of Science and Technology

P08074 Characterization of an Arabidopsis mutant with altered *NCED3* expression induced by drought stress
Kim, Ji Hong-presenter biomoon@ijnc.inje.ac.kr(b) Kang, In-Soon (b,a) Moon, Byoung Yong (b) Moon, Yong-Hwan (a) Lee , Chin Bum (c)
"Abscisic acid (ABA) plays a pivotal role in the abiotic stress-induced signal transduction pathway and *NCED3* (*9-cis-epoxycarotenoid dioxygenase 3*) is one of key genes responsible for ABA biosynthesis. In the present study we aimed at identifying upstream genes regulating ABA biosynthesis involved in abiotic stress-responsive signal transduction. We obtained several Arabidopsis mutants with changed promoter activity of *NCED3* from EMS-mutagenized seed pools of *NCED3::LUC* transgenic Columbia. We screened those mutants whose *NCED3* expressions were down-regulated after drought stress using LUC bioluminescence imaging technique, and denoted them as *lenc* (for *low expression of NCED3*) mutants. We selected *lenc2* which showed pale-green phenotype together with lowered transcript level of *NCED3* and *RAB18* in normal condition. The mutant was further analyzed in terms of growth, pigment compositions, photosynthetic performances as well as the membrane fatty acid composition. *lenc2* was sensitive to salinity stress, but was more resistant to oxidative stress. Chlorophyll content of *lenc2* was very low, in contrast to that of carotenoids which was quite high as compared to the wild-type plants. When chloroplasts were isolated from leaves and the PS II-mediated electron transport was measured, *lenc2* showed higher activity, indicating its photosynthetic machinery was normally operating in spite of decreased chlorophyll content. Moreover, when the membrane lipids and the fatty acid compositions were analyzed, those of phospholipids, namely phosphatidylglycerol and phosphatidylcholine were quite different as compared to the wild-type plants. We suppose that LENC2 has a role in the positive regulation of *NCED3* expression during drought-induced signaling."
(a) Pusan National University (b) Inje University (c) Dong-eui University

P08075 The role of ABA-regulated RNA-binding proteins in ABA-dependent germination and growth of *Arabidopsis thaliana*
Hyun Ju, JUNG-presenter dokgu82@gmail.com(a) Hunseung, KANG (a)
"In recent years, it is increasingly evident that abscisic acid (ABA) is one of regulatory factors in the control of posttranscriptional RNA metabolism. RNA-binding proteins (RBPs) are implicated to be regulated by ABA in a variety of cellular processes, but the roles of ABA-regulated RBPs in the control of RNA metabolism remain largely unknown. Here, we identified two RBPs in *Arabidopsis thaliana*, Mei2 C-terminal RRM like protein1 (MCT1) and ABA-regulated RBP (ARP), as highly ABA-regulated genes, and investigated their roles in the response of plant to ABA. The expression of *MCT1* was markedly up-regulated, while *ARP* expression was highly down-regulated by ABA. *MCT1* and *ARP* were predominantly expressed in the flowers and buds compared with other tissues, and ARP was also strongly expressed in the roots. Analysis of GFP-fusion protein confirmed that MCT1 and ARP are localized to the nucleus in Arabidopsis and tobacco plants. MCT1 and ARP proteins harboring a conserved RNA-recognition motif were able to bind different mRNA targets. Loss-of-function mutants of ARP lead to ABA hypersensitivity, such as delayed seed germination and green cotyledon formation, and MCT1 RNAi lines and overexpression plants showed different ABA response. This results show that MCT1 and ARP affect ABA-regulated seed germination and seedling growth of *Arabidopsis*, and suggest that the control of posttranscriptional RNA metabolism is important for the response of plants to ABA. "
(a) Chonnam National University

P08076 *LENC1* and *CENC1* are involved in the regulation of *NCED3* gene expression under osmotic stress in Arabidopsis
Woo, Dong-Hyuk-presenter humblewoo@hanmail.net(a) Park, Hee-Yeon (a) Kang, In Soon (a) Moon, Byoung Yong (c) Lee, Chin Bum (b) Moon, Yong-Hwan (a)
"A plant hormone, abscisic acid (ABA), is known as a main signal transducer that confers abiotic stress tolerance to plants. In this study, to identify upstream genes regulating ABA biosynthesis involved in abiotic stress signal transduction, Arabidopsis mutants with changed promoter activity of *9-cis-epoxycarotenoid dioxygenase 3* (*NCED3*), a key gene in ABA biosynthesis, were identified and characterized. Among these mutants, *lenc1* (for *low expression of NCED3 1*) showed lower *NCED3* promoter activity compared with wild-type after dehydration treatment and *cenc1* (for *constitutive expression of NCED3 1*) showed constitutively high *NCED3* promoter activity without dehydration treatment. RT-PCR analysis indicated that the transcript level of *NCED3* gene in *lenc1* was lower under dehydration treatment but in *cenc1* was high without dehydration treatment. *lenc1* mutant was sensitive to LiCl and showed different responses to MV depending on developmental stages. On the other hand, *cenc1* mutant was resistant to NaCl and its root elongation was more suppressed by KCl treatment. The size of *lenc1* was small, but *cenc1* was almost same as wild-type on soil. The aerial part of *lenc1* lost water faster than wild-type possibly due to larger opening of stomata. Our results suggest that *LENC1* and *CENC1* might act as a positive regulator and a negative regulator, respectively, in the regulation of *NCED3* gene expression under osmotic stress. "
(a) Pusan National University (b) Dong-eui University (c) Inje University

P08077 Root development strongly enhanced by stoller's bioforge
Liptay, Albert-presenter aliptay@hotmail.com(a) Salzman, Ron A (b) Stoller, Jerry H (a)

"BioForge, a di-formyl urea product, has strong root-producing effects on crop plants, used either as a seed treatment or applied to the furrow in which the transplant is placed to establish crops. BioForge enhances seed germination of seedling development with more and larger root systems. In gene expression studies, BioForge up-regulates the master gene dreb1a, outlined by Kasuga to enhance drought tolerance, salt tolerance and frost tolerance. BioForge suppresses ethylene production in plants and also has a positive effect on nodule development in legumes as attested by field and gene regulation studies. The resulting combined effects is enhancement of yields of a number of crops."
(a) Stoller Enterprises Inc (b) Texas A&M University

P08078 Studies on the functions of HMA3 as a Cd/Pb transporter in *Arabidopsis thaliana*
Ahn, Sung-Ju-presenter asjsuse@chonnam.ac.kr(a) Jang, Ha-Young Jang (c) park, Won (a) Park, Ha-Na (a) Chung, Gap-Chae (b) Bae, Hyun-Jong (a)
"Heavy-metal P1B-type ATPases (HMAs) are transmembrane proteins that contribute to metal homeostasis in plants. HMA3 is one of P1B-ATPase of *Arabidopsis thaliana* which transports and/or stores Cd and Pb in the cell. To test whether AtHMA3 can be used to generate resistant plant grown at the contaminated soil with heavy metals, we cloned and overexpressed AtHMA3 in *Arabidopsis* (Col-0) using a pBI121 vector containing 35S promoter. Wild type, three different T3 homozygous AtHMA3 over lines (HMA3-1~3) and T-DNA insertion lines (*hma3-1~3*) were tested. When grown under 50μ;M Cd for 9 days, roots of transgenic lines grew better than wild type and knock-out mutant. In the 500μ;M Pb and combination of 50μ;M Cd+500μ;M Pb mediums, AtHMA3 overlines also showed characteristics of Cd and Pb resistance compared to wild type and knock-out mutant. Chlorophyll content in overline was not affected by the presence of higher amount of Cd and Pb, indicating the effective transport system from root to shoot. In addition, AtHMA3 gene transformed and expressed in *E. coli* improved the resistance to Cd and Pb. Further research on the determination of the cellular and subcellular localization of HMA3 using the GUS reporter gene and GFP fusion protein in *Arabidopsis* is under way."
(a) Bioenergy Research Institute (b) Agricultural Plant Stress Research Center (c) Interdisciplinary Program of graduate School for Bioenergy and Biomaterials

P08079 Functional characterization of a chloroplast antioxidant 2-Cys Prx induced by low temperature in mungbean (*Vigna radiata* L.)
So, Hyun-A (a) Cho, Chang-Woo -presenter ccw3307@daum.net(a) Lee, Jai-Heon (a) Chung, Eunsook (a) Choi, Hong-Kyu (a) Heo, Jee-Eun (a) Jeon, Eun-Hee (a) Kim, Kyoung-Mee (a) Kwack, Yeon-Joo (a) Kim, Kyoung-Sook (a) Chung, Young-Soo (a)
"We isolated several low temperature inducible genes using suppression subtractive hybridization (SSH) method and were able to obtain to clone MLT107 gene encoding 2-cys peroxiredoxin. The full-length cDNA of MLT107 is 1,049 bp with an open reading frame (ORF) consisting of 261 amino acid (aa). Genomic southern blot confirmed that mungbean genome has one copy of MLT107 gene. Northern blot analysis was carried out for the gene expression during low temperature, ABA, NaCl, drought, wounding and H2O2 stresses. The RNA expression of MLT107 gene was significantly decreased by ABA, NaCl and drought stress, but wounding, low temperature and H2O2 stresses significantly induced MLT107 RNA expression. It was shown that MLT107-GFP was targeted to chloroplast and the N-terminal chloroplast transit peptide is required for its targeting to the chloroplast in tobacco protoplasts. For the functional analysis of MLT107, MLT107 recombinant protein was heterologously expressed in E. coli. The MLT107 recombinant protein showed moderate antioxidant activity compared to other antioxidant enzymes. The role of MLT107 was investigated using MLT107 overexpressing Arabidopsis during environmental stresses. "
(a) Dong-A University

P08080 Molecular characterization of DEAD-box RNA helicase isolated from soybean
So, Hyun-A (a) Chung, Eunsook -presenter grace77@dau.ac.kr(a) Cho, Chang-Woo (a) Choi, Hong-Kyu (a) Kim, Kyoung-Mee (a) Kwack, Yeon-Joo (a) Kim, Kyoung-Sook (a) Lee, Jai-Heon (a)
"DEAD-box RNA helicase plays a crucial role in RNA processing and gene regulation. We isolated a low temperature-inducible DEAD-box RNA helicase from soybean. The full-length cDNA of LT248 contains an open reading frame of 1,617 nucleotides of 538 amino acids containing a DEAD-box RNA helicase motif. Genomic DNA blotting showed that there are two copies of LT248. Northern blot showed that LT248 mRNA was induced by low temperature at early time or NaCl, but not by ABA and drought stress. Based on GFP targeting experiment, LT248(1-355)-GFP fusion protein was localized to the nucleus, but LT248(130-355)-GFP fusion protein to the cytoplasm. This shows that the N-terminal region of LT248 is necessary for the nuclear targeting of LT248. To examine the function of LT248, LT248 was expressed in Escherichia coli as GST fusion protein. Purified GST-LT248 showed RNA helicase activity in vitro. We propose that LT248 plays an important role in RNA process regulating gene expression during abiotic stresses. "
(a) Dong-A University

P08081 Functional analysis of mungbean ubiquitin conjugating enzyme induced by salt stress and abscisic acid
So, Hyun-A-presenter love2284@yahoo.co.kr(a) Kang, Jee-Sook (a) Chung, Eunsook (a) Cho, Chang-Woo (a) Choi, Hong-Kyu (a) Kim, Kyoung-Mee (a) Kwack, Yeon-Joo (a) Kim, Kyoung-Sook (a) Lee, Jai-Heon (a)
"A low temperature-inducible cDNA designated as MLT113 from mungbean was isolated by suppression subtractive hybridization method. By rapid amplification of cDNA end technique, the full-length cDNA of MLT113 was obtained. The full-length cDNA of MLT113 contains an open reading frame of 444 nucleotides in length and capable of specifying a 16.5-kDa protein of 148 amino acids (aa) with an isoelectric point of 7.72. MLT113 mRNA was induced by NaCl and ABA, but not by wounding and low temperature stress. To examine the localization of MLT113, MLT113-GFP was expressed in tobacco cells. It was shown that MLT113-GFP was localized to the cytoplasm in tobacco cell. To examine the function of MLT113 as ubiquitin-conjugating enzyme E2, MLT113 was expressed in Escherichia coli as His-fusion protein. Purified MLT113-His recombinant protein was shown to have ubiquitination activity in vitro. For the in vivo functional analysis of MLT113, MLT113 was expressed in yeast ubc9 knock-out mutant. Stress tolerance will be tested in the MLT113 overexpressing Arabidopsis transgenic plants. We propose that MLT113 play an important role in protein degradation processes during abiotic stress in plants. "
(a) Dong-A University

P08082 Metabolome analysis of oxidative stress response in rice suspension cells overexpressing cell death suppressor Bax inhibitor-1
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"Bax inhibitor-1 (BI-1) is an endoplasmic reticulum-localized cell death suppressor widely conserved in higher plants and animals. Although it is well-known that overexpression of BI-1 in plants leads to enhanced tolerance to various stresses, physiological aspects of BI-1 overexpressing plants are still unclear except for the cell death suppressing activity. Here we report effects of BI-1 overexpression in rice on metabolic responses to oxidative

stress. Rice suspension cells overexpressing Arabidopsis BI-1 (AtBI-1) displayed enhanced resistance to cell death induced by menadione-mediated oxidative stress. Capillary electrophoresis-mass spectrometry (CE-MS)-based metabolome analysis revealed that cell death-inducible oxidative stress caused dynamic alteration in primary metabolism in rice cells, such as a shift of carbon flow from glycolysis to oxidative pentose phosphate pathway, imbalance in redox state and energy charge, and accumulation of amino acids. In normal growth condition and an early response to oxidative stress, there was no significant difference in metabolite composition between vector control and AtBI-1 overexpressing rice cells. Meanwhile, at 24 h after menadione treatment, AtBI-1 overexpressing rice cells exhibited a distinctive metabolic profile, in which disturbance of redox state and energy charge was recovered. These results suggest that AtBI-1-mediated cell death suppression leads to enlarged capacity for metabolic acclimation to oxidative stress."
(a) Graduate School of Science and Engineering, Saitama University (b) Japan Science and Technology Agency (JST), Core Research for Evolutional Science and Technology (CREST) (c) Institute of Molecular and Cellular Biosciences, University of Tokyo (d) Iwate Biotechnology Research Center

P08083 Alterations in gene expression due to the overexpression of LOV kelch protein 2 (LKP2) in transgenic Arabidopsis
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"The LOV kelch protein 2 (LKP2) comprises three functional domains: the LOV, F-box, and kelch repeat.The LOV domain is involved in blue light perception and protein-protein interaction. The F-box motif is included in SCF complex, and the kelch repeat is a protein-protein interaction module. Arabidopsis plants that overexpressed LKP2 cDNA exhibited elongated hypocotyl under light conditions, delayed flowering time under long-day conditions, and arrhythmia under continuous light or dark conditions. In this stydy, whether overexpressor of LKP2 affect other phenomena, we used DNA microarray analysis to identify genes with altered expression levels in the LKP2 overexpressor. Microarray analysis revealed that the expression levels of some of the genes were altered in the LKP2 overexpressor as compared to the expression levels in the wild type. In addition, the upregulated genes included some plant hormone-responsive and stress-responsive genes. In order to examine the effect of LKP2 overexpression in stress responses, we assessed the expression levels of stress-responsive genes and stress tolerance in the LKP2 overexpressor."
(a) United Graduate School of Agricultural Sciences, Ehime University (b) Faculty of Science, Gakushuin University (c) RIKEN, Bio Resource Center (d) RIKEN, Plant Science Center

P08084 Characterization of Arabidopsis RopGEF Family Genes in Response to Abiotic Stresses
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"Rho-related GTPase of plants (ROP) plays an important role in plant growth and development as a signaling protein. Plant RopGEFs are recently identified ROP activator proteins in Arabidopsis. In this study, we cloned 14 RopGEFs in Arabidopsis and characterized their expression patterns in response to abiotic stresses. Fourteen RopGEF genes were categorized into three groups based on their amino acid homologies and molecular sizes. Most RopGEFs were expressed predominantly in flower but some RopGEFs displayed a tissue specific expression pattern. RopGEF1, 4, 5 and 11 were expressed in all tissues including roots and leaves whereas RopGEF7, 8, 9 and 13 were expressed only in flowers. Histochemical analysis of transgenic plants using GUS tagged RopGEFs showed the GUS activity of four RopGEFs (1, 4, 5 and 6) in roots, leaves, flowers and young seedlings. However, RopGEF10 and RopGEF12 were expressed in the roots and flowers and RopGEF9 was not expressed in any tissues except of flowers. The transcript levels of 14 RopGEFs were changed significantly depending upon abiotic stresses such as cold, heat, drought and salts. RopGEF5 transcription was up-regulated by salt and drought treatment but down-regulated by heat. RopGEF14 transcript level was also increased by salt but decreased by heat stress. The transcript levels of RopGEF1, 7, 9, 12 were enhanced in response to heat stress but no changes to cold stresses. Drought stress activated group3 RopGEFs such as RopGEF5 and 7. Taken together, 14 RopGEFs are responding to the abiotic stresses individually or as a group."
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P08085 Expression analysis of genes for development of bladder cells in an epidermal bladder-cell-less mutant of the common ice plant *Mesembryanthemum crystallinum*
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"The common ice plant *Mesembryanthemum crystallinum* L. develops specialized trichome cells called epidermal bladder cells (EBCs) on all aerial surfaces. Physiological analysis of this EBC-less mutant clearly showed that EBCs contribute to salt tolerance by maintaining ion sequestration and homeostasis within photosynthetically active tissues, while also serving as a water storage organ (Agarie et al., (2007). J. Exp. Bot. 58:1957). We compared transcript abundance of trichome-related genes such as *TTG1*, *GL2*, *TRY*, *CPC*, *AN*, *TFCA*, *WRM* and *CRK* and fiber-related genes such as *RDL*, *MYB2*, *SUT1*, *EXP1*, *ABP*, *MAPK*, *RAC1*, *ACT1*, *PFN1*, *CER6*, *EF1A4*, *ACY*, *FDH*, *SCP*, *TUA6*, *TUB1*, *ACT*, *CesA* and *Susy* between the EBC-less mutant and wild-type plants. Among these genes, *GL2* (contributes to trichome development) and *MYB2* (a seed-trichome production-related gene) showed reduced steady-state transcript abundance and *TRY* (limits trichome initiation) and *CPC* (inhibits trichome initiation in cells surrounding trichome precursors) showed increased transcript abundance in the mutant. Seventeen unknown genes were obtained from cDNA-based suppression subtractive hybridization (SSH) PCR. Five genes and two genes of these genes were expressed highly in the mutant and wild-type plants, respectively. Sequencing analysis of SSH-derived cDNAs showed that the two highly expressed genes in the mutant have homologies with a tobacco cysteine protease and a grape protein kinase, respectively. One gene that was expressed highly in wild-type plants has homology with a gene encoding a jasmonate-inducible protein. The molecular mechanisms associated with epidermal bladder cell development in the common ice plant *M. crystallinum* will be discussed."
(a) Saga University (b) University of Nevada, Reno

P08086 Dual action of the ethylene for antioxidative responses with a low threshold level during stress response in plants
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"Reactive oxygen species (ROS) play a crucial role in many cellular responses and signaling pathways. ROS are inevitable by-products of many redox reactions in eucaryotic cells, and are also generated in the reactions catalyzed by NADPH oxidase. The interactions among ROS, ethylene and antioxidant enzymes in leaves of tobacco (*Nicotiana tavaccum* L.) plants under the environmental stress are studied. The treatment of ACC, a precursor of ethylene biosynthesis, produced a biphasic ethylene production peaked at 3 h and 24 h in concentration-dependent manner. After treatment with low level of ACC at 1 μ;M concentration, gene expression of antioxidative enzymes such as cytosolic *ascorbate peroxidase*, *CuZnSOD*, and *MnSOD* was induced at highest level than other higher concentrations. These results that the highest capability of ROS detoxification was appeared at 1 μ;M level of ACC implied that efficiency for ROS detoxification can be significantly responsible for signaling in further tolerant or harmful response. In plants treated under the threshold level of stress, ethylene produced at moderate level, and then ROS detoxification machinery induced efficiently, which might result in tolerant response. However, in plant treated with serious level of stress, which resulted in higher production of ethylene than endurable level, meet an ultimate death by less efficient of ROS detoxification. The level of ethylene and ROS accumulation at early

stage might determine whether cells live or die, by inducing further responsive level for ethylene synthesis and ROS detoxification at later stage. The tolerant response is in inverse proportion to the amount of ethylene production and ROS detoxification at higher level of threshold during stress response."
(a) Sunchon National University

P08087 Arabidopsis thioredoxin reductase type C (ANTR-C) functions as an electron donor of 2-Cys peroxiredoxin in chloroplast
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"2-Cys peroxiredoxins (Prxs) play important roles in the antioxidative defense systems of plant chloroplasts. In order to determine the interaction partner for these proteins in Arabidopsis, we used a yeast two-hybrid screening procedure with a C175S-mutant of Arabidopsis 2-Cys Prx-A as bait. A cDNA encoding an NADPH-dependent thioredoxin reductase (NTR) isotype C was identified and designated ANTR-C. We demonstrated that this protein effected efficient transfer of electrons from NADPH to the 2-Cys Prxs of chloroplasts. Interaction between 2-Cys Prx-A and ANTR-C was confirmed by a pull-down experiment. ANTR-C contained N-terminal TR and C-terminal Trx domains. It exhibited both TR and Trx activities and co-localized with 2-Cys Prx-A in chloroplasts. These results suggest that ANTR-C functions as an electron donor for plastidial 2-Cys Prxs and represents the NADPH-dependent TR, Trx system in chloroplasts.[Supported by EB-NCRC & BK21 program] "
(a) Division of Applied Life Science (BK21 Program), EB-NCRC and PMBBRC

P08088 Knock-down of a thioredoxin m in rice by RNA interference (RNAi)
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"Plant cells contain several thioredoxin isoforms which are generally characterized according to their subcellular localization and substrate specificity. Here we describe the functional characterization of an Oryza sativa thioredoxin m isoform (Ostrxm) using a reverse genetics technique. Ostrxm showed green tissue-specific and light-responsive mRNA expression. Ostrxm was localized in chloroplasts of rice mesophyll cells, and the recombinant protein showed DTT-dependent insulin β-chain reduction activity in vitro. RNA interference (RNAi) of Ostrxm resulted in rice plants with developmental defects including semi-dwarfism, pale green leaves, abnormal chloroplast structure, and reduced carotenoid and chlorophyll (Chl) contents. Ostrxm RNAi plants showed remarkably decreased Fv/Fm values under high irradiance conditions (1,000 μ;mol m&−2 s&−1) with delayed recovery. Two-dimensional electrophoresis and MALDI-TOF analysis showed that the levels of several chloroplast proteins critical for photosynthesis and biogenesis were significantly decreased in Ostrxm RNAi plants. Furthermore, 2-Cys peroxiredoxin (2-Cys Prx), a known target of thioredoxin m, was present in oxidized forms, and hydrogen peroxide levels were increased in Ostrxm RNAi plants. The pleiotropic effects of Ostrxm RNAi suggest that Ostrxm plays an important role in the redox regulation of chloroplast target proteins involved in diverse physiological functions. [Supported by EB-NCRC & BK21 program]"
(a) Division of Applied Life Science (BK21 Program), EB-NCRC and PMBBRC

P08089 Characterization of the role of putative Arabidopsis stress granule markers in translational regulation during hypoxia
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"Abiotic stresses that reduce ATP availability, such as hypoxia, rapidly and selectively reduce the translation of mRNAs as an energy conservation mechanism (Branco-Price *et al.*, 2008. *Plant J.* 56:743-55). The mRNAs that are poorly translated during the stress are remobilized to active translation complexes within minutes of reoxygenation. We hypothesize that translationally arrested mRNAs are relocated to processing bodies (PB) or stress granules (SG), the large cytoplasmic complexes known to store, sort, or degrade mRNAs in eukaryotes. Recent studies confirm the formation and function of PBs in plant cells and suggest that SGs are also conserved. *Arabidopsis thaliana* encodes at least 63 orthologs (19 gene families) of mammalian SG and PB proteins that coordinate both mRNA binding and protein aggregation. By use of ectopically expressed fluorescent-protein-tagged fusions in stable transgenics, we visualized dynamic changes in subcellular localization of the DHH1-like AtRH6, -8, and, -12, three DEAD-box RNA helicases similar to human DDX6, as well as the PB-localized decapping complex subunit AtDCP2 in response to hypoxia, heat stress and cycloheximide. The TIA1-like SG protein AtUBP1A also formed cytoplasmic foci when transiently expressed in leaf mesophyll protoplasts. To further characterize these complexes and their passenger mRNAs, we plan to immunopurify epitope-tagged versions of these proteins using endogenous promoters in mutant backgrounds, followed by mRNA and protein analysis. The elucidation of these lesser known mRNPs promises to shed light on the role of SGs in the coordination of cellular energy conservation via enhanced sequestration and selective translation during stress."
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P08090 Comparative Evaluation of Physiological Post-Harvest Root Deterioration of 25 cassava accessions
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"Cassava is the most important root crop in the tropics and is consumed by 500 million people daily. Due to its drought tolerance, ability to grow in poor soils and resistance to herbivory cassava is well suited for cultivation by subsistence farmers particularly in Africa. However its use and expansion is constrained by rapid physiological post harvest deterioration (PPD), which often starts within 24 hours after harvest, renders the root unpalatable and affects the economic value of the crop significantly. PPD is a complex process that involved changes in metabolic process and accumulation of secondary metabolites. Those metabolites include hydroxycoumarins, such as scopoletin, esculin and scopolin. The quantification of their emitted florescent has been proposal as an objective tool to evaluate PPD response in cassava. Traditionally, the evaluation of PPD has been performed by more subjective method based on the analysis of deterioration visually. Here we present data on the use of a standard visual methodology in comparison to an image analysis of hydroxycoumarins florescent accumulation. Ten month old storage roots from the Puerto Rican cassava germplasm which comprise of 25 accessions from Africa, Caribbean, Central America and South America, grown the Northwest Puerto Rico were analyzed for PPD. Our findings suggest that there was no correlation (r = 0.14) between the florescent accumulation of hydroxycoumarins and the visual symptoms five days after harvest. We concluded that the accumulation of hydroxycoumarins is not a reliable marker for evaluation of PPD response. Furthermore we were able to identify the accessions with high- and low-levels of PPD in the Puerto Rican cassava germplasm based on visual symptoms."
(a) Department of Biology, Uni. of Puerto Rico Mayaguez (b) Department of agronomy and Soils, University of Puerto Rico Mayaguez

P08091 Discovery and characterization of regulatory factors associated with drought tolerance in soybean
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Satish (a) Quach, Truyen N. (a) Tran, Huong N.T. (a)
"Recurring and prolonged periods of drought is one of the major factors affecting crop yield in many regions of the country in recent years. It is reported that the average yield losses are more than 40% in soybean due to drought stress. Plant root system plays an important role in adaptation and productivity under water stress environments. Deeper and proliferate root system help extract enough water under these environmental conditions. Research efforts to understand the physiological mechanisms and the genetic dissection of drought responses in legumes, especially in soybean, are still in the early stages. In the signal transduction cascades from perception of stress signals to stress-responsive gene expression, various regulatory factors including transcription factors (TFs) function not only as molecular switches for gene expression but also as interacting partners in the network. Focus on these networks is essential to understand the transcriptional regulatory mechanisms of root growth and plasticity under water deficits. Our overall goal is to understand functions of these regulatory factors and to dissect their specific role in plant development and stress tolerance. We have utilized functional genomics tools to discover and characterize these factors. The results reveal several stress induced and root related TFs and their *in-planta* characterization is in progress. For example, we have identified 111 NAC transcription factors, a plant specific TF family, and 31 unigenes with complete open reading frames encoding GmNAC proteins were cloned and characterized. The results of this systematic analysis of regulatory factors provide novel tools for the development of soybean plants with enhanced drought tolerance."
(a) National Center For Soybean Biotechnology and Division of Plant Sciences, University of Missouri

P08092 "Unique metabolic regulation of citrulline, a defensive compatible solute in a xerophyte wild watermelon"
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"Citrulline is an intermediary metabolite in the biosynthetic pathway of arginine, and functions as a unique compatible solute in wild watermelon, a xerophyte inhabiting in the Kalahari Desert, Africa. Citrulline has a potent activity for scavenging hydroxyl radicals, and the accumulation of citrulline in the leaves is triggered by the onset of drought stress in the presence of high light. However, the mechanisms for the accumulation have remained to be characterized. In this study, we performed comprehensive analysis on the regulation of citrulline/arginine biosynthetic pathway in wild watermelon. Enzyme assays for all the 12 metabolic enzymes revealed that activities for the first and second steps of this pathway, *N*-acetylglutamate synthase and *N*-acetylglutamate kinase, were significantly up-regulated in the leaves under the stress. Activity for carbamoyl phosphate synthetase, which supplies carbamoyl-moiety for the synthesis of citrulline, was also enhanced under drought. In contrast, argininosuccinate synthase, which catalyzes the conversion of citrulline to argininosuccinate, were down-regulated under the stress. The corresponding cDNAs for these enzymes were isolated from wild watermelon, which revealed that citrulline anabolic enzymes may be targeted to the chloroplasts, whereas the catabolic enzymes may localize in the cytosol. Analysis of the mRNA abundance suggested that expression of several citrulline metabolic enzymes were regulated at the transcriptional level. Taken together, these observations suggested that wild watermelon possesses unique regulatory mechanisms of the citrulline metabolic pathway, to achieve massive accumulation of this compatible solute under severe environmental conditions."
(a) Nara Institute of Science and Technology

P08093 Improving tolerance to water deficit stress in crops by expression of pSARK-IPT
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"Arcadia Biosciences is testing technology with the aim of making crops more tolerant of water deficit stress. This research program is based on the expression of a cytokinin biosynthesis protein, isopentenyltransferase (IPT), at the onset of leaf senescence driven by the SARK (Senescence Associated Receptor Kinase) promoter isolated from bean. Previous work in tobacco showed that transgenic plants containing pSARK-IPT had elevated cytokinin levels, showed reduced dark induced senescence and were more tolerant to drought (Rivero et al, 2007). Transgenic canola and rice are being evaluated under both normal and stress (dark induced senescence and water deficit) conditions to examine the phenotypic effects of pSARK-IPT in these crops. In transgenic canola, expression of IPT and SAG12-1 (endogenous senescence marker) are being examined. Preliminary analysis indicates that expression of IPT, as driven by the SARK promoter, increases with leaf age. In addition, the expression of SAG12 is much reduced in the transgenic lines as compared to controls. Experiments are ongoing to test whether SARK driven IPT expression leads to a delay in leaf senescence and improved drought tolerance in canola and rice."
(a) Arcadia Biosciences

P08094 BON1-associated protein LPK4 regulates temperature-dependent plant growth and cell death in Arabidopsis
Yang, Shuhua-presenter yangshuhua@cau.edu.cn(a) Wang, Zheng (a)
"As an important environmental factor, temperature is one of the major environmental factors that regulate plant growth, distribution, and survival. The molecular mechanisms of plant responses to extreme temperatures including cold acclimation, vernalization and high temperature response have been extensively studied. Ambient temperatures also affect various processes of growth and development. However, not much is known about the molecular mechanism of growth response to ambient temperature. Our previous studies revealed that temperature-dependent plant growth homeostasis was regulated by a copine gene *BONZAI1 (BON1)*, which encodes a calcium-dependent membrane binding proteins highly evolutionarily conserved. We also found that BON1 and its homologs BON2 and BON3 act together to regulate programmed cell death likely through negatively modulating multiple R genes in temperature-dependent manner. To further investigate the biological and biochemical function of BON1, we performed the screening of BON1-associated proteins using yeast two hybrid assay. One of them was pridicted to be a leucine-rich repeat receptor like protein kinase, which was designated as LPK4. LPK4 was co-localized with BON1 at the plasma membranes when expressed in Arabidopsis protoplasts and *Nicotiana benthamiana* leaves. BIFC analysis verified that LPK4 interacted with BON1 in vivo. The *lpk4* loss-of-function mutant conferred temperature-dependent cell death and seedling lethal phenotype, resembling *bon1bon2* and *bon1bon3* double mutants. Further charaterization of LPK4 is in progress. This study will gain insights into the molecular mechanism of temperature-dependent plant growth and cell death controlled by BON1 and LPK4. "
(a) State Key Laboratory of Plant Physiology and Biochemistry, China Agricultural University, Beijing 100193, China

P08095 Characterization of an Arabidopsis mutant with altered *CBF3* gene expression
Park, Joo-hyuk-presenter oilo28@naver.com(a) Lee, Byeong-ha (a)
"In plants, cold stress is one of the challenges that interfere with growth and development. The temperate zone plants have mechanisms that adapt themselves to low temperatures. Many of these plants can acquire freezing tolerance by being exposed to non-freezing temperatures prior to freezing temperatures. This process is called cold acclimation. The cold-inducible DREB/CBF (dehydration-responsive element/C-repeat-binding factor) proteins are transcription activators that induce many cold responsive gene expression, hence the freezing tolerance. After mutagenizing Arabidopsis plants expressing the *CBF3* promoters driven luciferase (*CBF3-LUC*), we isolated a mutant that showed altered *CBF3-LUC* expression and named it *e1*. Compared to the wild type (*CBF3-LUC*), the *e1* shows higher expression of *CBF3-LUC* luminescence, but lower expression of the endogenous

CBF3. The expression of *CBF1* and *CBF2* genes and the CBF target genes were not affected as much. The *e1* mutants will provide the genetic tool to study the promoter activity differences between the transgene and the endogenous gene."
(a) Sogang University

P08097 Infrared thermographic analyses of leaf surface temperature for studying salt responses in Arabidopsis and ice plant
Yang, Jyisy-presenter jyisy@dragon.nchu.edu.tw(a,b) Huang, Yuan-Chang (a,b) Yen, Hungchen E (a,c)
"Continuous monitoring the response of plants to salt stress was performed using infrared thermoimager. Thermography is a complementary technique to visualize leaf surface temperature, and is a proxy to stomatal conductance. Different developmental stages of Arabidopsis (glycophyte) and ice plant (halophyte) were salt-stressed and the fluctuations of leaf surface temperature were monitored, along with the changes in relative water content and protein profile. Based on the recorded fluctuations of the leaf surface temperature, Arabidopsis and ice plant showed different patterns in responses. In Arabidopsis, the fluctuations of leaf surface temperature showed similar trends in both control (irrigated water) and salt-treated (irrigated 100 mM NaCl) plants, yet the leaf surface temperature was 0.6oC higher in salt-stressed plants than control plants. The elevated leaf surface temperature was maintained for 10 h after salt stress indicating that stomates were rapidly closed upon addition of high salt in Arabidopsis. In 3-wk-old C3 mode of ice plant, leaf surface temperature dropped instantaneously upon irrigating water and slowly restored to pre-watering temperature 6 h after. On the other hand, no significant fluctuation of leaf surface temperature was detected in salt-stressed ice plant suggesting stomates remained opened under saline environment. The different stomatal responses to salt stress between glycophytes and halophytes are discussed."
(a) National Chung Hsing University (b) Dept of Chemistry (c) Dept of Life Sciences

P08098 Proteomics analysis of *Synechocystis* sp. strain PCC6803 in response to high light
Higashitani, Nahoko-presenter naho@ige.tohoku.ac.jp(a) Higashitani, Atsushi (a)
"Cyanobacteria are photosynthetic prokaryotes able to exist in a wide range of ecological environments, and are useful to characterize effects of several environmental stresses on photosynthesis as a model system. In order to study the mechanism of acclimatization to high-light (HL) conditions in the cyanobacterium Synechosystis sp. PCC6803, we have performed a comparative proteomic analysis of soluble proteins (ex1) and insoluble proteins (ex2) using 2-DE coupled with MALDI-MS. Approximately 700 total protein spots were identified and 20% of these proteins were altered in expression levels between control and HL conditions. Under HL conditions, Rubisco protein content increased but oppositely, several proteins involved in antenna pigments decreased. In addition, certain proteins in response to oxidative stress increased and cell proliferation related proteins were reduced. These results indicate that HL conditions leads to the induction of oxidative stress with simultaneously reducing cell growth rate. In the ex2 fraction, some proteins annotated as unknown periplasmic proteins increased under HL conditions. We are now attempting to observe whether structural alterations in periplasmic space with electron microscopy in response to HL."
(a) Graduate School of Life Sciences, Tohoku University

P08099 "Analysis of the Rice Plasma Membrane-Localized Receptor Kinase like Protein, OsCMPK1"
Sasaki, Tadamasa-presenter sasakic@rs.noda.tus.ac.jp(a,b) Hiroki, Kobayashi (a) Yukiko, Nishino (a) Yasuko, Watanabe (a) Hiroaki, Shimada (a,b)
"Rice OcCMPK1, a plasma membrane-localized receptor kinase like protein, composed of 833 amino acid residues. A signal peptide sequence, transmembrane domain and Ser/Thr protein kinase domain are exist in its N-terminal, middle and C-terminal domain, respectively. *OsCMPK1* is found only one copy in rice genome. By the q-PCR analysis, this gene is weakly expressed in whole plant tissues and has photoresponse expression pattern, however the expression pattern is disrupted by drought stress. *OsCMPK1* suppressed transformants created by RNAi showed abnormal growth, and almost all plants could not survive although under the normal conditions. Whereas over expression of C-terminal domain showed apparent tolerance for drought stress. These results suggest that OsCMPK1 is necessary for plant development and is involved in drought tolerance mechanism. Investigation of subcellular localization of OsCMPK1 with sGFP fusion protein revealed that full-length protein localize at plasma membrane. Partial length protein which does not contain N-terminal or transmembrane domain showed cytoplasmic distribution. Interestingly, fusion protein with portion of C-terminal kinase domain showed nuclear localization. Nuclear localization signal was found by detailed analysis of this region. These results supposed that OsCMPK1 was usually localized at plasma membrane through transmembrane domain at N-terminal, however, structural change was induced by the stresses (e.g. drought), and C-termial kinase domain was transfer into nucleus where this domain may function as a signal for environmental stresses."
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P08100 Identification of stress tolerance related genes via FOX hunting system of *Thellungiella halophila* full-length cDNA library
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"*Thellungiella halophila* has been identified as a model system for understanding abiotic-stress tolerance, and it shows extreme salt tolerance. *Thellungiella* is closely related to Arabidopsis, and its genes show 90% identity to those of Arabidopsis. Recently, full-length cDNA library with a total of 9,569 unique genes were constructed from *Thellungiella* plants treated with salinity, cold, freezing stresses or ABA treatment (Taji *et al.*, 2008). Using ectopic expression of full-length cDNAs, a novel gain-of-function system, termed the FOX hunting system (Full-length cDNA Over-eXpressing gene hunting system) was developed. To identify the genes conferring salt tolerance to Arabidopsis plants, we developed two strategies of FOX hunting, a whole genome FOX hunting and several mini-scale FOX hunting. In the mini-scale FOX hunting, we extracted genes by their functions such as transporters, transcription factors and abiotic stress inducible genes from the cDNA library. We will summarize the progress of all our FOX huntings."
(a) Department of BioScience, Tokyo University of Agriculture (b) RIKEN Plant Science Center

P08101 A calcium sensor AtCBL5 regulates osmotic and drought responses in Arabidopsis
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"Calcium serves as a critical messenger in many adaptation and developmental processes. Cellular calcium signals are detected and transmitted by sensor molecules such as calcium-binding proteins. In plants, the calcineurin B-like protein (CBL) family represents a unique group of calcium sensors and plays a key role in decoding calcium transients by specifically interacting with and regulating a family of protein kinases (CIPKs). We report here that the CBL protein AtCBL5 functions as a crucial regulator of salt and drought tolerance in Arabidopsis. AtCBL5 gene is expressed in green tissues, but no significant amounts of AtCBL5 mRNA were observed in roots. AtCBL5 was not induced by abiotic stress conditions such as high salt, drought or low temperature. To determine whether the AtCBL5 gene plays a role in stress response pathways, we ectopically expressed the AtCBL5 protein in

transgenic Arabidopsis plants (35S-AtCBL5) and examined the effects on the stress response in these plants. AtCBL5-overexpressing plants display enhanced tolerance to high salt and drought stress. Also, AtCBL5 overexpression rendered plants more resistant to high salt and hyperosmotic stress during early development (i.e., seed germination) but did not alter their ABA response. Furthermore, Overexpression of AtCBL5 alters the stress induction of stress gene markers, such as RD29A, RD29B and Kin1 etc. This result suggests that AtCBL5 functions as a positive regulator of salt and drought responses in plants. [This work was in part supported by a grant from BioGreen 21]"
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P08102 Characterization of rice group 3 late embryogenesis abundant (LEA) genes expression during development stages and stresses
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"Late embryogenesis abundant (LEA) proteins were initially found in the stage of embryo maturation. According to amino acid sequence similarities and conserved motifs, the LEA proteins can be separated into more than five groups. LEA proteins have been shown to involve in binding and replacement of water molecule, ion sequestration, maintaining of protein or cell membrane structure, and development regulation. Group 3 LEA proteins are characterized by 11-mer amino acid conserved tandem repeats (TEAAKQKAAET) and thought to be as the stress proteins. Barley HVA1, a member of group 3 LEA protein, has been reported that can improve stress tolerance in transgenic plants. However, its exact physiological functions still remain unclear. In this study we used the sequence BLAST to search Group 3 LEA protein orthologues in rice genome database and identify five lea3-like genes. They are AP004018/BAD19162, AP003381/BAB86507, AC073556/AAL84288, AC098833/AAU43988, AP000836/BAD81113 (gene/protein accession number). Here we were trying to characterize their gene sequence organization and expression patterns during development stages and environment stresses. The results showed that these LEA genes played important roles on regulation of plant growth and response for varied stresses, and tissue-specific expression in root, stem, leaf, and seed. Furthermore, analysis for cellular localization of the AP004018-, AP003381-, AC073556-, AC098833-, and AP000836-GFP fusion proteins in onion epidermal cells indicated that rice LEA 3 proteins can move to nucleus, cytoplasm, or membrane structure under stress conditions, whereas these fusion proteins localize in nucleus or cytoplasm under non-stress conditions."
(a) Department of Life Science (b) Institute of Systems Biology and Bioinformatics

P08103 Characterization of a CBF cold-response pathway in petunia.
Walworth, Aaron E (a) Warner, Ryan M-presenter warnerry@msu.edu(a)
"Freezing tolerance of many plant species increases following exposure to low, non-freezing temperatures, a process termed cold acclimation. In the model plant species *Arabidopsis thaliana*, the CBF family of transcriptional activators plays an important role in cold acclimation. The CBF-regulon is conserved in a number of species, including members of the family Solanaceae. Constitutive expression of the *CBF3* gene from *Arabidopsis* imparted constitutive freezing tolerance in *Petunia hybrida* cv. Mitchell. This, together with the identification of four genes encoding putative CBFs in the petunia genome, suggests that a functional CBF-regulated cold acclimation pathway is present in petunia. Expression analysis of the putative petunia CBF transcription factors (*PhCBF1-4*) indicated that all four genes are cold-inducible, and two of the four (*PhCBF3* and *PhCBF4*) remain highly expressed after 24 h at 3 degrees C. To identify potential members of the CBF regulon in petunia, sequences of known CBF-responsive genes from other Solanaceous species were used to search the DFCI Petunia Gene Index. From this, two dehydrin-like genes (*PhDHN2* and *PhDHN10*) were identified that exhibit increased expression in response to either cold temperatures or constitutive *AtCBF3* expression, suggesting that they are downstream components of the CBF cold response pathway in petunia. "
(a) Michigan State University

P08104 A comparative metabolite profile assessment of dehydration tolerance strategies in two species of *Sporobolus*: the desiccation tolerant *S. stapfianus* and the desiccation sensitive *S. pyrimidalis*.
Oliver, Melvin J-presenter Mel.Oliver@ars.usda.gov(a) Alexander, Danny (b,b)
"An understanding of plant responses to dehydration has important consequences for plant biology in general and directly for agriculture. Over 10% of arable lands are affected by drought, declining average yields for most crops by more than 50%. Thus, improving drought tolerance is a priority area for agricultural research agencies. Understanding how plant cells tolerate water loss is a vital prerequisite for developing strategies for improving drought tolerance. We used the desiccation tolerant grass *Sporobolus stapfianus* and the desiccation sensitive *S. pyrimidalis* to form a sister-group contrast to reveal adaptive metabolic responses to dehydration. Leaf extracts from plants at various stages of dehydration for both species were analyzed using three independent platforms, LC-MS/MS (+ESI), LC-MS/MS (- ESI), and GC-MS. The design and statistical analysis of the data allowed for a robust assessment of the levels of 167 individual metabolites. Significant changes in metabolite levels did not occur in *S. pyrimidalis* in response to severe water deficit. *S. stapfianus* however exhibits significant increases in anti-oxidant relate compounds, amino acids, and sugars. The most significant are the gamma-glutamyl peptides that presumably serve an anti-oxidant role within the cell. The accumulation of amino acids occurs during the later stages of drying indicating that they may play a role in nitrogen storage during desiccation. The accumulation of sugars during drying is not unexpected, however the fact that tri- and tetrasaccharides exhibit the largest increase is unusual in desiccation tolerant plants. These studies help to form a framework for further functional studies into the mechanisms of dehydration tolerance in plants. "
(a) USDA-ARS Plant Genetics Research Unit (b) Metabolon Inc, Durham NC

P08105 RAV transcription factors positively effect ABA responses
Mittal, Amandeep-presenter amandeep.mittal@ttu.edu(a) Luo, Qingjun (a) Gampala, Srinivas (b) Stidger, Dustin (a) John, Burke (c) Rock, Christopher D (a)
"Transcription factors (TFs) are proteins having DNA binding domains that regulate the expression of other genes. ABA (Abscisic acid) is a plant hormone that mediates growth and development in response to various abiotic stresses. The RAV (Related to ABA-insensitive3/Viviparous1) family of transcription factors (six members in Arabidopsis) contain two separate DNA binding domains- AP2 and B3, that function to promote high-affinity and specific DNA binding. In a maize mesophyll protoplast transient gene expression assay, we show RAV1 and RAV2 are sufficient to trans-activate ABA-inducible Em-GUS reporter activity and can synergize with co-transformed VP1 and with the bZIP Transcription Factors ABI5 or ABF3. Arabidopsis RAV1 and RAV2 overexpression lines under the control of CaMV 35S promoter show enhanced seed dormancy and ABA hypersensitivity to inhibition of seed germination and root growth. The RAV1 transcript is unstable (Gutierrez et al. PNAS 99: 11513 [2002]) and we are interested to understand the molecular mechanisms and genetic requirements of its post-transcriptional regulation in response to stresses, as well as functional interactions with other TFs. Compelling evidence of ABA hypersensitive phenotypes in Arabidopsis provoked our interest in developing transgenic cotton overexpressing RAV1 and RAV2. Results of experiments on ABA physiology of seed, root, and photosynthesis in RAV-overexpressing Arabidopsis and cotton lines and/or crossed to ABI5- and ABF1- overexpressing lines will be presented. Drought stress results in more than a billion dollars loss to cotton industry every year. We will conduct controlled field experiments in the future to critically test the utility of RAVs and their interactions in

conferring drought tolerance to cotton and other crops."
(a) Department of Biological Sciences, Texas Tech University, Lubbock TX 79409 U.S.A (b) Edenspace systems corporation, Manhattan, KS 66502 (c) USDA-ARS Plant Stress & Water Conservation Laboratory, Lubbock TX 79415 U.S.A.

P08106 Large scale evaluation of salt tolerances among *Arabidopsis thaliana* accessions
Katori, Taku-presenter 56080001@nodai.ac.jp(a) Ikeda, Akiro (a) Satoshi, Iuchi (b) Masatomo, Kobayashi (b) Kazuo, Shinozaki (c) Yoichi, Sakata (a) Shigeo, Tanaka (a) Teruaki, Taji (a)
"Much effort has been directed toward understanding the molecular mechanism for plant salt tolerance, with the eventual goal of improving salt tolerance of crop plants. However, it is poorly understood how salt tolerant plants acquire the remarkable tolerance. There are approximately 1,000 accessions (ecotypes) in Arabidopsis. Although there are few differences among their nucleotide sequences, these subtle differences induce large genetic variation in phenotypes such as flowering time or stress tolerances. Here, we performed a large scale evaluation of salt tolerance among 354 accessions. The evaluation revealed a wide variation in the tolerance among them. Several accessions including Bu-5, Bur-0, Ll-1, Wl-1 and Zu-0 exhibited remarked stress tolerance compared with the experimental accession, Col-0. To confirm the reliability of this assay, the tolerant accessions were also evaluated their tolerance by different assays. The data obtained by the large scale evaluation was in good correlation with the result of salt acclimation assay, in which plants were transfered to high salinity medium following the placement on moderate salinity medium for 7 days, rather than that of another salinity shock assay. Genetic analyses of salt tolerant accessions indicate that the salt tolerance without salt acclimation is a quantitative trait under polygenic control, whereas it with salt acclimation is regulated by monogenic control located in chromosome 5. We also analyzed the expression profile during salt acclimation between a tolerant accession and the sensitive one using microarray. In this conference, we will summarize the progress."
(a) Department of Bioscience, Tokyo University of Agriculture (b) RIKEN Bio Resource Center (c) RIKEN Plant Science Center

P08107 Gating of cold-regulated gene expression by the circadian clock in Arabidopsis
Dong, Malia A-presenter dongmali@msu.edu(a) Thomashow, Michael A (a)
http://https://www.msu.edu/~mftlab/index.html
"Many plants increase in freezing tolerance in response to low non-freezing temperature, a phenomenon known as cold acclimation. In Arabidopsis, cold acclimation is associated with the induction or repression of over a thousand genes. The CBF cold response pathway has a central role in this process. Within minutes of transfer to low temperature, genes encoding three closely related transcription factors, CBF1, 2 and 3, are induced and alter expression of more than one hundred target genes, which impart freezing tolerance. Recently, it has been shown that cold-induction of the CBF1-3 genes is gated by the circadian clock. In these free run experiments, plants were grown under a photoperiod, moved to constant light, then tested for cold-induction of CBF1-3 at various times. When plants were exposed to low temperature at ZT4 (4 hours after dawn), CBF1-3 induction is greater than if plants were exposed to low temperature at ZT16 . In this project, we are addressing three questions: (1) Does the cold-regulated gating of CBF1-3 induction involve positive or negative regulation? (2) Are other rapidly cold-induced genes gated similarly to CBF1-3? (3) What are the transcription factors involved in cold-gating? The results of the proposed experiments should provide answers to these questions and insight on the role of the clock in the process of cold acclimation."
(a) Michigan State University

P08108 Regulation of dehydrin expression in soybean
Yamasaki, Yuji-presenter yujyamas@iupui.edu(a) Stephen, Randall K (a)
"We are interested in understanding the limited ability of soybean to respond to environmental stresses. Abiotic stress responses in Arabidopsis are mediated in part by C-repeat / DRE-transcriptional activating Factors (CBF/DREB). These transcription factors are regulated by an upstream stress responsive transcriptional activators, ICE1 resulting in regulation of many genes in the CBF regulon. ABA responsive pathways also contribute stress responsive at transcription level. One component of protection against environmental stresses is the acquisition of increased levels of dehydrin proteins. In Arabidopsis a response to stress usually involves accumulation of an acidic subclass of dehydrins which are primarily expressed in vegetative tissues. A sole member of the vegetative tissue specific acidic dehydrin family is present in Soybean (*Glycine max*). Interesting, this dehydrin is not up-regulated in response to cold, drought, or salt stress at the RNA or protein levels. This lack of accumulation may contribute to the decreased stress tolerance of soybean. By examining the genomic sequence encoding this protein, we observed possible DREB/CBF binding domains and AREB binding domains in the promoter region (within 2,000bp upstream of the coding region). Our current research effort has focused on cold specific transcription factors in soybean. We have searched for Arabidopsis CBF-like genes in the soybean genomic sequence, identifying as many as five distinct genes. Using semi-quantitative RT-PCR, we are currently examining transcript levels of CBF-like genes, as well as SCOF-1 in young soybean seedlings following exposure to cold stress."
(a) Indiana University Purdue University at Indianapolis

P08109 Post-transcriptional regulation of Rubisco activase gene expression in response to heat stress in Cotton and Arabidopsis
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http://www.grinnell.edu/academic/biology/faculty/deridder/
"Inhibition of photosynthesis by heat stress involves deactivation of Rubisco caused by the low thermal stability of Rubisco's chaperone, activase (RCA). Regulation of RCA gene expression has been proposed as a potential means by which photosynthesis may acclimate to heat stress. Our recent work in cotton (*Gossypium hirsutum*), suggested RCA transcript levels rapidly declined at the onset of heat stress, but returned to near normal levels during extended exposure to elevated temperature. Acclimation of RCA transcript levels was not associated with changes in the rate of transcription, but rather with changes in mRNA stability and differences in the length of RCA transcript 3'-untranslated regions that putatively lacked instability elements. These results suggested that activase gene expression is influenced by post-transcriptional mechanisms that may contribute to acclimation of photosynthesis during exposure to elevated temperature. Current experiments indicate a similar stabilization of steady-state activase mRNA levels in *Arabidopsis thaliana* during short term heat stress. Using a newly-characterized RCA mutant of Arabidopsis as a genetic background, we are utilizing transgenic plants to examine the influence of 3'-UTR elements on the stability of activase transcripts and the rate of activase protein turnover during heat stress *in vivo*."
(a) Grinnell College

P08110 Comparative Analysis of Protein and Transcripts Associated with Cold Response in Cultivated Strawberry
Koehler, Gage-presenter kgk@iupui.edu(a) Alsheikh , Muath (b) Wilson, Rob (c) Rohloff, Jens (d) Randall, Stephen (a)
"Robust winter survival is necessary for efficient production of commercial strawberries. To identify proteins responsible for freezing tolerance in strawberry we have examined alterations in protein levels in strawberry varieties that differ in cold tolerance. Short and long term cold response was

examined in mature plants by treating for 48 hours and 6 weeks at 4C. We made whole extracts from the crown tissue, as this is a major overwintering structure. Protein profiles were evaluated by using either 2-DE gel analysis followed by MALDI-TOF and LC-MS/MS analysis or a shotgun MS/MS approach. The 2-DE results suggest about 50 proteins out of 1000 are altered significantly in the cold tolerant variety. The shotgun results for the 48 h time point showed 44 significant changes of the 2017 proteins. We also identified proteins with distinct levels in the different varieties. Additional analysis includes functional categorization and principal component analysis. Transcriptional analysis was done on known abiotic (cold/freezing) regulated genes, where varietal differences were found. The knowledge attained from these endeavors is expected to expedite breeding of strawberries to achieve freezing stress tolerant lines."
(a) Indiana/ Purdue University (b) Graminor Breeding Ltd. (c) Hedmark College (d) Norwegian University of Science and Technology

P08111 Genetic evidence of a link between chloroplast EF-Tu and the heat tolerance phenotype in maize
Fu, Jianming-presenter jianming@ksu.edu(a) Habben, Jeffrey (b) Hulbert, Scot (c) Fritz, Allan (a) Ristic, Zoran (d)
"Previous studies have suggested that a gene encoding the maize (*Zea mays* L.) chloroplast protein synthesis elongation factor, EF-Tu, may play a role in the development of heat tolerance. However, there was no genetic evidence linking the EF-Tu protein to the heat tolerance phenotype. We conducted a genetic study and investigated possible co-segregation between the heat-induced accumulation of EF-Tu and the heat tolerance phenotype in maize. In addition, we also investigated the number of copies of maize EF-Tu gene and determined their sequence. For the genetic study, we used a homozygous maize EF-Tu mutant (*Zmeftu::mum540*) that displays both reduced capacity to synthesize and accumulate EF-Tu and reduced tolerance to heat stress. We crossed *Zmeftu::mum540* with the maize genotype homozygous for the non-mutant EF-Tu allele (*Zmeftu-wt*) and investigated heat tolerance and accumulation of EF-Tu in F2 progeny. Heat tolerance and endogenous levels of EF-Tu were investigated in four-week-old plants following exposure to 45oC for 42 h. Heat tolerance was assessed by examining damage to thylakoid membranes and measuring shoot dry mass after recovery from stress. A significant positive correlation was observed between the endogenous levels of EF-Tu and plant tolerance to heat stress; F2 plants with higher levels of EF-Tu displayed better recovery. Genomic DNA blot analysis revealed two copies of maize EF-Tu gene. The sequence analysis of the two genes, which included both coding and promoter region, revealed no introns. The two genes, a and b, encode polypeptides of 466 and 465 amino acids, respectively, and the two polypeptides share 96% identity and 97% similarity. This study provides genetic evidence linking chloroplast EF-Tu to the maize heat tolerance phenotype. "
(a) Kansas State University (b) Pioneer Hi-Bred International, Inc (c) Washington State University (d) USDA-ARS, Plant Science and Entomology Research Unit, Manhattan, KS 66506

P08112 Salinity alters floral volatiles in *Iris hexagona*
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http://ulceet.com
"Coastal landscapes worldwide experience saltwater intrusion caused by human impact and climate change. Salt is a major cause of abiotic stress in freshwater plants and can affect survival, growth, and reproduction. Plants that require insect pollination depend upon floral volatile organic compounds (FVOCs) to ensure outcrossing, yet little is understood about how abiotic salt stress affects these compounds. We used solid phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS) to study the effect of salinity on FVOCs produced by *Iris hexagona*, a freshwater wetland plant. In our study, 50% of 22 measured FVOCs were altered significantly by 4-8 parts per thousand NaCl, with the majority of compounds declining (7/11). Furthermore, floral size was reduced three-fold. Because FVOCs and floral size are necessary to attract pollinators, salinity-induced changes in these traits can affect sexual reproduction, and have important consequences for the population dynamics and evolutionary biology of freshwater plants."
(a) University of Louisiana

P08113 Carotenogenesis and stress response in maize and Arabidopsis
Cuttriss, Abby-presenter abby.cuttriss@gmail.com(a,b) Li, Faqiang (b,c) Christopher, David (a) Wurtzel, Eleanore (b,c)
"Carotenoid pigments have roles in photosynthesis, plant protection and as precursors for the stress hormone abscisic acid. Thus carotenoid pigments are critical for plant survival and present a target for manipulation in order to increase plant stress tolerance and yield. The ultimate aim of the proposed research is to identify and manipulate carotenoid regulators, which will lead to enhanced root carotenoid composition, improved stress tolerance and plant yield. Here we analyze the regulation of root carotenogenesis in response to abiotic stress in maize, as a model for grasses. Phytoene synthase (PSY) is the first step of carotenogenesis and a key regulatory step in determining carotenoid content. In maize, PSY is encoded by a family of three paralogous genes, a pattern that is conserved between different grass species, but not Arabidopsis, which has a single *PSY* copy. Maize *PSY3* mRNA elevation correlates with carotenoid accumulation, and novel maize mutants with blocked carotenoid biosynthesis exhibit altered stress-induced abscisic acid accumulation. Maize *PSY3* transcript abundance correlates with carotenoid abundance and is upregulated in response to abiotic stress, including salt and drought. *PSY3* is thus a regulator of abiotic-stress-induced root carotenogenesis. Here we compare *PSY* stress response in maize and Arabidopsis. Examination of *PSY3* in the Grasses will enable us to understand and manipulate the root-specific stress responses that impact plant yield."
(a) Molecular Biosciences & Bioengineering, University of Hawaii at Manoa (b) Biological Sciences, Lehman College, The City University of New York (c) The Graduate School and University Center, CUNY

P08114 Characterization of the abiotic stress-responsive *arabidopsis thaliana RD29A* and *RD29B* genes and evaluation of transgenes
Msanne, Joseph-presenter jmsanne@huskers.unl.edu(a,b) Lin, Jiusheng (a,c) Stone, Julie (d,e) Awada, Tala (a,b)
"Drought, salt and freezing are abiotic stresses have adverse effects on plant growth and productivity. *RD29A* and the homologous *RD29B* genes are reported to be exquisitely sensitive to various abiotic stressors. Therefore their upstream promoter sequences have been used to confer distinct abiotic stress resistance in some plants by driving expression of transcription factors that control stress regulons. However, virtually nothing is known about the physiological functions of the proteins encoded by *RD29A* and *RD29B*. To understand how these proteins function, we are using reverse genetic approaches, including identifying *rd29a* and *rd29b* T-DNA knockout mutants, and examining the effects of complementing transgenes with the genes under control of their native promoters and chimeric genes with the native promoters swapped. We cloned four binary vectors having the promoters from *RD29A* and *RD29B* upstream of the *RD29A* and *RD29B* cDNAs respectively, and two chimeric genes with promoters from the two aforementioned genes swapped upstream of the cDNAs. These were used to transform *rd29a* and *rd29b* plants. Cold, drought, and salt induce both genes; the promoter of *RD29A* is more responsive to drought and cold stresses while the promoter of *RD29B* is highly responsive to salt stress. Studying the possible utility of *RD29A* and *RD29B* genes for conferring abiotic stress resistance in plants is of great importance for many crop species grown in arid and semi-arid regions. Results from our experiments show that *rd29a* and *rd29b* knockout mutants are actually less responsive to salt-mediated inhibition of primary root growth. Therefore, the RD29A and RD29B proteins may serve not as protective molecules, but rather as warning signals for abiotic stress responses."

(a) University of Nebraska Lincoln (b) School of Natural Resources (c) Department of Biochemistry (d) Center for Plant Science Innovation (e) Redox Biology Center

P08115 Increased ER calcium confers better stress tolerance through enhanced CIPK6 expression in Arabidopsis

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"Ca2+ supplementation can reduce the deleterious effects of NaCl in plants. To further test this effect, we generated transgenic Arabidopsis lines that constitutively express a low affinity, high capacity Calcium Binding Peptide (CBP) localized to the endoplasmic reticulum (ER). Each of four independent 35S-GFP-CBP or 35S-CBP lines contained up to 10% more total calcium than GFP-control and wild-type plants. CBP-transgenic lines also showed increased K+ compared to controls, which was balanced by decreased Na+ accumulation. Furthermore, CBP transgenic plants exhibited better salt and osmotic tolerance, and survived longer under intermittent drought conditions in soil. Only one member of the CIPK (CBL-Interacting Protein Kinase) gene family, CIPK6, showed higher transcript levels in CBP-transgenic lines. When CBP-transgenic plants were crossed with cipk6 mutants, the increased tolerance to salt and osmotic stress was lost. To determine whether [Ca2+]cyt was altered in the CBP-transgenic plants, we used two methods, confocal ratio imaging of Indo-1 and cytosol-targeted aequorin luminescence, to measure transient changes in [Ca2+]cyt. There were no significant differences in response to a short-term salt treatment (~ 20 min) when measured by either method. Under prolonged stress conditions (3-5 days) there were significantly different changes suggesting that the extra ER Ca2+ in CBP-transgenic plants was used to maintain [Ca2+]cyt signaling. To our knowledge, this is the first correlation of a genetic response (increased CIPK6 expression) to alterations in ER Ca2+. Although ER Ca2+ participates in signal transduction under stress, [Ca2+]cyt is not changed, even after a 10-fold increase in ER Ca2+, under normal conditions. This study is now being repeated in rice. "

(a) North Carolina State University (b) Grand Valley State University

P08116 Interaction of high temperature and drought stress on physiology and plant yield of spring wheat

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http:// http://www.agronomy.ksu.edu/pages/vara

"High temperature (heat) and drought stress are among the two most important environmental factors influencing crop growth, development, and yield processes. These two stresses commonly occur in combination. Objectives of this research were to investigate the interaction effects of high temperature and drought stress during reproductive development on physiological, vegetative, and yield traits and expression of a chloroplast protein synthesis elongation factor (EF-Tu) of wheat (Triticum aestivum L.). Two spring wheat cultivars (Pavon-76 and Seri-82) were grown at optimum temperatures (OT; day/night, 24/14 C) from sowing to flowering. Thereafter, plants were exposed to high temperature stress (HT; 31/18 C, Exp. 1 and 34/22 C, Exp. 2), drought stress (withholding water for 15 d), or a combination of both HT and drought stress. There were significant influences of HT and drought stress on physiological, growth, and yield traits. The interaction between HT and drought stress was significant for total dry weights, harvest index, and spikelet fertility, particularly when HT stress was severe. For leaf chlorophyll content and reproductive growth and processes such as spikelet fertility, grain yield, and harvest index, the combined effects of HT and drought were more severe than additive effects of HT or drought alone. High temperature stress and combination of HT and drought stress but not drought stress alone resulted in over expression of EF-Tu in both spring wheat cultivars."

(a) Kansas State University, Manhattan, Kansas (b) USDA-ARS, Manhattan, Kansas (c) Institute of Biological Research, Belgrade, Serbia

P08117 Wheat leaf and crown tissues show differential expression patterns of *COR* genes upon exposure to low temperature

Ganeshan, Seedhabadee-presenter pooba.ganeshan@usask.ca(a) Fowler, Brian (a) Chibbar, Ravindra N (a)

"The low temperature (LT) tolerance of winter wheat (*Triticum aestivum* L.) needs to be improved to prevent winter kill and maximize its yield potential. Therefore more detailed understanding of molecular mechanisms underlying LT tolerance is required. Thus, the objective of this study was to determine the patterns of expression of cold-regulated (*COR*) genes in leaf and crown tissues of LT-exposed wheat. Survival of crown tissues after exposure to freezing temperatures is a reflection of the level of LT tolerance of a genotype. Insight into the patterns and levels of *COR* gene expression in the crown tissue will help to further understand LT tolerance mechanisms in wheat. Materials used for this study included a winter hardy cultivar, Norstar, a tender spring cultivar, Manitou and two-near-isogenic lines with the *Vrn-A1* (spring Norstar) and *vrn-A1* (winter Manitou) alleles of Manitou and Norstar, respectively. The dominant Vrn-A1 locus confers spring habit and therefore flowering occurs without vernalization. Quantitative real-time polymerase chain reaction for several *COR* genes indicated that the Vrn-A1 locus determined the level of expression in leaf tissue, being higher in the lines having the recessive *vrn-A1* allele compared to the dominant *Vrn-A1* allele lines after two days of cold exposure. In the crown tissue, the Norstar genetic background appears to influence higher level of expression of the *COR* genes than in the Manitou background. Furthermore while exposure to low temperature for 98 days show maximum peak expression at two days, in short term 24 hour exposure such peaks were generally between 7 and 12 hours, depending on the *COR* genes."

(a) Department of Plant Sciences, University of Saskatchewan

P08118 Characterization of CBL-CIPK in Stress Signaling in Populus

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"Calcineurin B-like (CBL) and CBL-interacting protein kinases (CIPKs) proteins are involved in calcium signal transduction under stress. We identified 10 potential CBL and 27 CIPK genes in Populus genome. Comparative genomics analyses in Populus and Arabidopsis showed that the two families in poplar appear to have more paralogous gene pairs. Furthermore, we presented a study of CBL-CIPK Signaling in Populus euphratica, a mostly salt- and drought-tolerant Populus species. Seven CBL gene members in Populus euphratica (PeCBL1, 2, 3, 4, 5, 9, and 10) can be regulated in correspondence to specific external stress. PeCBL4 can interact with PeCIPK24, 25, 26 under yeast-2 hybrid and may play an important role under salt stress. It has been demonstrated in Arabidopsis that AtCIPK23 and AtCIPK24 (homologous to PeCIPK24/25 and PeCIPK26 respectively) can interact with AKT1 and SOS1 and regulate the low K+ and salt stress pathway, we deduced that PeCBL4-PeCIPK24, 25, 26 may also play a crucial role in keeping the ion homeostasis in Populus euphratica. We identified 10 KC (shake-like potassium channel family) members, 13 ECT members and 7 NHX (Na+/H+ channel) members in Populus genome. PeKC2 can interact with PeCIPK10 and 23, and PeECT4 can interact with PeCIPK24 by yeast-2 hybrid methord. Taken together, we identified the pathway of PeCBL4-PeCIPK24-PeECT4, PeCBL4- PeCIPK24, 25, 26 pathway and the PeCIPK10, 23- PeKC2 pathway in Populus euphratica. And also we found that these pathways may play an important role in corresponding to stress signal transduction pathway. Our results will provide an important foundation for further functional dissection of the CBL-CIPK signaling network especially in woody plant. "

(a) College of Biological Sciences and Biotechnology, Beijing Forestry University (b) National Engineering Laboratory of Forest Genetics and Tree Breeding

P08119 Large scale Q-PCR reveals maize transcription factors that are regulated under water deficit in a tissue-specific manner
Cho, In-Jeong-presenter choin@missouri.edu(a) Srivastava, Gyan Prakash (b) Joshi, Trupti (b) Xu, Dong (b) Hearne, Leonard B (b) Sharp, Robert E (b) Oliver, Melvin J (a)
"Overcoming the effects of field water deficits to improve yield and ensure food security is a major challenge for crop improvement strategies. Maize is a crop for which the meeting of this challenge is of critical importance given its significance to world food supplies. Part of the challenge is to fully understand the complex maize response to water deficits and for this the primary need is to identify the important gene networks and processes involved that lead to tolerance. With the advent of high throughput technologies and the maize genome we have addressed this need by targeting the water deficit response of a large number of maize transcription factors (TFs), which we envision as being among the first responders and indicators of processes important for stress tolerance. We have used high throughput qPCR to evaluate the expression of over 1000 putative maize TF transcripts, 384 of which have homology to known-rice TFs, in various tissues following exposure to precise water deficits. We used a vermiculite-based system to tightly control the water status of maize seedlings. Seedlings were exposed to mild (water potential of -0.3 MPa) or moderate (water potential of -1.6 MPa) stress levels for 5 h, 26 h and 44 h. Seedlings were separated into root tip, the rest of the root, and shoot. qPCR profiling reveals that 5 h of exposure to mild stress is sufficient to trigger the expression of some TFs in all tissues. The majority of TFs that respond to the mild stress treatment respond within 26 h. Under moderate stress, the majority of TFs that respond do so within the first 5 h. Individual TF expression profiles under water deficit treatments reveal a mosaic of temporal and tissue specific expression patterns that offer new insights into stress adaptation mechanisms. "
(a) USDA-ars (b) University of Missouri

P08120 Molecular characterization and functional analysis of the *OsAR5* gene in rice
Huang, Wen-Lii-presenter wlhuang@mail.ncyu.edu.tw(a) Lin, Wei-Chih (a)
"Aldose reductase (AR) is NADPH-dependent and widely distributed in animals and plants. It has been considered for function in cellular detoxification, sugar alcohol biosynthesis, and stresses tolerance. We cloned and identified the aldose reductase5 gene (*OsAR5*) from T-DNA insertional rice mutants. The gene expression patterns and physiological functions of *OsAR5* gene were further studied. It showed that *OsAR5* gene was strongly induced by ABA in root tissues. Besides, the homozygous individual of *OsAR5* knockout mutant was identified by genotyping analysis. It showed lower germination frequency, less tillers, growth retardation, and lower fertility. To clarify the physiological function, we construct the over-expressing plasmid that use ubiquitin promoter to drive *OsAR5* coding sequence. The plasmid was transformed into rice by *Agrobacterium*-mediated transformation. T0, T1, and T2 generation were further propagated and identified to get the homozygous lines. According to the germination and seedling morphology under stress treatment, we found T1 and T2 transgenic lines represent highly tolerant to osmotic, salinity, and drought stress. Besides, the transgenic lines, OsAR5ox5-7, OsAR5ox5-8, OsAR5ox7-7, maintain higher photosynthetic rate during stresses treatment and recover stage. These results suggested that *OsAR5* would be closely related to stress tolerance in rice. Further studies are necessary to unravel the possible roles of OsARs genes on stress resistance and rice development. "
(a) Dept. of Agronomy, National Chiayi University

P08121 The Wheat VERNALIZATION 2 (*TaVRN2*) delays flowering and enhances freezing tolerance in *Arabidopsis*
Sarhan, Fathey-presenter sarhan.fathey@uqam.ca(a) Diallo, Amadou Oury (a) Kane, Ndjido Ardo (a) Agharbaoui, Zahra (a) Badawi, Mohamed (a)
http:// http://www.er.uqam.ca/nobel/sarhan
"**The Wheat VERNALIZATION 2 (*TaVRN2*) delays flowering and enhances freezing tolerance in *Arabidopsis*. Diallo, A.O.,Kane, N.A. Agharbaoui, Z. Badawi, M. and F. Sarhan.** Departement des Sciences biologiques Universite du Quebec a Montreal CP 8888, Succursale Centre-Ville Montreal, Quebec H3C 3P8 CANADA. The vernalization gene 2 (*VRN2*), is a major flowering repressor in temperate cereals that is regulated by low temperature and photoperiod. In this report, we show that *VRN2* gene from *Triticum aestivum* (*TaVRN2*) are also regulated by salt, heat shock, dehydration, wounding and abscissic acid. Promoter analysis indicates that *TaVRN2* regulatory region possesses all the specific responsive elements to these stresses. This suggests pleiotropic effects of TaVRN2 in wheat development and adaptability to environment. To test if TaVRN2 can act as flowering repressor in species different from the temperate cereals, the gene was ectopically expressed in the flowering model plant *Arabidopsis*. Transgenic plants showed no alteration in morphology, but their flowering time was delayed compared to controls plants, indicating that *TaVRN2*, although having no ortholog in Brassicaceae, can act as a flowering repressor outside the cereals group. The delay in flowering was associated with the up-regulation of *Arabidopsis* flowering genes *FLC* and *FCA* and the down regulation of *FT* and *SOC1*. Furthermore, transgenic plants showed enhanced freezing tolerance, likely due to the accumulation of *CBF2*, *CBF3* and the *COR* genes. Overall, our data demonstrate that TaVRN2 could be used to manipulate flowering time and enhance freezing tolerance in other species. "
(a) Universite du Quebec a Montreal,Departements des sciences biologiques

P08122 Effect of cold acclimation on *O*-Methyltransferases (OMTs) in wheat leaves
Moheb, Amira-presenter amiramoheb@yahoo.com(a,b) Kanapathy, Francesca (c) Ibrahim, Ragai (c) Roy, René (a) Sarhan, Fathey (d)
"In plants, *O*-methylation is mediated by an enzyme family of *O*-methyltransferases (OMTs) that transfer the methyl groups from the methyl donor, *S*-adenosyl-L-methionine (AdoMet) to suitable phenolic acceptor molecules. *O*-Methylation of flavonoids increases their lipophilicity, and reduces their mutagenic activity via decreasing the reactivity of their phenolic hydroxyl groups. In a previous study*, a flavonoid OMT (*TaOMT2*) was isolated and characterized from wheat (*Triticum aestivum L.*) leaves. Its novel gene product catalyzes three sequential O-methylations of the flavone tricetin to its 3' -mono →3',5'dimethyl ether (tricin) →3',4',5'-trimethyl ether derivatives. TaOMT2 activity was measured using tricetin and 5-hydroxyferulic acid as substrates and was found to be up-regulated by cold in both winter and spring wheat. This increase in activity was associated with increase of the protein level as measured by immunoblot analysis using TaOMT2 antibody. To determine if this increase in TaOMT2 activity is associated with the accumulation of the product tricin, we identified and quantified tricin using HPLC and LC/MS techniques. The preliminary data showed higher accumulation of tricin in the winter variety Clair compared to the spring variety Bounty. Work is in progress to determine the function and the biological importance of tricin in wheat, during cold acclimation. * J. Zhou, N. D. Gold, V. J.J. Martin, E. Wollenweber, Ragai K. Ibrahim ; Biochim Biophys Acta (2006), 1760, 1115. "
(a) PharmaQAM, Département de chimie, Université du Québec àMontréal (b) Department of Chemistry and Biochemistry, Concordia University (c) Plant Biochemistry Laboratory and Centre for Structural Functional Genomics, Concordia University (d) Département des Sciences biologiques, Université du Québec àMontréal

P08123 Molecular analysis of heat shock transcription factors in soybean
Hanumappa, Mamatha-presenter hanumappam@missouri.edu(a) Aldrich, Donavan (a) Joshi, Trupti (b) Srivastava, Gyan Prakash (b) Xu, Dong (b) Nguyen, Henry T. (a)
"Agriculture is the biggest user of fresh water among human activities and water availability is the key factor determining crop productivity

worldwide. While soil and water management and conservation practices can help to some extent, we need stronger and parallel solutions such as developing drought tolerant varieties. Genetic manipulation and direct introduction of one or more genes of known function is a rapid and effective way to achieve drought tolerance and stack other desirable traits. Recent advance in soybean genome sequencing has enabled candidate mining by comparison with other species where gene function in stress regulation is determined. Alongside, candidate gene family profiling in tissue subjected to different abiotic stress conditions is useful to identify regulators of multiple stress tolerance. Heat shock transcription factors (Hsf), highly conserved among eukaryotes, play a major role in regulating heat stress with accumulating evidence pointing to involvement in drought tolerance also. Specifically, we are interested in root related Hsf and other genes that may be involved in stress regulation via modulation of root growth and architecture which contributes to drought tolerance. Simultaneously, we are interested in tissue specific and stress inducible promoter identification. We are developing the hairy root method in soybean for preliminary analysis of such promoters prior to full characterization. Progress in tissue specific Hsf profiling under multiple stress conditions, comparative genome analysis of the Hsf family among important crop species, characterization of key Hsf and other genes involved in root growth maintenance under stress, and development of the hairy root method for promoter analysis will be discussed."
(a) National Center for Soybean Biotechnology and Division of Plant Sciences, University of Missouri, Columbia, MO 65211 (b) Digital Biology Laboratory, Department of Computer Science, University of Missouri, Columbia, MO 65211

P08124 *AtPUB18* and *AtPUB19* Are Involved in ABA-dependent Drought Stress Signal Transduction as a Negative Regulator in *Arabidopsis*.
Seo, Dong Hye-presenter es1106@yonsei.ac.kr(a) Kwak, June M. (b) Kim, Woo Taek (a)
http://biology.yonsei.ac.kr/wtklab/
"Plants have a large number of U-box E3 ubiquitin (Ub) ligases compared to humans and yeasts. This implies that U-box E3 Ub ligases have plant-specific functions. The phytohormone abscisic acid (ABA) is well known as a mediator in responses to stresses in higher plants. We identified two ABA-induced genes, *AtPUB18* and *AtPUB19*, which encode putative U-box-containing E3 Ub ligases in *Arabidopsis*. Both genes were induced not only by ABA treatment but also by broad spectrum of abiotic stresses, including high salinity, cold, and drought. To explore gene expression patterns in more detail, each promoter of *AtPUB18* and *AtPUB19* was fused to a *β-glucuronidase* (GUS) gene and introduced into *Arabidopsis*. GUS expression was detected in stomatal guard cells under drought stress and ABA treatment in transgenic *Arabidopsis*. For loss-of-function assay, we obtained *atpub18* and *atpub19* single knock-out mutant lines and subsequently generated *atpub18atpub19* double knock-out mutant plant. These mutant plants displayed ABA hypersensitive phenotypes, such as increased stomatal closure and decreased germination ratio, relative to wild type plant. In addition, mutant plants were significantly tolerant to severe drought stress. These results indicate that *AtPUB18* and *AtPUB19* are involved as a negative regulator in ABA-dependent drought signaling pathway in *Arabidopsis*. To address more detailed functions of these two proteins, we are carrying phenotypic analysis of *AtPUB18* and *AtPUP19* over-expressing transgenic plants under various abiotic stresses."
(a) Department of Biology, College of Life Science and Biotechnology, Yonsei University, Seoul 120-749, Korea. (b) Department of Cellular Biology and Molecular Genetics, University of Maryland, College Park, Maryland 20742.

P08125 Isolation and characterization of BrMDR1 a novel MDR-type ATP-binding cassette (ABC) transporter in ''*Brassica rapa* ' L.
Jung, Yu Jin (a) Kang, Kwon Kyoo-presenter kykang@hknu.ac.kr(a) Nou, Ill Sup (b) Singh, Narendra (c)
"A cDNA clone encoding a MDR-like ABC transporter protein was isolated from ''*Brassica rapa* ' seedlings, through rapid amplification of cDNA ends (RACE). This gene (named as Brmdr 1; GenBank accession no.: DQ296184) had a total length of 4222 bp with an open reading frame of 3900 bp, and encoded a predicted polypeptide of 1300 amino acids with a molecular weight of 143.1 kDa. The BrMDR1 protein shared 71.0, 62.5, 60.0 and 58.2% identity with other MDR proteins isolated from ''*Arabidopsis thaliana* ' (AAN28720), ''*Coptis japonica* ' (CjMDR), ''*Gossypium hirsutum* ' (GhMDR) and ''*Triticum aestivum* ' (TaMDR) at amino acid level, respectively. Southern blot analysis showed that Brmdr1 was a low-copy gene. Expression pattern analysis revealed that Brmdr1 constitutively expressed in the root, stem petals and stamens, but with lower expression in leaves and open flowers. The domains analysis showed that BrMDR1 protein possessed two transmembrane domains (TMDs) and two nucleotide binding domains (NBDs) arranging in 'TMD1-NBD1-TMD2-NBD2' direction, which is consistent with other MDR transporters. Within NBDs three characteristic motifs common to all ABC transporters, 'Walker A', 'Walker B' and C motif, were found. These results indicate that BrMDR1 is a MDR-like ABC transporter protein that may be involved in the transport and accumulation of secondary metabolites. Key words: ABC transpoter, ''*Brassica rapa* ' , Gene expression, MDR, Secondary metabolism. "
(a) Hankyong Nat'l University (b) Sunchon Nat'l Univ (c) Auburn University

P08126 Isolation and characterization of the *BrCP*1 gene encoding cysteine protease inhibitor from *Brassica rapa* L.
Nou, Ill Sup-presenter nis@sunchon.ac.kr(a) Jung, Yu Jin (b) Kang, Kwon Kyoo (b) Singh, Narendra (c)
"Cystatins are proteins that inhibitors of cysteine proteases by direct interaction with the active site, have been well characterized in animals as well as in plants, and more than 30 peptidase families have been identified among different plant species. Although these proteins are potentially involved in a number of diverse plant processes. This studies were investigated molecular characterization of *BrCP*1 gene encoding cysteine protease inhibitor from *Brassica rapa* L. A cDNA clone encoding a cysteine protease inhibitor (*BrCP*1), was isolated from an flower tissue cDNA library of Chinese cabbage (*Brassica rapa* L.) and characterized. *BrCP*1 was 881bp long, with 609bp open reading frame (ORF) encoding 135 amino acid residues including a putative N-terminal signal peptide. Other relevant regions found its sequence included the G and PW conserved aa motifs, and the consensus LARFAV sequence for phytocystatins and the reactive site QVVAG. The predicted amino acid sequence for *BrCP*1 gene showed significant sequence similarity to other plant cystatins. Gene expression analyses revealed that *BrCP*1 was a tissue-specific expressing gene during reproductive growth and strongly expressed at juvenile seedling stages. Furthermore, overexpression of *BrCP*1 cDNA in transgenic Arabidopsis enhanced tolerance to high-salt stresses. Meanwhile the juvenile seedling of *BrCP*1 transgenic plants was not affected by various concentrations ABA in MS medium. Taken together, the results showed that *BrCP*1 functioned as a cysteine protease inhibitor and it exhibited a protective agent against diverse types of abiotic stress, which induced this gene in a tissue- and stress-specific manner. Key words: Abiotic stress, *Brassica rapa*, Cysteine protease inhibitor, Overexpression, Gene expression. "
(a) Sunchon National University (b) Hankyong National University (c) Auburn University

P08127 Combating abiotic stresses by boosting the plants defence mechanism
Marks, David (a) Papadopoulos, Apostolos-presenter apostolos@plantimpact.com(a)
http://www.plantimpact.net
"Abiotic stresses are responsible for significant reductions in crop yields worldwide. Although the types of stresses vary widely, most result in excess production of toxic reactive oxygen species (ROS) which damage plant cell structure. Plants do have stress tolerance mechanisms for such circumstances; however, these do not prevent loss of agronomic yield and are too complex, involving more than one metabolic pathway and are regulated by many genes, and therefore difficult to optimise by breeding or other means. This paper presents a patented new innovative technology

called Alethea developed by David J. Marks of Plant Impact plc which combines a benzoic acid donor with a novel molecule, magnesium dihydrojasmonate, used in conjunction with Arginine in mineral fertiliser formulations. The benzoic acid donor encourages maintenance of cell-wall integrity during stress conditions by suppressing ethylene production and the magnesium dihydrojasmonate accelerates production of plant antioxidants resulting in improved ability to neutralise ROS, therefore, reducing damage and maintaining yield. Alethea technology has produced significant benefits to a variety of stresses including heat, cold, salinity, drought and light intensity. Application rate and dosage varies with crops and stresses although typically it is applied at minute quantities and at low frequency. Overall, Alethea technology has proven to be highly effective of supporting the stress tolerance mechanism of plants to combat prolonged abiotic stress conditions and maintain crop quality and yield. Extensive experiments using Alethea chemistry on cocoa plantations consistently provided >50% yield increase. "
(a) Plant Impact plc, Gordon Manley Building, Lancaster University, Lancaster, LA1 4YQ, United Kingdom.

SESSION P09 – AGRICULTURE & CROP BREEDING

P09001 Pale-green leaves for *japonica* sub-species rice breeding
Zhu, Xudong-presenter ricezxd@126.com(a) Chen, Hongqi (a) Ni, Shen (a) Wang, Yuexing (a)
"There are two sub-species of *Oryza sativa* L., *indica* and *japonica*. The planting of *japonica* rice occupies about 30 % of the total sowing area in China. The taste quality with soft and sticking traits of *japonica* has been generally accepted by consumers in China. One of many differences between *indica* and *japonica* is the leaf color, the former of natural green and the later of dark-green. Considering the fact that there are relatively fewer chances for the pale-green leaf to be eaten or laid eggs upon by rice insects, two pale-green leaf mutants, spontaneous one L64-QR and *γ*-ray induced one ZH-Danlv were chosen to be used in *japonica* rice improvement. These two mutants possessed of erect and thick leaves. And the photosynthesis of the mutants, which vegetated normally, was roughly the same as that of the dark-green leaf *japonica* rice. L64-QR and ZH-danlv were controlled by different gene(s), respectively."
(a) China National Rice Research Institute

P09002 Genetic control and breeding application of rice grain filling and broad-spectrum blast resistance
Wang, Ertao (a) Deng, Yiwen (a) Li, Qun (a) He, Zuhua-presenter zhhe@sibs.ac.cn(a)
"Rice grain-filling, a process controlling the weight of each grain after pollination, has been a bottleneck in rice breeding. Based on screening rice genetic resources and mutants, we identified the rice gene GIF1 as a key regulator of the entire grain-filling process, which encodes a cell-wall invertase required for carbon partitioning during early grain-filling. We provided genetic evidence that GIF1 was a target of rice domestication selection. Artificial selection led to the GIF1expression pattern facilitating sucrose unloading favoring grain-filling. GIF1 could be used to increase grain production in transgenic rice. We further show that functional evolution of duplicated genes contribute to genetic novelty and morphological difference in genus Oryza through GIF1 and OsCIN1 divergence These findings strongly suggest that such a domestication-selected gene can be used for further crop improvement in a modern variety, providing a new approach for high-yielding molecular design. Rice blast is one of the most serious diseases leading to large loss of grain production. We identified the Pigm locus with broad-spectrum and durable resistance to blast, which contains an NBS-LRR resistance gene cluster, allelic to Pi2 and Pi9. The locus contains sequence difference to and broader-spectrum resistance than Pi2 and Pi9. In comparison with the Pi2 and Pi9 clusters, the Pigm cluster performed gene duplication and unequal cross. Broad-spectrum resistance might be attributed to the more R genes pyramided in the Pigm locus. Subclones were constructed and transformed to the susceptible variety Nipponbare to confirm the functional R gene(s). Elite resistant lines carrying Pigm were developed by marker-aid selection, indicating good potential of the gene in rice breeding. "
(a) Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences,CAS

P09003 Embryo Rescue in Zizyphus spina-christi L.
Al-Agamy, Samir Z.-presenter samiragamy@hotmail.com(a) El-Sese, M. A. (a) Hashem, Amal S. (a)
"Hozaien is a genic mutation of *Zizyphus spina-christi* originated in the early 1950's in Assiut , Egypt. Seeds of such mutant is unable to germinate therefore its use in further improving programs was hindered. So, the target of this study was to rescue embryo. The objectives of this study were to: a) study the influence of embryo age and medium type on survival and germination of Hozaien embryo and b) study the influence of benzylaminopurine on survival and germination percentages, proliferation and plantlet growth characteristics for mass production of the mutation. Until the 7th week beyond fruit set there was no survival for embryo on MS and N&N media .Survival % increased gradually starting from week 7 and a dramatic rise in survival percentage on both media occurred starting from the week 12 of embryo age. The maximum survival% was 63.33% on either medium. In general, MS medium induced relatively higher germination percentage compared to NN one. Germination ceased at embryo age of 20 and 21 weeks for NN and MS, respectively. BAP improved survival and embryo germination; the maximum survival percentage (80%) occurred during the 18th week of embryo age on both media when BAP was used at 5.00 mg/l compared to 63.33% in media devoted BAP, BAP at 1.00 mg/l had an intermediate value (73.33%). BAP at 5.0 mg/l induced a great promotion in Hozaien nabaq embryos germination compared to the other treatments regardless of medium type used. "
(a) Genetic Engineering and Molecular Biology Research Centre

P09004 Impact of Tannery Effulents on Growth Performance and Carbohydrates Status in Wheat
Hussain, Mian W-presenter wajahathussain@fccollege.edu.pk(a)
"Present study deals with the effect of tannery effulents on the growth performance and carbohydrate status of wheat (*Triticum aestivum* L.). The effulents were diluted to 25, 50, 75 and 100% concentrations and soil was treated with these concentrations prior to sowing. The result of season-long effects of effulents indicated that specific leaf area (SLA), net assimilation rate(NAR), relative growth rate (RGR) and total nitrogen significantly decreased ($p<0.01$)in higher concentrations as compared to control. During the vegetative growth leaf sucrose and starch concentrations decreased with increasing effulents concentrations. During reproductive growth stage sucrose and starch contents did not show significant differences in low effluents concentrations, but in high effluents concentrations sucrose content increases as compared to starch. The data suggests that in lead blades the priority between the partitioning of carbon into sucrose or starch changed with developmental stage and effulents concentrations."
(a) Forman Christian College, Lahore

P09005 Development of a new variety of rice for effective prevention of people and their environment from arsenic contamination
Mandal, Abul K-presenter abul.mandal@his.se(a) Mirtahery, BentolHoda (a) Nahar, Noor (a,b) Lundh, Dan (a)
http://www.his.se/mana
"Arsenic poisoning through consumption of cultivated crops is a severe health problem in many countries in South Asia especially in India,

Bangladesh, Burma and Thailand. For instance, in Bangladesh more than 30 millions people are affected from rice-derived arsenic contamination leading to severe damage of kidney, liver, lungs, bladder etc and many other neurological and vascular disorders. To solve this severe problem we aim to generate a genetically modified variety of rice either by inhibiting/activating native gene(s) responsible for arsenic uptake or by insertion of foreign genes responsible for arsenic metabolism *in planta*. For identification and characterization of genes responsible for arsenic uptake or metabolism of arsenics *in planta* we have employed data mining, an in silico analysis based on searching of the existing genomic databases. Data mining experiments resulted in identification of four candidate genes that are involved either in uptake, transport or cellular localization of arsenic in plants. However, there is only one candidate gene that might be involved in arsenic metabolism in rice. As an alternative to in silico analysis we have also screened available T-DNA insertion mutants for identification of the candidate genes. Results obtained in both in silico analyses and screening of T-DNA insertion mutants were then utilized for cloning of the candidate genes. To date we have cloned and characterized two candidate genes *ADC1* and *ADC25* from the genomic DNA of Arabidopsis thaliana by PCR using database sequences as primers. We are now studying these genes in heterologous systems such as the yeast or E. coli and the results will be discussed. Vectors containing the target genes will be constructed for transformation of rice. "
(a) University of Skovde, Sweden (b) University of Rajshahi, Bangladesh

P09006 Mapping of the stunted lemma/palea (*slp*) mutant in rice
Wang, Sheng-Shan-presenter r94621118@ntu.edu.tw(a) Chen, Kai-Yi (a)
"We characterized a sodium azide-induced rice mutant, stunted lemma/palea (*slp*). Genetic analysis showed that *slp* is recessive for sterility and dwarf but is semidominant for stunted lemma/palea. Furthermore, genetic mapping based on 42 F2 plants derived from the cross between *SLP/slp* (*Oryza sativa* subsp.*japonica* cv Tainung 67) and Taichung native 1 (*O. sativa* subsp.*indica*) suggested that *slp* was located between markers RM23477 and RM23557 in the distal region of the long arm chromosome 8. Moreover, high-resolution mapping using 982 F2 plants delimited the *slp* gene in a 53 kb genomic region containing two annotated putative genes. We aim to positional cloning the *SLP* gene."
(a) National Taiwan University, Department Of Agronomy

P09007 Croplife-B Increases the extension of green beans shelf life by nitrous oxide and carbon dioxide
Al-Jamali, Abbas F.-presenter jamali@just.edu.jo(a) Bani Hani1, Majde T. (a)
"Croplife-B is said by the manufacturer to stimulate the production of phytoalexins and to reduce fungal growth in sprayed plants. To investigate the effects of Croplife-B preharvest sprays and increased postharvest carbon dioxide (CO_2) and nitrous oxide (N_2O) concentration on pod quality and shelf life, off-season green bean plants, winter-grown in the warm Jordan Valley, were sprayed twice at a rate of 270 mL/hectare Croplife-B. Green bean pods were kept after harvest in 100 kPa N_2O, 20 kPa CO_2 or 60g dry ice in 120 L barrels for twenty days. Results indicated that, Croplife-B accelerated the production time and reduced mold appearance and development during and after storage when used alone or in combination with CO_2, N_2O or dry ice treatments. Nitrous oxide and CO_2 treatments resulted in decreased water loss from the pods and maintained regular pod shape throughout the storage period, with N_2O being the most efficient treatment. Nitrous oxide and CO_2 resulted in improved bean pod coloration at the end of storage time. Firmness and titratable acidity decreased while the pH increased more when beans were treated with N_2O and CO_2 during the storage period than in the dry ice treatment or control. White mold did not appear for 20 days on pods treated with N_2O and CO_2 when stored at 16-23C and 88% relative humidity. With dry ice treatment the white mold appeared at 10 days of storage. Dry ice treatment reduced the appearance of mold, maintaining it at under 50 % for 20 days. The beneficial effect of N_2O and CO_2, coupled with Croplife, in inhibiting the mold continued for 10 days after the bean pods were removed from storage on day 20. The results of these experiments are consistent with Croplife-B acting as an elicitor of phytoalexins. "
(a) Jordan University of Science & Technology

P09008 Identification of the molecular basis of the seed low phytic acid phenotype in soybean line CX1834
Gillman, Jason D-presenter gillmanj@missouri.edu(a) Pantalone, Vince R (b) Bilyeu, Kristin (a)
"Plant seeds accumulate phosphorus in the form of *myo*-inositol-1,2,3,4,5,6 hexa*kis*phosphate, commonly referred to as phytic acid. Phytic acid is complexed with cationic mineral species in the form of phytate, which is not well digested or absorbed by monogastric species such as humans, poultry and swine. As a result, soybean has an effective deficiency of phosphorus and other minerals, despite high levels of these components in the seed. Excreted phytate can also contribute to phosphorus contamination of groundwater and eutrophication of freshwater lakes and streams. In maize, a recessive mutation in a conserved region within the *low phytic acid 1 (lpa1)* gene is responsible for the low phytic acid phenotype. We have identified recessive mutations in two soybean homologues of the maize *lpa1* gene in CX1834, a soybean line with a low phytic acid phenotype derived from EMS mutagenesis of a breeding line with normal phytate levels. In three populations analyzed, we identified complete association between homozygosity for mutant alleles of the two *lpa1* homologues and the low phytic acid phenotype. Molecular marker assays were designed that can be used to directly select for the mutant alleles that control the phenotype. The identification of the molecular basis for the low phytic acid phenotype will dramatically ease the introgression of the low phytic acid trait into elite soybean cultivars. The ultimate goal of such introgression is soybean-derived food and feed which require less nutrient supplementation, are more nutritious, and are more environmentally friendly."
(a) Agricultural Research Service, USDA (b) Dept. of Plant Sciences, University of Tennessee

P09009 Evaluation of a Gene Specific Marker in Populations of $F_{2:3}$ Families for Resistance to Aflatoxin Accumulation in Maize
Mylroie, J. Erik (a) Warburton, Marilyn L. (b) Windham, Gary L. (b) Williams, W. Paul (b) Wilkinson, Jeff R.-presenter jwilkinson@bch.msstate.edu(a)
"*Aspergillus flavus* is an opportunistic fungal pathogen that infects maize and other oil seed crops and can produce a toxic and carcinogenic metabolite, aflatoxin. The FDA has imposed limits of 20 ppb for all food products destined for human consumption. High heat and drought conditions, common in the South, favor *A. flavus* infection and aflatoxin contamination, often resulting in large economic losses for Southern farmers. One promising area of research to respond to these economic and health effects is the development of maize lines resistant to fungus or toxin accumulation. However, development of a viable resistant commercial line has proven difficult, in part due to a lack of gene specific genetic markers for resistance breeding. The objectives of this study were to develop gene specific markers from candidate genes selected from a previous microarray study, and confirm that these candidate genes lower aflatoxin in lines containing them. Candidate genes were sequenced in both resistant and susceptible lines to identify polymorphisms. A gene encoding a chloroplast precursor was found to contain different alleles in resistant and susceptible lines. These alleles were developed into a marker that was subsequently used to screen two populations of $F_{2:3}$ families; Mp313E (resistant) x B73 (susceptible) and Mp313E x Va35 (susceptible). Individuals with one or two copies of the resistant allele were found to have accumulated significantly less aflatoxin than homozygous susceptible plants. The allelic marker was mapped to chromosome 4 and found to have significant phenotypic effects for aflatoxin resistance. This marker will be a valuable addition to existing breeding programs aimed at incorporating resistance into elite maize production lines."
(a) Department of Biochemistry and Molecular Biology; Mississippi State University (b) USDA-ARS Corn Host Plant Resistance Research Unit

P09010 Breeding maize and sorghum for improved adaptability and yield for acid soil of Western Kenya
Gudu, Samuel-presenter samgudu2002@yahoo.com(a) Ligeyo , Dickson Were, Beatrice Onkware, Augustino Too, Emiliy Ringo, Justin
"MAIZE AND SORGHUM BREEDING FOR IMPROVED YIELDS UNDER ACID SOILS OF WESTERN KENYA Samuel Gudu, B. A. Were, A. Onkware, E. J. Too, G. O. Dangasuk, J. Ringo (Moi University, P.O. Box 3900, Eldoret, Kenya and Dickson Ligeyo (Kenya Agricultural Research Institute, Kitale, Kenya, (KARI)). Maize and sorghum are staple food crop for in the East Africa particularly, Kenya. The two crops are often grown in acid soils of east Africa, but the yields are low partly due to many factors including soil acidity that results in aluminium toxicity and low phosphorus availability. Moi University and KARI embarked on breeding sorghum and maize for adaptability to acid soils of western Kenya. The objective of the breeding program was to screen maize and sorghum aceessions for tolerance to Al toxicity and improved P uptake efficiency; and also to introgress Al toxicity into the Kenyan maize and sorghum from standard materials from ICRISAT (for sorghum) and Brazil. The screening for tolerance to Al toxicity was carried in solution culture and P uptake using standard procedures. Variability to Al tolerance and P uptake was found among Kenyan germplasm. In addition, highly tolerant maize and sorghum landrace populations were found, some of which had tolerance compared to ACRISAT and Brazil standars, for sorghum and maize. Crosses made to introgress Al toleranc or P uptake; and to combine Al tolerance with P-uptake efficiency were successful and segregating populations, particularly in sorghum expressing tolerance to Al toxicity and/or P uptake efficiency, have been obtained. More interestingly, some of the segregating populations in sorghum have shown high yields that surpass the highest yielding parents by as much as 60 – 70 % in F3 populations. In maize, some of the inbred lines obtained from Brazilian single crosses or Brazilian Kenyan top cross or landrace populations are extremely tolerant to Al toxicity. These inbred lines are undergoing tests for general and specific combining ability including hybridity. For sorghum, ISSR molecular markers that co-segregate with Al tolerance have been identified in one of the crosses and more of such markers are being sought to support marker – assisted selection. It is hoped that this breeding program will be able to produce varieties that could show improved grain yield in acid soils of Kenya and other East African countries. Key Words: soil acidity, al tolerance, P-uptake efficiency. "
(a) Moi University (b) kari

P09011 EFFECTS OF SYNTHETIC BRASSINOSTEROIDS IN CRIOLLO MAIZE (*Zea mays* L.).
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"One of the alternatives to increase the yield of maize is the use of plant hormones. In our laboratory we are working with different analogues of brassinosteroids in order to quantify the effect of this in mono- and dicotyledonous plants including hybrid and criollo maize, and bean. Some analogues are being synthesized in our research group from natural diosgenin. Brassinosteroids promote cell elongation and cellular division and therefore increase biomass; other processes as seed germination, root production, flowering, senescence, abscission and maturation are also influenced. We present here the results of the activity of four analogues: BSS1, BSS2, BSS3, BSS4, in criollo maize known as creamy. After seven days seedlings were evaluated in length, fresh and dry weight. Germination trials were held in a germinator at 25 oC using 3 different concentrations (0.1mg/l, 1.0mg/l, and 10.0mg/l) and four replicates. After 7 days digital images were taken and measured, the plants were dried at 70 oC for 48 h, and weighed. The results showed a clear positive effect on germination and growth. BSS1 and BSS4 increased biomass by 15% with 1.0mg/l, while BSS2 and BSS3 increased 10% biomass when using 0.10 mg/l and 1.0 mg/l respectively. The results showed relatively good biomass increases, but better ones were obtained in the hybrid variety TL5B, which reached more than 20% in the same concentration. Testa hardness and imbibition time are hypothesized as factors influencing results. "
(a) Herbario y Jardin Botanico. BUAP (b) Escuela de Biologia. BUAP (c) Facultad de Ciencias Quimicas. BUAP

P09012 Morphological Characters of Panicle and Seed Mutants of Rice
Kang, Sang Gu-presenter kangsg@ynu.ac.kr(a) Matin, Mohammad Nurul (a) Pandeya, Devendra (a)
"Rice with white pericarp is the world most important crop as food sources. Whereas, beside food value, rice having purple, brown and red colored pericarp could be useful for its medicinal value and antioxidant activity that might help human health and could protect from diseases. Color formation in the rice seed is usually caused by the accumulation of several pigments in the hull and pericarp. The pigmentation in the rice bran is genetically determined by the presence of the Rc locus which recessive allele rc form white bran. For the better understanding of the rice panicle development, and colorization in the hull and pericarp in the various rice varieties, we have analyzed the phenotypes of panicle, hull and seed of several rice varieties. Panicle mutants were classified in 4 groups with their internode length of main rachis, primary rachis, secondary rachis and pedicel. Hull and seed mutants were grouped into 12 based on their mutant characters in shape, size and seed coat color. These natural and spontaneous mutant collections showed distinct phenotypes to wild type rice. The linkage analysis in the F1 and segregating population of the cross between *Oryza sativa* japonica cv. (RdRc, red pericarp) and *O.sativa* Indica cv Kumgangbyeo (white pericarp) indicated that the seed color of F1 was determined by its female parent. Based on seed color and plant segregation of F2 and F3 generations the red phenotype of the seed was determined as dominant as it was segregated according to Mendelian 3:1 (red pericarp: white pericarp) ratio. This information might be useful for the identification of the functions of genetic factors in the Mendelian inheritance. [This research was supported by the Grant funded by Agricultural R&D Promotion Center, ARPC (108091-05-1-CG000)]. "
(a) Yeungnam University

P09013 Effect of Deep Sea Water Treatments on the Growth and Metabolites in Dropwort (*Oenanthe javanica* DC.) Plants
Lee, Taek-Jong (a) Shrestha, Surendra Lal (a) Choi, Seul-Gi (a) Jo, Hyeok-Jin (a) Lee, Jong-Ho (a) Kang, Won-Hee-presenter whkang@kangwon.ac.kr(a)
"This experiment was conducted on Dropwort (*Oenanthe javanica* DC.) plants to study whether treatments with sea water (SW) result in enhancement of the growth, antioxidant activity, and quantity of phenolic contents, and to identify the optimum concentration of SW. Experiments were laid out in completely randomized design (CRD) with three replications with ten treatments; DSW (deep sea water 5%, 10%, 20%), SSW (surface sea water 5%, 10%, 20%), RO (reverse osmotic 5%, 10%, 20%), and DW (distilled water). In experiment 1, 1 cm long nodes and in experiment 2, only leaves were mass cultured in above mentioned concentrations for 168 hours and 336 hours respectively at 25&176C under continuous light. Then leaves were harvested from the nodes and measured antioxidant activity with DPPH free radical scavenging activity method and quantities of phenolic compounds with HPLC analysis. Results showed that all concentrations of DSW, RO, and 10% SSW treatments had improved the shoot and root growth from nodes as compare to other SSW and DW treatments. Second experiment of water mass culture showed that 5% RO, 5% SSW, and all of the DSW treatments had improved growth as well as antioxidant activity and phenolic compound. In both of the experiments, only DSW treatments were not observed contamination caused by microorganisms during culture period. Hence, 5% DSW was the best treatment."
(a) Department of Horticulture, Kangwon National University, Chuncheon 200-701, Korea

P09014 "SolCAP: translating solanaceae sequence diversity and trait variation into applied outcomes through integrative eesearch, education, and extension"
Douches, David (a) Buell, C. Robin (a) Francis, David (b) Van Deynze, Allen (c) De Jong, Walter (d) Mueller, Lukas (d) Stone, Alexandra (e) Zarka, Kelly-presenter zarka@msu.edu(a)
"The USDA-CSREES National Research Initiative (NRI) awarded a Coordinated Agricultural Project (CAP) for the improvement of Solanaceae crops which began in October 2008. The project is called SolCAP and it focuses on the two most important vegetable crops in the Solanaceae: potato and tomato. Our vision is to move translational genomics beyond commodity boundaries toward an emphasis on taxonomic groups and DNA sequence homology, leveraging knowledge and resources across species. Sequencing efforts in Solanaceae have lead to extensive expressed EST resources and genome sequence is emerging for both potato and tomato. Ultimately, understanding variation at the DNA sequence level is useful in crop improvement only to the extent that it helps us understand and/or predict phenotypic variation for agriculturally important traits. The primary research objective of this proposal is to provide the infrastructure to link allelic variation in genes to valuable traits in cultivated germplasm of potato and tomato. Focusing on elite breeding material will increase the probability that these solanaceous crops will benefit from genotype-based selection. The extension and education components will integrate training in genomics and plant breeding with curriculum aimed at students and existing breeders seeking to make better use of sequence data for crop improvement. To foster interaction across plant translational genomics CAPs we have invited other projects into our eXtension community of practice, created a Plant Breeding and Genomics workspace to leverage Web 2.0 interactive functions, and we are developing content for publication to eXtension.org. "
(a) Michigan State University (b) Ohio State University (c) University of California - Davis (d) Cornell University (e) Oregon State University

P09015 Tuber physiological aging of potato clones produced during fall and spring growing seasons and stored at different temperatures
Pereira, Engil I -presenter isapereira@ucdavis.edu(a) Freitas, Sergio T (a) Bisognin, Dilson A (b)
"Potato is the fourth food crop in the world with an annual production of more than 300 million tonnes in more than 140 countries. The physiological age of potato tubers is characterized by the stages of dormancy, apical dominance, multiple sprouting, and senescence. These developmental stages are used to monitor physiological aging and quality of potato tubers, either for processing or seed. The genetic background, growing season, and storage temperatures are important factors that affect potato tuber aging and quality after harvest. The objective of this work was to determine the effect of different storage temperatures on tuber physiological aging of three potato clones produced during fall and spring growing seasons. The experiment was carried out as factorial of three clones (Asterix, SMIJ461-1 and SMINIA793101-3) by four storage temperatures (4, 8, 12 and 25 C) and two growing seasons (fall and spring) in a random design with four replications. At 30-day intervals, tubers were evaluated from the beginning to 180 days of storage. Cold storage increased dormancy period, reduced sprout number and tuber rotting. Tubers produced during fall season did not sprout at the storage temperatures of 4 and 8 C. Tuber fresh weight loss and respiration increased with storage period and temperature. Crop growing season changes tuber physiological aging during storage. Storage in low temperature (4 and 8 C) conditions is efficient to slow down tuber aging."
(a) Plant Sciences Department, University of California - Davis (b) Federal University of Santa Maria, Brazil

P09016 Assessment of genetic diversity of pawpaw (*Asimina triloba*) varieties and native Kentucky populations with Simple Sequence Repeat markers
Lu, Li-presenter li.lu@kysu.edu(a) Pomper, Kirk (a) Crabtree, Sheri (a) Lowe, Jeremy (a) Schneider, Kyle (a) Tidwell, James (a) Dutta, Shandeep (a)
"The North American pawpaw [*Asimina triloba* (L.) Dunal] bears the largest edible fruit native to the United States and is in the early stages of commercial production. There have been continuous efforts to select new pawpaw cultivars from the wild and through breeding. However, many cultivars have been lost from cultivation, especially during the beginning of the early twentieth century, through neglect, abandonment of collections, and loss of records necessary for identification. This may have eroded the genetic diversity of currently available selections. In 1994, Kentucky State University was designated as a satellite repository for *Asimina* preservation in the U.S. Department of Agriculture, National Plant Germplasm System. Germplasm evaluation, preservation, and dissemination have been high priorities at KSU since that time. The objective of this study was to examine the genetic diversity displayed in 41 pawpaw varieties and six native Kentucky populations using simple sequence repeat (SSR) markers. Leaf samples were collected from 28 cultivars and 13 advanced selections from the PawPaw Foundation (PPF) breeding program, and six native Kentucky populations for genomic DNA isolation. Four SSR primer sets B3, B103, B129, and G119 were selected and labeled with 6-FAM for use in amplification of SSR-PCR products. The PCR products were separated with an Applied Biosystems capillary electrophoresis system, 3130 Genetic Analyzer. Each primer yielded approximately 10 alleles for each primer combination. The results showed significant genetic variation among the pawpaw cultivars and native populations, suggesting that the genetic base of currently available cultivars is diverse. "
(a) Kentucky State University

P09017 Effects of long-day treatment on flowering and maturity in Korean soybean cultivars
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http://www.yari.go.kr
"The discovery of flower induction by short day lengths has brought many investigators to study its mechanism. In spite of those researches, previous studies on long-day treatment suggested that there were not enough evidences to predict grain filling condition in some varieties. Soybean is divided into several ecotypes according to its photoperiods and temperature responses. It is important to distinguish the response of the varieties to day length on flowering behavior and fruiting to obtain high quality soybean grains. This study was conducted in order to determine flowering behavior, maturity and grain characteristics of several soybean cultivars under 16 h long-day treatments at two growth stages (V2 and R5), which were determined in our previous studies. Five cultivars with different ecotypes were selected, treated and examined on their growth, development, and seeding responses under controlled green house conditions. In general, most of the cultivars treated at V2 stage showed delayed flowering and maturity. However, other cultivars varied in their responses to other agronomic characteristics, such as ripening period and plant height. In R5 stage day-length treatment, two cultivars showed an increase in grain weight and longer maturity period. Yield per plant and grain weight were associated with varying maturity periods."
(a) Department of functional crop, NICS, RDA (b) Rural Development Administration

P09018 Isolation and molecular characterization of a putative ascorbate peroxidase gene from citrus
Louzada, Eliezer S-presenter elouzada@ag.tamu.edu(a) Kunta, Madhurababu (a) Del Rio, Hilda Sonia (a)
"Reactive oxygen species (ROS) are continuously produced during normal aerobic metabolism and also under environmental stress conditions. They are the major damaging factors to the photosynthetic machinery under stress conditions and need to be scavenged for normal plant growth.

Ascorbate peroxidase (APX) is the key enzyme in detoxifying H_2 O_2 and other ROS from chloroplast and cytosol. A cDNA encoding a putative APXcit was isolated from mature Dancy tangerine (Citrus reticulata Blanco) juice vesicles using differential display reverse transcription-polymerase chain reaction (DDRT-PCR). Subsequently, the full-length APXcit cDNA and genomic clone were obtained and sequenced. The full-length APXcit sequence was composed of 1082 bp nucleotides, including an open reading frame (ORF) of 753bp, putatively encoding a protein of 250 amino acids with a predicted molecular mass of 27 kDa. The 5' untranslated region (5' UTR) consisted of 90 nucleotides and the 3' UTR of 239. The genomic clone of 2457 bp was composed of 8 introns and 9 exons. Expression of APXcit in Escherichia coli (E. coli) cells was drastically increased upon isopropyl-β-D-thiogalacto-pyranoside (IPTG) induction. A homology search for APXcit at the GenBank database showed high similarity to APX from several plant species. Challenging of a susceptible citrus species with *Phytophthora nicotianae* showed a reduced expression of APX while challenging of a resistant species showed increased expression of APX."
(a) Texas A&M University-Kingsville

P09019 Identification of Differentially Expressed Stress Related Genes Using 3 Prime Untranslated Region Sequencing in *Malus x domestica*
Schaeffer, Scott M-presenter smschaeffer@wsu.edu(a) Krishnan, Vandhana (b) Kalyanaraman, Ananth (b) Dhingra, Amit (a)
"The expression of stress related genes significantly impacts the shelf life of apples. To understand the genetic factors involved in post harvest storage of apples, a transcriptomics approach was used to identify differentially expressed genes in developing apples. Two apple cultivars, Honeycrisp and Golden Delicious, were chosen for this study due to their opposing phenotypic qualities with respect to post harvest shelf life. High throughput sequencing of the 3 prime untranslated region (UTR) was performed to identify differences in transcript levels in peel and core tissues of each variety. Transcripts in peel tissue and core tissue have been identified that are expressed at significantly different levels between the two cultivars. Of these transcripts only 20 percent had a significant alignment with sequences from the NCBI Nucleotide Database. Several of the characterized transcripts have been identified as stress related genes that may prove useful for enhancing apple shelf life. "
(a) Department of Horticulture and Landscape Architecture, Washington State University, Pullman, WA 99164 (b) School of Electrical Engineering and Computer Science, Washington State University, Pullman, WA 99164

P09020 Identification and Functional characterization of CBL1 gene promoter in Ammopiptanthus mongolicus
Yin, Weilun -presenter yinwl@bjfu.edu.cn(a,b) Xia , Xinli (a,b) Guo, Lili (a,b)
"Ammopiptanthus mongolicus is an evergreen broad-leaf shrubs growing in the desert region in northwest of China with extremely strong drought and cold tolerance. CBL1 (Calcineurin B-like) is a calcium sensor that differentially regulates drought, cold and salt signals in Arabidopsis. A 1683bp 5-flanking region (AmCBL1P) of CBL1 was isolated from Ammopiptanthus mongolicus by anchored PCR walking, and a 124bp 5-untranslated region (5-UTR) of AmCBL1 gene was obtained by RACE strategy. Analysis of the promoter sequence exhibited the existence of a 690bp intron and basic cis-acting elements related to multiple environmental stresses. Five plant expression vectors containing GUS gene driven by fragments of AmCBL1 promoter at different length from -1659, -1414, -1048, -296 to -167bp relative to the transcriptional start site were constructed and transferred to tabacum.L.cv.89 for checking the functional region of AmCBL1P. Functional properties of each promoter segment were examined by GUS historical staining and fluorescence quantitative analyses using at least five PCR-positive transgenic tobacco plants treated with multiple stresses for different times. The results showed that AmCBL1P was a vascular-specific and a multiple environmental stress inducible promoter; the smallest promoter fragment B1S3 possesses all the essential cis-acting elements, 129bp sequence in -881/-752 contains cis-regulatory element and the intron in the 5-UTR plays a role as an enhancer in stress. The allocation and quantification of AmCBL1 promoter segments for expression levels in different tissues provides further insight into the regulation of stress-inducible expression in desert plants."
(a) College of Biological Sciences and Biotechnology, Beijing Forestry University (b) National Engineering Laboratory of Forest Genetics and Tree Breeding, Beijing Forestry University

P09021 Protection and conservation of Caricaceae germplasm with PRSV resistant transgenic papaya
Matsumoto, Tracie K.-presenter tracie.matsumoto@ars.usda.gov(a) Zee, Francis Y. P. (a) Suzuki, Jon Y. (a) Tripathi, Savarni (a) Mackey, Bruce (b) Hollingsworth, Robert (a) Shintaku, Michael (c) Lisa, Keith M. (a)
"Papaya ringspot virus (PRSV) is a devastating disease that has a detrimental impact on both commercial papaya production and Caricaceae germplasm conservation. The PRSV coat protein transgenic line 55-1 and derived progeny are resistant to PRSV and have saved the papaya industry in Hawaii. Here we present preliminary information on a method to protect susceptible Caricaceae germplasm using PRSV resistant transgenic papayas as border planting to limit aphid transmitted infection. Similar to transgenic crops throughout the world there is public concern on cross contamination of transgenic material into non-transgenic lines. As the designated germplasm repository for Caricaceae we are responsible for maintaining the genetic integrity of each accession. Therefore, we have also developed a protocol utilizing polymerase chain reaction (PCR) for the detection of adventitious presence of transgenic material in both the parental plants and the resulting seed population by testing for the 55-1 transformation event to assure a 99.9% chance of obtaining greater than 99.5% transgene free seeds. The protocol developed in this study is not typical for most seed validation techniques since there is a higher than normal rejection rate for rejecting seed lots; however, we believe this is necessary to ensure the genetic integrity of seeds stored in the repository."
(a) USDA, ARS, Pacific Basin Agricultural Research Center (b) USDA, ARS, Pacific West Area (c) University of Hawaii at Hilo, CAFRNM

P09022 High-Throughput Genotyping of Rice Recombinant Inbred Lines by Next-Generation Sequencing
Han, Bin-presenter bhan@ncgr.ac.cn(a,c) Huang, Xuehui (a) Zhao , Qiang (a) Wang, Ahong (a) Guan, Jianping (a) Feng, Qi (a) Weng, Qijun (a) Fan , Danlin (a) Sang, Tao (a) Qian, Qian (b)
"Recombinant populations were the basis for Mendel's first genetic experiments and continue to be a key to the study of genes, heredity, and genetic variation today. The rice recombinant inbred lines (RILs) that were derived from the Oryza sativa indica 93-11 and japonica Nipponbare are an ideal genetic resource. The RILs will be genotyped only once, and this initial investment will be recovered with every new experiment. A limitation to speeding linkage mapping is often the ability to resolve genotype. To overcome the limits of conventional genotyping methods, we have now employed next-generation sequencing as a rapid, cost-efficient, and highly accurate way to genotype a RIL population in rice. We applied the short reads from the Illumina Genome Analyzer to accurately identify the genotype, according to known single nucleotide polymorphisms (SNPs) between two parent lines. With an average resolution of at least 1 SNP marker per 100 kb for each RIL, we then determined the precise locations of 2,432 recombination breakpoints of the population. With this set of breakpoint locations, we constructed a bin map, which was used for the quantitative trait loci (QTL) analysis. These results give a powerful and promising approach to speed up genetic dissection of complex traits and characterization of functional genes."
(a) National Centre for Gene Research & Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy (b) State Key Lab of Rice Biology, China National Rice Research Institute, Chinese Academy of Agricultural Sciences (c) Beijing Institute of Genomics, Chinese Academy of Sciences

SESSION P10 – BIOENERGY CROPS & BIOFUELS

P10001 "Directed Evolution of Polyhydroxalkonate Biosynthesis Amy C. Schroeder, Rebecca E. Cahoon, Jan G. Jaworski, Edgar B. Cahoon, Joseph M. Jez, Donald Danforth Plant Science Center, 975 N. Warson Rd, St. Louis, MO 63132 USA"
Schroeder, Amy C-presenter aschroeder@danforthcenter.org(a) Cahoon, Rebecca E. (a,b) Jaworski, Jan G (a) Cahoon, Edgar B (a,b) Jez, Joseph M. (a,c)
"Polyhydroxyalkanoates (PHA) are aliphatic polyesters that naturally accumulate as storage materials in certain bacteria; however, their physical properties are suitable for use as a renewable source of biodegradable thermoplastics. The directed evolution of PHA synthesis can determine how to best enhance the enzymes to produce the polymer. By engineering a polycistronic vector encoding 3-ketothiolase (phaA), acetoacetyl-CoA reductase (phaB), and PHB synthase (phaC) from R. eutropha, we can evolve each individual enzyme or the entire operon. For rapid screening of PHA-producing, a sensitive staining method using Nile red was developed. The oxazine dye Nile blue A and its fluorescent oxazone form, Nile red, are a simple and sensitive staining method to detect poly(3-hydroxybutyric acid) and other polyhydroxyalkanoic acids in bacterial colonies. To produce a library of mutants, PCR-based random mutagenesis was used to generate an initial library of mutant phaC variants. Using a polycistronic vector, screening experiments will be performed on each of the three biosynthetic genes and on the entire operon to identify clones with improved production. To evaluate how mutations affect molecular function, clones conferring the largest increases in PHA yield will be isolated, sequenced, and the evolved enzyme(s) overexpressed, purified, and kinetically characterized. In the long-term, directed evolution of PHA synthesis will develop the technological foundation for efficiently producing bioplastics in bacterial and non-food crops. "
(a) Donald Danforth Plant Science Center (b) University of Nebraska Lincoln (c) Washington University in St. Louis

P10002 "Cellulose microfibrils: biosynthesis, molecular structure, and dynamics in the processes of deconstruction to sugars for biofuels production"
Ding, Shi-You-presenter shi_you_ding@nrel.gov(a)
"Biofuels produced from cellulosic materials are considered today to be a highly promising technology for meeting the energy challenges of the 21st century. The crucial challenge faced in producing lignocellulosic biofuels economically is to overcome the recalcitrance of plant cell walls to the processes of being deconstructed to simple sugars that can be fermented to transportation fuels. Increasing the efficiency of this deconstruction step will require deeper understanding of the way in which plant cell walls are synthesized and the structural dynamics of plant development and growth, as well as of the structural and chemical changes brought about during the conversion processes for biofuels production. Advanced imaging techniques have been developed and applied to characterize the plant cell walls at the nanometer scales under non-destructive conditions. These techniques include scanning probe microscopy, non-linear optical microscopy, and single molecule spectroscopy. In this presentation, I will summarize our recent findings in the imaging of cellulose microfibrils from fresh, naturally-senescent, and chemically/biologically-pretreated cell walls."
(a) National Renewable Energy Laboratory

P10003 Starch to Oil: Engineering an Efficient Biofuel Currency
Dehesh, Katayoon-presenter kdehesh@ucdavis.edu(a) Hayden, Daniel M (a) Eckman, Asa (b) Stymne, Sten (b)
"The project aims to understand the interplay between oil and starch biosynthesis during endosperm development in oat seed with the goal of using the gained knowledge to modify starchy energy crops. Cereals and tuberous crops yield significantly more biomass than oilseed crops providing a unique opportunity to generate dual-use energy crops that can provide both a high-energy density biodiesel fraction while preserving the starch component that is an essential feedstock for the starch-based ethanol industry. Oat seed has evolved the unique ability to store the fixed carbons as a commingled fraction of oil and starch in developing endosperm. By using oat seed as a model system, the molecular switches of carbon allocation can be elucidated, thereby opening the possibility to redirect carbon flux from starch to oil in cereals and tubers through genetic engineering. Data discussed will detail carbon partitioning between oil and starch at different developmental stages of endosperm, in conjunction with bioinformatics complexities and strategies concerning 454 pyrosequencing of oat endosperm transcripts. The efficacy of a novel method of gene silencing within oat endosperm will also be presented. "
(a) Plant Biology, UC Davis (b) Department of Plant Breeding and Biotechnology, Swedish University of Agricultural Sciences, Alnarp, Sweden.

P10004 Hydrothermal conversion of biomass into fuels and chemicals: Technology that mimics nature
Jin, Fangming-presenter fmjin@tongji.edu.cn(a)
"A serious global energy crisis and environmental problems is due to imbalance between the slow formation of fossil fuels and rapid consumption by human activities. To diminish the imbalance, a rapid conversion of biomass into fuel and chemicals is important. Hydrothermal reaction has acted as a very important role during formatting fossil fuels. Thus, if humans could simulate the natural phenomena of the formation of fossils, then, it should quickly turn organic waste and biomass into fuels and chemicals. Such a process has the potential to efficiently and harmoniously circulate carbon resources on the earth, thus helping to maintain the needed balance of energy and resources. With this in mind, we have conducted a series of research of application of hydrothermal reaction to conversion of biomass into fuels and chemicals. The presentation gives an overview of some recent studies of hydrothermal conversion of biomasses, mainly including cellulosic and lignocellulosic biomasses, into chemicals, such as such as, acetic acid, lactic acid and formic acid. Keywords: Hydrothermal reaction; biomass; lignocelluloses; cellulose; fuels; chemicals; formic acids; acetic acid; lactic acid "
(a) School of Environ Sci and Eng., Tongji University

P10005 Development of *Dunaliella* strains for enhanced biofuel feedstock production.
Hernandez-Gomez, Leyla T.-presenter leylathg@yahoo.com(a) Lemos, Mark S. (a) Albion, Rebecca L. (a) Shintani, David K. (a) Harper, Jeff F. (a) Cushman, John C. (a)
"Halophytic unicellular green algae within the genus *Dunaliella* could serve as alternative feedstocks for the generation of biofuels, because they contain high amounts of triacylglycerols (TAGs) and starch, which could be extracted, processed and refined to meet the global demand for transportation fuels. The overall goal of our project is to develop *Dunaliella* strains that contain elevated TAGs or starch content in order to increase their feedstock potential for biodiesel or ethanol production, respectively, and to reduce production costs. In order to create an algal strain with improved traits, *Dunaliella* cells were chemically mutagenized using ethyl methyl sulfonate (EMS) and mutant cells were selected reiteratively, by continuous percoll density gradient centrifugation, for cells populations containing high oil/starch amounts. After only 10 rounds of density selection there were significant qualitative differences observed in buoyant density between mock selected and reiteratively selected cell populations. Furthermore, the selected populations displayed quantitative increases in either TAG or starch production illustrating that alterations in feedstock characteristics can be derived in relatively short time frames. In addition, a stable transformation strategy to establish genetic manipulation tools is

being developed that will allow for the engineering of algal strains with improved feedstock traits."
(a) University of Nevada, Reno

P10006 Endosperm targeted expression of the CBHI exocellulase in maize
Vicuna Requesens, Deborah V.-presenter dvicuna@astate.edu(a,b) Hood, Elizabeth E (a,b)
"One of the most important steps in the conversion of biomass into fermentable feedstocks for production of fuels is the ability to break down cellulose into glucose molecules. Increasing the amount of cellulases to efficiently digest cellulose will have an enormous impact in the production of biofuels. The plant production system for industrial enzymes has been successfully utilized as demonstrated by transgenic maize. In order to minimize the cost of cellulases by maximizing protein accumulation, CBH1, an exocellulase from *Trichoderma reesei*, was expressed in transgenic corn kernels. It was cloned under the control of various seed-specific promoters. Previously, this cellulase has been expressed using the embryo-preferred globulin-1 promoter from maize, with expression of up to 17.9% total soluble protein (%TSP) in first generation seed (Hood et al., 2007). To further increase seed-based protein accumulation, two endosperm-specific promoters from rice (a globulin and a glutelin promoter) as well as four maize Zein promoters are being employed. They have been tested using the GUS gene as a reporter in maize. Using X-gluc staining, the promoters were shown to have pronounced expression of the reporter gene in endosperm. In transient expression experiments on maize endosperm, the rice promoters were also active in expressing CBHI, up to 1.7% TSP. Constructs containing CBHI under the control of these endosperm-specific promoters have been transformed into corn using co-cultivation with immature zygotic embryos. Transgenic maize plants resulting from these experiments will be crossed to those already over-expressing the embryo-specific promoter from maize in order to achieve a synergy in expression of this exocellulase, providing larger volumes of this enzyme at lower costs"
(a) Arkansas State University (b) Arkansas Biosciences Institute

P10007 A functional genomics approach to understanding and remodeling plant cell walls of bioenergy crops
Chen, Fang (a,c) Wang, Huanzhong (a,c) Zhao, Qiao (a,b) Shen, Hui (a,c) Zhou, Rui-presenter rzhou@noble.org(a) Jackson, Lisa (a) Shadle, Gail (a) Hernandez, Tim (a,c) Qi, Liying (a) Dixon, Richard A (a,c)
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" The composition and structure of lignified cell walls has a significant impact on the value of plant-derived raw materials. We have previously demonstrated clear relationships between lignin content/composition and the efficiency of saccharification by both chemical pre-treatment and enzymatic hydrolysis using stably transformed alfalfa lines. It is clear that lignification is critical in plant support, water transport and, in some cases, disease resistance. Therefore, technology for tissue-specific modification of lignin biosynthesis is highly desirable. To obtain a genetic and biological understanding of the mechanisms that regulate cell wall biosynthesis, we developed a method for the large scale screening of M. truncatula transposon insertion lines for discovery of novel genes affecting spatial patterns and amounts of lignin deposition in Medicago (~10,000 plants with multiple insertions). Twenty five particularly interesting mutant lines were obtained with altered patterns of lignification and cell wall structure. Some of these mutants have intact vascular systems but have altered fiber cell walls and lignin content with no visible defects in plant growth and development. Cloning and characterization of the genes affected in these mutants will enable us to confirm their biological functions and provide a better understanding of the biochemical and regulatory pathways that determine cell wall phenotypes. Candidate genes were also selected from microarray and comparative genomic studies in M. truncatula and switchgrass. We anticipate that this approach will ultimately provide a technical platform for the remodeling of plant cell wall structure of bioenergy crops. "
(a) The Samuel Roberts Noble Foundation (b) Oklahoma Bioenery Center (c) DOE BioEnergy Science Center

P10008 Comparing components of CO_2 assimilation in a variety of willow species
Andralojc, John-presenter john.andralojc@bbsrc.ac.uk(a) Bencze, Szilvia (a) Guinard, Jeremy (a) Shield, Ian (a) Karp, Angela (a) Parry, Martin A.J. (a)
"We need to know the extent to which photosynthetic capacity varies between willow (*Salix*) genotypes in order to assess the potential for increasing carbon capture through selection of trait associated (photosynthetic) QTLs. If sufficient natural variation in photosynthetic capacity is apparent, then subsequent attempts to identify the underlying processes and genes may be justified. This preliminary work demonstrates the existence of significant variation in photosynthetic capacity (expressed on a leaf area or leaf dry matter basis) attributable to metabolic and/or anatomical characteristics within willow leaves, as distinct from stomatal limitations. We are currently attempting to identify metabolic processes which contribute to these differences in photosynthetic capacity. Within this diverse group of *Salix* species, the variation in above ground biomass (leaves + wood) was more closely correlated to total leaf area per plant, than to assimilatory capacity per unit leaf area. Nevertheless, the observed variation in assimilatory capacity per unit leaf area (of between 2 and 3-fold) imply that considerable gains in biomass production could be achieved by increasing the photosynthetic capacity per unit area in species which already produce many leaves. "
(a) Rothamsted Research

P10009 Genetic engineering of switchgrass to improve renewable energy production
Yin, Xiaoyan-presenter yinxi@missouri.edu(a) Zhang, Zhanyuan (a)
http://www.plantsci.missouri.edu/muptcf
"Genetic engineering plays a unique and important role in improving crop traits. Teaming up with several other laboratories in the state of Missouri, we are developing an engineering approach to improve biofuel production as an alternative source of energy. One of the most important crops to be engineered in this project is switchgrass (Panicum virgatum). To be successful in this project, it is essential to develop an efficient Agrobacterium-mediated transformation process in switchgrass. The Agrobacterium-mediated T-DNA transfer offers several advantages over other transformation systems. However, in spite of previous reports, Agrobacterium-mediated transformation of this crop has been proven to be very difficult. Therefore, since our project started we have optimized a number of critical conditions affecting switchgrass transformation. These conditions included the switchgrass genotypes, cocultivation temperatures and medium salt concentrations, Agrobacterium strains, transformation vectors, selection system and selective agents. Our results show that the optimal Agrobacterium infection and subsequent selection conditions led to rapid and consistent transgenic recovery of switchgrass plants. These works will lay a good foundation for efficient engineering of switchgrass via Agrobacterium for improved renewable energy production. "
(a) University of Missouri-Columbia

P10010 Evaluation of oil production and growth in several Mexican Jatropha species from arid regions.
Xicotencatl-Lozano, Michelle-presenter micxi_86@yahoo.com.mx(a) Rodriguez-Acosta, Maricela (b) Zeferino-Diaz, Reyna (c) Sandoval-Ramirez, Jesus (d)
"*Jatropha curcas* has been widely grown for oil production but in arid areas the yield is reduced considerably. There are, however, many species of

Jatropha native to Mexico that occur in dry areas and have not been evaluated for oil production. This research work aims to evaluate six endemic Jatropha species of Mexico that grow mainly in semi-arid lands in Puebla state. The main goal is to find suitable species compatible with Jatropha curcas in order to create new hybrids that improve fruit production and oil quality. Several aspects of Jatropha species were evaluated: rootability, response to growth hormones and shoot production. Germination trials in laboratory and greenhouse conditions were also carried out, as well as determination of seed oil content and chemical composition. The results show that growth hormones increase the rootability in the stakes, and protect them from pests and diseases. The appearance of vegetative parts was not a good indicator of good root mass production. There are great differences between species in both root and shoot production. Germination in the laboratory for three species started at 5 days and was mostly complete in 2 weeks. *J. elbae* had a germination rate of 75% and did not show susceptibility to pest contamination. *J. oaxacana* had a germination rate of only 16%, much lower than that of *J. elbae*. *J. andrieuxii* was very susceptible to pest contamination and none of the seeds germinated. Oil content in **J. elbae** is as high as that reported for *J. curcas*, however seed size and fruit production vary widely in the six species studied. "
(a) Escuela de Biologia (b) Herbario y Jardin Botanico (c) Ingenieria Ambiental (d) Facultad de Ciencias Quimicas, BUAP.

P10011 "Properties of culm strength, lignin content and cinnamyl-alcohol dehydrogenase in the rice cultivar, Leaf Star with high lodging resistance -Comparison with parents and *gold hull and internode* mutants"
Ookawa, Taiichiro-presenter ookawa@cc.tuat.ac.jp(a) Inoue, Kazuya (a) Sunaga, Kaoruko (a) Tanaka, Satomi (a) Tojo, Seisyu (a) Hirasawa, Tadashi (a)
"Lignin modification in feed and bioenergy crops has been the main target for breeding to improve forage digestibility and energy yield. However, improvements in forage digestibility resulting from reduced lignin content are often accompanied by reduced lodging resistance. The rice *gold hull and internode2* (*gh2*) has been identified to be a lignin-deficient mutant. The *GH2* gene has been mapped to the short arm region on chromosome 2 and encodes a cinnamyl-alcohol dehydrogenase. In the *gh2* mutant, conifenyl alcohol dehydrogenase (CAD) activity is reduced and sinapyl alcohol dehydrogenase (SAD) activity is not detectable. The new forage cultivar, Leaf Star has high lodging resistance, and has a reddish-brown pigmentation in the hull and internode similar to that of its parent, Chugoku 117 and *gh* mutants. We compared the location of the *gh* locus, the properties of CAD and SAD activities, and the lignin content and culm strength of Leaf Star with those of its parents and *gh* mutants. By QTL analysis using parents, the *gh* locus was located to the same region on chromosome 2 with *gh2*. Leaf star exhibited similar substrate specificity for CAD and SAD activities as Chugoku 117 and the *gh2* mutant. The stem lignin content of Leaf Star was 20% lower than that of its parent, Koshihikari. The *gh2* mutant, SG0207, which had low lignin content, was susceptible to lodging due to its weak and fine culms. In contrast, Leaf Star has high culm strength due to its thick and strong culms. Our results suggest these improvements in forage digestibility and energy yield can be combined with high lodging resistance by utilizing the genetic resources of rice that confer thick and strong culms, such as in the *gh2* cultivar, Leaf Star. "
(a) Tokyo University of Agriculture and Technology

P10012 Improved methods for tissue culture and genetic transformation of switchgrass
Nelson, Kimberly (a) Hague, Joel (a) Deresienski, Adam (a) Kausch, Albert-presenter akausch@etal.uri.edu(a)
"The use of dedicated energy crops, such as switchgrass and other perennial grasses, as a source of biomass for renewable biofuels is of great relevance to global economic and ecological issues. Currently, strategies are being developed using plant genetic engineering approaches for enhancement of biofuel production. We have evaluated tissue culture parameters for nine varieties of switchgrass (*Panicum virgatum*) and Atlantic Coastal Panic grass (*Panicum amarum*) for their ability to produce embryogenic and regenerable cultures that would be useful for genetic transformation. An increase in seed germination and callus formation in culture was achieved via the use of PPM and stratification. We have isolated embryogenic callus lines for the different varieties and have evaluated and optimized regeneration media. Several hundred regenerates per cultivar have been recovered and are currently being evaluated for somaclonal variation. Kill curves for both glufosinate and hygromycin have been done to optimize selection criteria. We have used a gene construct consisting of an ubiquitin promoter from rice driving expression of the bar gene for glufosinate resistance. Using Agrobacterium-mediated transformation (LBA 4404) we have successfully introduced this gene construct into switchgrass (cv Alamo), producing a total of over 125 stably transformed individual events. The calculated transformation efficiencies per experiment, using the number of transgenic colonies derived by the number of embryogenic calli infected had an average of about 6.5%. We have analyzed these T_0plants for three characteristics, including; herbicide resistance in paint assays, PCR, and Southern blots to confirm transformation."
(a) University Of Rhode Island

P10013 Male sterilty as a method for constructing wide crosses and for gene confinement in switchgrass and other biofuels crops
Deresienski, Adam (a) Hague, Joel (a) Nelson, Kimberly (a) Kausch, Albert-presenter akausch@etal.uri.edu(a)
"Energy from biomass, particularly cellulosic biomass from dedicated energy crops, will have tremendous economic, environmental and national security benefits. Biofuels will help alleviate our national dependency on fossil fuels, with a cost savings of an estimated 20 billion USD per year based on 2050 estimated fuel costs. Trait improvement of switchgrass and other perennial grasses via genetic engineering has barely begun but is important to the development of the renewable energy industry and the environment. However, the possibility of gene flow to wild and non-transformed species raises commercial and ecological concerns. Male sterility provides an effective strategy for interrupting gene flow through the pollen. In addition, male sterility may allow for the recovery of rare wide crosses. Promoters from male gametophyte-specific genes, such as *Zm13* from maize and *rts* from rice, can be used to induce male sterility. We selected a gene construct consisting of a rice tapetum-specific promoter *rts*, fused to the ribonuclease gene *barnase* and linked to a constitutive *bar* cassette for glufosinate resistance. Using *Agrobacterium*-mediated transformation, we have successfully introduced this gene construct into switchgrass (*cv* Alamo), producing a total of over 125 stably transformed individual events. The vegetative phenotype of the transgenic plants was identical compared with the control wild-type plants indicating that expression of tapetum-specific *barnase* did not affect normal plant development. T_0 plants have been evaluated for herbicide resistance in paint assays; PCR and Southern blots have confirmed transformation. Male sterility is currently being evaluated. This strategy may be useful for recovery of wide crosses and as a gene confinement approach."
(a) University Of Rhode Island

P10014 Sixty percent more productive than maize in the Midwest! How does Miscanthus do it?
Dohleman, Frank G.-presenter dohleman@illinois.edu(a) Long, Stephen P (a)
http://www.miscanthus.uiuc.edu
"An economically and energetically favorable bioenergy crop must be able to produce large quantities of biomass with minimal inputs. The C_4 species Miscanthus (*Miscanthus* x *giganteus*) and maize (*Zea mays*) are being used or considered as energy crops. Until now their productivity has not been directly compared. In side-by-side large scale field trials in the Corn Belt of central Illinois, USA, Miscanthus was 60% more productive than maize ($p<0.0001$). The total productivity of a crop species is determined by the product of the total amount of solar radiation which is incident on an area of land (Q_{tot}) efficiencies of light interception (ε_i) and conversion into biomass (ε_c). Understanding the basis for higher productivity in Miscanthus will show how corn and other C4 crops could be engineered to increase yield. Averaged over two complete growing seasons, ε_i for Miscanthus was 61%

higher than in maize ($p<0.0001$) accounting for the difference in biomass accumulation. This was because Miscanthus developed a green leaf canopy earlier and maintained it later than maize. Conversion efficiency was not different between species ($p=0.5$). In 2007 and 2008, the diurnal course of photosynthesis was measured on sunlit and shaded leaves of each species on 26 dates throughout the two growing seasons. The daily integral of photosynthetic CO2 uptake was up to 60% higher in maize, during mid-summer, however when integrated across the two complete growing seasons, there was no difference in leaf-level assimilation ($p=0.4562$). Green Leaf Area Index (GLAI), was measured destructively on the same dates as gas exchange, and when integrated over two full growing seasons, GLAI was more than double for Miscanthus when compared to maize ($p<0.0001$). By combining information on photosynthesis of different leaf layers with canopy size and structure, total photosynthesis of both crops were calculated. Canopy photosynthesis across both growing seasons was 44% higher in Miscanthus than in maize ($p<0.0001$), corresponding closely with the difference in peak biomass. Finally to determine the basis for the superior leaf-level photosynthesis in maize at mid-season, light and CO2 responses were derived to determine *in vivo* biochemical limitations. These showed that in mid-season maize has a higher maximum velocity of PEP carboxylation (V_{pmax}) and a higher velocity of PEP regeneration (V_{pr}), as well as a higher light saturated rate of photosynthesis (A_{sat}) and higher maximum quantum efficiency of CO_2 assimilation ($\Phi_{CO2\ max}$). These biochemical differences, however, are compensated by a larger leaf area in Miscanthus, and its ability to maintain photosynthetically competent leaves at the cooler temperatures of late spring and fall."
(a) University of Illinois

P10015 Transcriptional regulatory network that controls secondary wall biosynthesis
Ko, Jae-Heung (a) Kim, Won-Chan (a) Kim, Hyun-Tae (a) Han, Kyung-Hwan-presenter hanky@msu.edu(a)
"Secondary cell wall of plant biomass is a major source of lignocellulosic material for liquid biofuel production. Better understanding of the molecular mechanisms underlying its biosynthesis will help us develop biotechnological means to genetically control key pathways that determine the quantity and quality of the biomass. In an effort to identify candidate genes involved in transcriptional regulation of secondary wall biosynthesis, we developed an experimental system that induces ectopic development of secondary wall in Arabidopsis thaliana. Using this system, we carried out Affymetrix GeneChip and Illumina Digital Gene Expression analyses to identify a battery of genes differentially expressed during secondary wall biosynthesis. A total of 292 genes were dramatically upregulated within 6-hrs of the secondary wall induction treatment. Most of the secondary wall biosynthetic genes (e.g., cellulose, hemi-cellulose and lignin genes) were represented in the list. We then identified 38 transcription factors whose expression is coincided or preceded with the induction of secondary wall biosynthetic genes. Based on these transcriptome analysis results, we constructed a tentative hierarchical transcriptional regulatory network leading to biosynthesis of secondary wall components. In order to confirm the relationship between transcription factors and their target genes, we are using both transient activation assay and electrophoretic mobility shift assay. This presentation will describe (1) our genomics approach for identifying a transcriptional regulatory network that control secondary wall biosynthesis and (2) functional characterization of selected candidate genes in the network."
(a) Michigan State University

P10016 TILLING for Altered Fatty Acid Profiles in Camelina sativa
Hutcheon, Carolyn-presenter chutcheon@targetedgrowth.com(a) Ditt, Renata (a) Kiser, Jack (e) Comai, Luca (b) Shewmaker, Christine (c) Nguyen, Thu (a) Schroeder, Jesara (a) Guillen-Portal, Fernando (d) Van Deynze, Allen (b) Kohl, Elizabeth (a) De Rocher, Jay (a)
"*Camelina sativa*, a member of the Brassicaceae family, has renewed interest as a crop due to its potential for biofuels applications. It has low input requirements allowing it to be grown under marginal growth conditions. Targeted Growth is developing Camelina for biofuel purposes using three parallel approaches: mutation breeding, classical breeding, and genetic modification. Mutation breeding provides an opportunity to develop new camelina varieties with modified fatty acid profiles that are more desirable for biodiesel use. We have created a population of ~ 8,000 mutants by EMS mutagenesis and taken advantage of Targeting Induced Local Lesions IN Genomes (TILLING) to identify individuals mutated in Fatty Acid Desaturase 2 (FAD2), a gene involved in converting oleic acid (18:1) to linoleic acid (18:2). We have previously identified 3 copies of FAD2 in *Camelina sativa*. Mutations that knock out or compromise FAD2 could lead to a decrease in the polyunsaturated fatty acids 18:2 and 18:3 and/or give a concomitant increase in the 18:1 monounsaturated fatty acid, improving fatty acid composition of the oil for biodiesel production. A pilot study determined that the mutation density of our mutant camelina population was 1/75 kb to 1/120 kb. TILLING of an initial 768 M2 individuals for FAD2 has identified 60 mutants, 60% of which are non-silent mutations. Of the non-silent mutations, about 30% are predicted to be severe missense or truncation mutations. Mutations were identified in all 3 copies of camelina FAD2. Our previous finding that *Camelina sativa* may be polyploid is further supported by the high density of lesions this plant is willing to tolerate in its genome. We are currently growing the mutant M3 plants and plan to analyze their fatty acid profiles by GC. "
(a) Targeted Growth Inc, Seattle, WA (b) University of California, Davis, CA (c) Blugoose Consulting, Woodland ,CA (d) Barkley Ag Enterprises, LLP, Bozeman, MT (e) Sustainable Oils, Davis, CA

P10017 Non-destructive continuous extraction of microalgal neutral lipids under simulated field conditions
Swanson, Andrew K-presenter Andrew.Swanson@phycal.com(a) Allnutt, Thomas FC (a) Sayre, Richard (a,b)
"Two of the major challenges facing the successful use of algae in the biofuel industry involve economical means of dewatering and extraction. Currently these processes are estimated to exceed 40% of an operations total costs, primarily due to the small cell sizes (~10 µ;m) of oleogenous microalgae, and the comparatively low working concentrations (~0.1% w/v) of cultures. Phycals non-destructive extraction process (the Olexal™ process) is capable of continuously removing up to 100% of the neutral lipids (TAGs) from living algal cultures without apparent harm. Further, Olexal extraction can be done in vivo with, or without partial dewatering. This extraction process was tested at significant culture volume scales (>1000 L), treatment intervals (>weeks), and on select species under simulated field cultivation conditions. Demonstrated advantages of the Olexal extraction process, including augmented total algal lipid production per unit area, elevation of cell density, improved culture integrity, and reduced contamination of recovered TAGs, will also be presented. "
(a) Phycal LLC (b) Donald Danforth Plant Science Center

P10018 Determination of photosynthetic enzymes activities' and carbon isotope labeling studies in Switchgrass cultivars
Soundararajan, Madhavan-presenter msoundar@unlnotes.unl.edu(a) Bundy, Robert (a)
"Switchgrass (Panicum variegatum L) is among the species selected by DOE for development as a bioenergy crop, to accelerate a sustainable production of cellulosic ethanol. Populations of switchgrass occur as lowland and upland ecotypes with divergent growth habits and yield potential. Selection and sustainably growing switchgrass requires a comprehensive understanding of its primary and secondary metabolism of its various cultivars. Previous studies have already established that Swtichgrass is a C4 plant with NAD-ME photosynthetic pathway type. The present study is however focused on characterizing a few of the field grown Switchgrass cultivars for their photosynthetic characteristics and their stable carbon isotope ratios. Cultivars of switchgrass showed: a) a range in activity of both phosphoenol-pyruvate carboxylase (67.1 - 231.4µ;mol mg-1 protein) and NAD-malic enzyme (220-317 µ;mol mg-1 protein) and b) a variation in their carbon isotope ratio values (12.4 to -13.3 per mil). Data will also be

presented for both Rubisco and PPDK enzyme activities in these cultivars along with their immunoblots. Preliminary results from 13CO2 pulse labeling of greenhouse grown switchgrass plants indicated an active carbon partitioning in their stem tissue over a period of 6 weeks than either in the leaf or root tissues. Results from further analyses of carbon partitioning into hemicelluloses, celluloses and lignins from the stem tissues of labeled plants will be presented."
(a) University Of Nebraska, Department of Biochemistry

P10019 Using als as a selectable marker for transformation of camelina sativa
Brost, Jennifer M-presenter Jennifer.Brost@targetedgrowth.com(a) Liu, Xunjia (a) Guilfoil, Robin (b) Hutcheon, Carolyn (b) Kiser, Jack (c)
"Camelina sativa, a member of the Brassicaceae family, is an alternative oilseed crop that is gaining interest as a feedstock for production of biodiesel and as an edible oil. Camelina has received limited improvement of its agronomic characteristics during its history as a crop, presenting an opportunity to achieve significant gains in productivity and other desirable traits. Targeted Growth and Sustainable Oils are developing camelina for biofuel applications by three parallel approaches: classical breeding, mutation breeding and genetic modification. Targeted Growth previously developed a floral dip method to transform camelina without a selectable marker. To improve the efficiency of the transformation process, we have developed a selection method based on the use of the acetolactate synthase (ALS) selectable marker. Kill curves were determined for three herbicides (Chlorsulfuron, Metsulfuron-methyl and Nicosulfuron). Effects of temperature, light intensity and plating methods on selection efficacy were tested. It was determined that growing seedlings at 28^{o}C, with 16/8hr photoperiod and light intensity of ~23000 LUX, in solid medium containing Chlorsulfuron is optimal. Positive seedlings grow straight up out of the selection medium, have the radicle embedded in the medium, have green cotyledons and continue to produce true leaves. Under this selection protocol, transformants are readily distinguished from non-tranformants as being positive for resistance to Chlorsulfuron. We are currently transforming camelina with genes to increase yield and selecting for transformants using the protocol developed above. Transformed plants carrying yield genes will be tested in field trials in spring 2010. "
(a) Targeted Growth Canada, Saskatoon, SK S7N 0W9 (b) Targeted Growth, Inc., Seattle, WA 98103 (c) Sustainable Oils, Davis, CA 95616

P10020 Carbon sequestration mediated by plant-soil-microbe interactions in tallgrass prairie communities
Davis, Sarah-presenter davissc@uiuc.edu(a) Yannarell, Tony (a) DeLucia, Evan (a)
"The conversion of the tallgrass prairie to annual row crops caused widespread loss of soil carbon and reduced the diversity of soil microbial communities throughout the Midwestern USA where biofuel feedstock croplands are proposed. We examined the interactive effect of plant species composition and soil microbial diversity on carbon storage of prairie communities that might either establish naturally on abandoned agricultural land or might be planted as high-diversity low-input biofuel crops. We then compared the relative carbon storage capacity of different plant-soil-microbial assemblages to determine the most efficient option for restoring carbon to the landscape. We found that some plant monocultures have 170% greater carbon storage capacity than mixed plant communities in sterile soils treatments, but mixed plant communities have more consistent carbon storage across a range of microbial communities. We employed a replicated block design to compare species assemblages in the field to the patterns we observed in a controlled greenhouse experiment, and found that mixed species prairie communities have soil carbon concentrations that are 120-183% greater than harvested monoculture grass plantings, but this difference depended on variation in climate and microbial communities among treatment blocks. Nitrogen use efficiency was an important determinant of harvested ecosystem carbon storage as evidenced by comparative biogeochemical cycles among crops. Results from this study serve as a baseline range of carbon storage that would be displaced by conversion of land use from livestock feed crops to biofuel agriculture. These results also may be used to inform prairie restoration efforts that would maximize carbon storage in an otherwise intensively harvested landscape."
(a) University of Illinois at Urbana-Champaign

P10021 Molecular genetic basis for variation in lignocellulosic biomass composition in segregating populations of shrub willow (*Salix* spp.) bioenergy crops
Serapiglia, Michelle J.-presenter mjserapi@syr.edu(a) Cameron, Kimberly D. (a,b) Stipanovic, Arthur J. (a) Smart, Larry B. (a,b)
"Rapid determination of biomass composition and the molecular basis for cell wall biosynthesis will greatly enhance the genetic improvement of fast-growing shrub willow (*Salix* spp.), a proven perennial bioenergy crop for temperate climates. High resolution thermogravimetric analysis (HR-TGA) was developed for rapid, low-cost trait phenotyping and selection of shrub willow genotypes with optimized biomass composition. To validate the HR-TGA method, a selection of 25 shrub willow clones was analyzed using traditional wet chemistry techniques. The hemicellulose, cellulose, and lignin content of the samples analyzed by wet chemistry correlated well with the HR-TGA results with R^2 values over 0.7. Furthermore, compositional differences among willow clones with a variation of 5 to 6 percent for all three components were determined. Increases in cellulose content and decreases in lignin content were observed as a function of the age of the stem. There was a strong impact of bark on biomass composition with a significant increase in lignin content and a significant decrease in cellulose content. Overall, the results indicate that HR-TGA is a reliable technique for the compositional analysis of biomass components in shrub willow. The expression patterns of 16 specific genes encoding enzymes involved in lignin biosynthesis and selected carbohydrate active enzymes are being investigated. Samples of stems were obtained from eight members of a hybrid shrub willow family throughout the 2008 growing season and are being used to examine the expression patterns of genes associated with stem growth throughout the growing season using real-time qPCR. This will provide insights into the genetic regulation of lignocellulosic deposition in this important bioenergy crop. "
(a) SUNY College of Environmental Science and Forestry (b) Cornell University

P10022 Genetic resources for functional genomics of maize cell wall biology
Penning, Bryan W (a,b) Hunter III, Charles T (c) McCann, Maureen C (b) Carpita, Nicholas C-presenter carpita@purdue.edu(a) Tayengwa, Reuben (d) Eveland, Andrea L (c) Olek, Anna T (a) Vermerris, Wilfred (d) Koch, Karen E (c) McCarty, Donald R (c) Davis, Mark F (e) Thomas, Steven R (e)
http://www.btny.purdue.edu/Faculty/Carpita/
"Grass species represent a major resource of our food, feed, and fiber crops and potential feedstocks for biofuel production. Most of the biomass is contributed by cell walls that are distinct in composition from all other flowering plants. Identifying cell wall-related genes and their functions underpins a fundamental understanding of growth and development in these species. Toward this goal, we are building a knowledge base of the maize genes involved in cell wall biology, their expression profiles, and the phenotypic consequences of mutation. Over 750 maize genes were annotated and assembled into 21 gene families predicted to function in cell wall biogenesis. Comparative genomics of maize, rice, and Arabidopsis sequences revealed differences in gene family structure between grass species and a representative dicotyledonous species. Deep sequencing of the maize unfertilized ovary showed that transcript abundance varies more than 100-fold between members of a single family of cell wall-related genes. When compared to expression for developing ovaries of rice and Arabidopsis, different sets of genes were expressed in the grasses compared to Arabidopsis. Over one-hundred new cell-wall mutants from a UniformMu population in both forward- and reverse-genetics approaches. A forward

screen of field-grown lines by near-infrared (NIR) spectroscopic screen of mature leaves yielded several dozen lines with heritable spectroscopic phenotypes. Pyrolysis-molecular beam-mass spectrometry confirmed that several NIR-mutants had altered carbohydrate-lignin compositions. The differences in gene family structure and expression between Arabidopsis and the grasses underscore the requirement for a grass-specific genetic model for functional analyses."
(a) Dept. of Botany & Plant Pathology, Purdue University (b) Dept. of Biological Sciences, Purdue University (c) Horticultural Sciences Dept., University of Florida (d) Genetics Institute and Agronomy Dept., University of Florida (e) National Bioenergy Center, National Renewable Energy Lab

P10023 Surveying and characterizing genomic repeats and small RNA production in *Miscanthus x giganteus*
Hudson, Matthew E-presenter mhudson@illinois.edu(a,b) Swaminathan, Kankshita (a,b) Rokhsar, Daniel (b,c) Ming, Ray (a,b) Varala, Kranthi (a) Moose, Stephen (a,b)
"*Miscanthus x giganteus (Mxg)* is a perennial grass that produces superior biomass yields in temperate environments. The triploid genome (3n = 57, x = 19) of *Mxg* is likely critical for the rapid growth of this vegetatively propagated interspecific hybrid. We conducted an initial survey of the complex Mxg genome using 454 pyrosequencing. The survey revealed that most of the *Mxg* genome consists of mobile repetitive sequences, including retrotransposons and other multi-copy elements such as MITES, along with highly abundant classes of structural repeats and rDNA. Using non-cognate assembly for de novo repeat detection, the most abundant sequences in the *Mxg* genome were predicted, many of which are retrotransposon related. Comparison of the abundant repeat sequences to a small RNA survey of *Mxg* performed using Illumina sequencing technology revealed that the majority of small RNA produced in three *Mxg* tissues is derived from the repetitive sequences identified using the genome survey. A strong, positive correlation was observed between copy number of repeats and small RNA production. Analysis of rDNA haplotype frequencies captured in the survey supports the hypothesized origin of *Mxg* as a recent hybrid between *M. sacchariflorus* and *M. sinensis* that has not yet experienced significant concerted evolution. These results indicate the feasibility of using next-generation sequencing technologies to characterize genome content and organization rapidly for Mxg and other large, complex and uncharacterized genomes."
(a) University Of Illinois, Urbana-Champaign (b) Energy Biosciences Institute (c) DoE Joint Genome Institute

P10024 Microbial contamination of Hawaiian E10 fuels
Kschowak, Max J (b,a) Rapolu, Madhusudhan-presenter rapolu@hawaii.edu(a) Poelman, Mary (a) Schneider, Kevin (a) Marrero, Glorimar (a) Paret, Mathews (a) Alvarez, Anne (a)
"A pink glutinous material has been observed in gasoline containing 10 percent ethanol (E10) in Hawaii, and microbial populations recovered from this material are implicated in slime production which plugs filters leading to motor failure. Microbial contamination has been linked to accelerated corrosion of metal parts of storage tanks for diesel and aviation fuel. The presence of pink material in the gasoline and rapid corrosion of metal in contact with E10 in Hawaii led to investigations of the possible role of contaminating bacteria in the fuel filters as well as the accelerated corrosion. In humid environments E10 gasoline entrains water forming a water:ethanol layer conducive to microbial growth. We have confirmed the presence of bacterial growth in E10 gasoline. Several genera and species of bacteria were cultured from pink glutinous materials, from a filter removed from an E10 gasoline dispenser, and from samples removed from corroded metal fittings on underground turbine pumps. Bacteria were characterized by light microscopy, scanning- and transmission-electron microscopy, and genera were identified by 16S rDNA sequence analysis. Several genera reproduced the pink slime typical of that observed in the ethanol-water phase of E10. These findings point to the risk of using ethanol fuels without a thorough study of potential microbial contamination and subsequent corrosion of ferrous metals which are an integral part of the fuel storage and fuel dispensing systems."
(a) Department of Plant and Environmental Protection Sciences, University of Hawaii, Honolulu, HI (b) Hochschule Mannheim-University of Applied Sciences, Germany

P10025 Expression of sucrose: sucrose-1-fructosyltransferase (1-SST) and sucrose: fructan-6-fructosyltransferase (6-SFT) under the control of aleurone specific promoters
Diedhiou, Calliste Jeremie J-presenter diedhiouc@agr.gc.ca(a) Liang, Yehong (a) Sun, Jeniu (a) Laroche , Andre (a) Gaudet , Denis (a)
"Fructans are linear or branched forms of fructose polymers, present in approximately 15% of the angiosperm flora, and are particularly widespread in grasses. Accumulation of fructans in plants has been found to be associated with tolerance of cold and drought and resistance to snow molds. Many prebiotic benefits of fructans to the human health have been reported. In this study, we report fructan accumulation in triticale seeds co-transformed with two natives genes, 1-SST (sucrose-sucrose 1-fructosyltransferase), and 6-SFT (sucrose-fructan 6-fructosyltransferase), both involved in fructan synthesis. The genes were cloned into transformation vectors under the control of barley amylase promoter a wheat lipid transfer protein promoter, both aleurone layer-specific. Southern blot hybridisation demonstrated the presence GFP and YFP reporter genes in different transgenic lines and PCR of plants carrying the encoded genes, showed a coordinate increase of 1-SST in 40% of the transformant and 6-SFT transcript in 28% of the transformants. Twenty percent of the transformants possessed both inserts. Fructan accumulation in the 1- and 2-gene transformants ranged from 4.4 to 10.4 % dry wt, representing a 2 to 6 fold increase in fructan content compared to the untransformed controls (1.7%). The results demonstrated that the carbohydrate metabolism within cereal seed can be modified to produce designer carbohydrates with benefits to human health. Key words: Fructan, sucrose: sucrose-1-fructosyltransferase (1-SST), sucrose: fructan-6-fructosyltransferase (6-SFT), seed, triticale "
(a) Lethbridge Research Centre

P10026 The introduction of freeze tolerance into a tropical *Eucalyptus* enables a new bioenergy crop for the Southeastern United States.
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"*Eucalyptus urophylla* (*E. grandis* x *E. urophylla*), a tropical tree with excellent biomass productivity, relatively low lignin content and a short rotation time, is an ideal energy crop. However, a major limitation to the expansion of this tree to the Southeastern United States is its high frost sensitivity. To increase the freeze tolerance of this tree, *Arabidopsis* CBF2 cDNA under control of a cold-inducible promoter was introduced via *Agrobacterium*-mediated transformation and multiple transgenic lines were obtained. A quick and reliable chamber test was developed to evaluate freeze tolerance in young, greenhouse-grown CBF2 eucalyptus trees. The chamber tests revealed that 72% of the CBF2 lines had better freeze tolerance than the control plants. The control eucalyptus was killed by 24 hrs of -7^{o}C while the CBF2 eucalyptus survived at -10^{o}C, suggesting that the CBF2 eucalyptus had at least a 3 degree improvement in freeze tolerance. Multi-year, multi-site field trials showed that the CBF2 eucalyptus was able to survive winter freezing temperatures of -9^{o}C while the control eucalyptus was killed to the ground. It was also observed that the growth rate, height, morphology, flowering time and flower patterns were similar between the CBF2 and control eucalyptus. However, the CBF2 eucalyptus did not produce pollen due to the presence of a pollen control gene. The CBF2 eucalyptus trees grew well, and achieved a height of 55ft and a diameter of 6in after 27 months field growth. This growth rate is anticipated to achieve a biomass productivity of 13-20 dry tons/ac/yr, which makes it a highly suitable and cost-

effective lignocellulosic feedstock for biofuels, bioenergy and industrial applications. "
(a) ArborGen, LLC

P10027 Strategy for Deconstruction of Biomass for Biofuels Production
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"Dedicated energy crops and agricultural waste are the preferred long-term solutions for renewable, cheap, and globally available biofuels as they avoid some of the market pressures and secondary greenhouse gas emission challenges currently facing corn ethanol. Typically lignocellulosic biomass are converted to fermentable sugars using a variety of chemical and thermochemical pretreatments, which disrupt cellulose and lignin cross-links, allowing exogenously added recombinant microbial enzymes to more efficiently hydrolyze cellulose for deconstruction into glucose. This process is plagued with inefficiencies, primarily due to the recalcitrance of cellulosic biomass, mass transfer issues during deconstruction, and low activity of recombinant deconstruction enzymes. One potential solution to these problems is found in synthetic biology. We have engineered plants that self-produce a suite of cellulase enzymes targeted to the apoplast for cleaving the linkages between lignin and cellulosic fibers; the genes encoding the degradation enzymes, also known as cellulases, are obtained from extremophilic organisms. These enzymes will remain inactive during the life cycle of the plant but become active during hydrothermal pretreatment. Deconstruction can be integrated into a one-step process, thereby increasing efficiency and reducing costs. The unique aspects of this technology are the rationally engineered, highly productive enzymes, targeted to specific cellular locations and their dormancy during normal plant proliferation, which become Trojan horses during pretreatment conditions. We discuss some of our initial results and possible implications of this work on developing dedicated energy crops and their advantage in consolidated bioprocessing. "
(a) Sandia National Laboratories (b) United States Department of Agriculture

P10028 Experiments on *Paulownia elongata* to establish it as a bioenergy crop
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"Georgia Forestry makes one of the largest contributions to the economy of the state equaling approximately 19.5 billion dollars. Nearly one in every nine people involved in manufacturing in Georgia is employed in the forestry sector. Georgia has led the United States in reforestation since 1982. Georgia plants and allows for the natural regeneration of an estimated 250 million seedlings every year, and over the past decade, has planted nearly 3 billion trees. New fast growing source of biomass will have to be integrated to assist the pine based biofuel production in order for a lasting sustainability of ethanol production. We are conducting research on such a fast growing paulownia tree that produces in 6-8 years 2-4 times more timber than most other commercial trees. Paulownia as a dedicated bioenergy crop can be harvested year round. Following harvesting, new paulownia shoots grow back from the stump and use the same well established root system by saving post-harvest clearing costs while controlling runoff and land erosion. The extensive tap root and the lateral root system of paulownia stabilizes soil and remediates land. At the Fort Valley State University experimental farm we are conducting tree spacing (12 x 12 ft and 8 x 8 ft) trials for timber and biomass production, respectively. In the lab, we have perfected rapid in vitro multiplication protocols and currently we are optimizing parameters for genetic transformation of paulownia germplasm. We determined that paulownia wood composition was 50.55% cellulose, 13.6% hemi-cellulose, 21.36% lignin, 14.0%, extractives, and 0.49% ashes. Additionally, it was learned that paulownia wood is also an attractive candidate for pyrolysis/gasification and that the resulting syngas is usually lower in tars. "
(a) Fort Valley State University, Fort Valley, GA 31088 (b) World Paulownia Institute, Lenox, GA

P10029 Selected gas exchange parameters as an important factor in selecting high yielding forms of energetic grasses from *Miscanthus* genus
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"Grasses from the *Miscanthus* genus are becoming a key renewable raw material for energy production. The presented paper is concerned the influence of photosynthesis under the field conditions on yielding of grasses from *Miscanthus* genus during first two years of cultivation. Experiments were conducted in a random block design in three repetitions and two localizations, lying 400 kilometres away from each other. Experimental material consisted of six genotypes from the *Miscanthus* genus that differed in yielding. Our aims were to evaluate the influence of selected gas exchange parameters on yielding of different *Miscanthus* genotypes and examine of genotype x environment interaction (ExG) for those factors. Photosynthesis rate (Pn), transpiration rate (E), stomatal conductance (GS), and internal CO2 concentration (Ci) were measured. The set of analyzed gas exchange parameters involved photosynthetic water use efficiency (WUE) and instantaneous photosynthetic water use efficiency (WUEI). The gas exchange parameters were measured using a gas exchange system (CIRAS-2). Each test plot measurement was conducted on two of the youngest leaves of five randomly chosen plants. Results showed large variability of analyzed genotypes in relation to gas exchange parameters and special to photosynthesis rate (Pn), transpiration rate (E), and the photosynthetic water use efficiency (WUE). A significant interaction was also observed between genotype and the environment in the relation to examined parameters. The correlation was also significant between biomass yield and gas exchange parameters. Obtained results can be valuable for selecting high yielding and efficient *Miscanthus* forms for energetic purposes. "
(a) Institue of Plant Genetics, Polish Academy of Sciences

P10030 Transcriptome discovery in Miscanthus x giganteus
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"The increasing desire to discover alternative energy sources has heightened interest in developing *Miscanthus x giganteus* (*Mxg*) as a biofuel. *Mxg*'s ability to quickly produce large quantities of biomass combined with the small amount of input required for the crop's growth makes *Mxg* an ideal biofuel candidate for many regions. *Mxg* is a sterile triploid hybrid (2n=57) perennial grass that is asexually propagated through rhizome planting. The plant itself is quite large, and easily reaches heights over 12 feet. A recent Illinois study has shown that its harvestable biomass can surpass that of other potential biofuel crops; *Mxg* produced around 29.6 Mg/ha, while, in comparison, corn stover produced biomass of 7.4 Mg/ha and switchgrass had 10.4 Mg/ha. In order to fully discern the biofuel potential of *Mxg*, a basic genomic and transcriptomic-level understanding of the crop must be acquired. Young root, apical meristem, inflorescence, and other tissues were collected at different developmental stages throughout the growing season of *Mxg* to produce a comprehensive set of mRNAs for transcriptome discovery. Combining paired-end Solexa sequencing on both non-normalized and normalized runs of these tissue samples along with a 454 sequencing run has granted us the first few bricks with which the foundation of the *Mxg* transcriptome can be constructed. Data gathered from these sequencing runs were assembled in order to both piece together the *Mxg* transcriptome and, at the same time, develop an expression profile of the various developmental stages. It is expected the draft genome sequence of sorghum will aid in further assembly. Ultimately, genes essential for *Mxg*'s beneficial biomass qualities will be identified and

characterized."
(a) Energy Bioscience Institute (b) Department of Crop Science, University of Illinois (c) Department of Plant Biology, University of Illinois

P10031 Attachment of Proteins to the Cell Wall Using an Anchor Motif
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"Lignocellulosic biomass requires pretreatment to access energy-rich cellulose and hemicellulose for breakdown and fermentation to ethanol biofuels. The pretreatment step is an energy-costly bottleneck in the biomass-to-biofuels process, but may be improved by anchoring pretreatment enzymes to the biomass cell walls. We hypothesize that short anchor sequences derived from leucine-rich repeat extensin (LRX) proteins found in plant cell walls can be fused to fluorescent proteins and can enable fluorescent proteins to withstand extraction from the cell wall. Through directional cloning methods, three anchor-fused fluorescent protein DNA sequences were constructed. Additionally, live onion epidermal cells were transiently transformed with positive control DNA encoding cytosolic green fluorescent protein and cell-wall associated fluorescent proteins using a biolistic particle delivery device. Future work will focus on testing anchor-fused fluorescent protein constructs for anchoring effectiveness and replicating the study with different test proteins secreted from microbes. "
(a) St. Olaf College (b) Department of Biology, Colorado State University (c) Colorado Center for Biorefining and Biofuels

P10032 Heterologous expression of galE changed the soluble carbohydrate composition and facilitated the growth and development in tobacco
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"*galE* gene, encodes UDP-galactose-4-epimerase (EC 5.1.3.2), catalyzes the reversible conversation of UDP-Glucose (UDP-Glc) to UDP-Galactose (UDP-Gal). Two homogenous overexpressing *galE* lines E54 and E41, together with line E23 that containing antisense *galE*, and a wild type line (WT) were analyzed. The heterologous overexpression of *galE* in E54 and E41 facilitated both growth and development of the transgenic plants than the WT, showing longer root system in seedling period, much leaf area in vigorous growing period due to longer and wider leaf blade, and taller plant height in maturation period. Compared to the WT, the initiation of blossom was earlier, and an obvious increase in net photosynthesis rate was observed in the two lines. However, the growth potential, initiation of blossom, and net photosynthesis rate of the antisense transgenic plants were not significantly different from the WT. The GalE activity in the transgenic plants was active between pH7 to pH9, with peak activity at pH7.5-8, and the optimal reaction temperature for the enzyme was 20 degree. The yield of UDP-Gal of the two sense transgenic lines was significantly higher (1.5-1.6 fold) than the antisense line and the WT. The content of total soluble carbohydrates in E54 and E41 was significantly higher than that of the WT and the levels of glucose, fructose, and sucrose in these two lines were all increased in considerable degrees. The content of UDP-Gal and UDP-Glc in E54 and E41 were significantly higher than that of the WT, the content of UDP-sugars, total soluble carbohydrates, and other carbohydrate composition in the antisense transgenic line E23 were not significantly different compared with the WT. "
(a) School of Life Science, Shandong University

P10033 Effects of overexpression of a cellulase gene on rice development
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"To generate a transgenic rice plant suitable for efficient bioethanol production by the use of a cellulase gene, we examined effects of overexpression of the cellulase gene on rice development. A rice cellulase cDNA driven by the maize ubiquitin promoter was introduced into the rice genome. Whereas no reduction of transformation frequency was observed, regenerated transgenic plants showed various physiological and morphological abnormalities. These results suggest that constitutive overexpression of cellulase can be used for improvement of rice plants, but induced expression of cellulase will be necessary to avoid unsuitable effects on rice development."
(a) Graduate School of Agricultural Science, Tohoku University

P10034 High oil lines enhance the accumulation and activity of cellulase in maize seed
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"Biofuels such as bioethanol may become a viable alternative to fossil fuels. Utilizing agricultural biomass for the production of biofuel has drawn much interest in many science and engineering disciplines. Effective digestion of cellulose to simple sugars is the key step in the production of cellulosic ethanol. Current methods of using microbial enzymes for cellulose digestion are expensive and it increases the costs of biofuel production. A newer approach of over-expression and engineering of enzymes in plants was found to be promising and improve the cost benefit ratio. Transgenic maize (Zea mays L.) seed containing cellulase protein, Endo-β-1,4-glucanase (E I) and cellobiohydrolase I (CBH I), in embryo was found to yield high amount of enzyme. The present study discusses the genetic approach taken to enhance the levels of cellulases in maize seeds and amount of cellulase enzyme in different transgenic maize lines. T5 generation of E I and CBH I crossed with high oil (HO) lines showed the highest cellulase accumulation of 0.15% and 0.57% of dry wt. of corn seeds respectively. A 2.5-fold and 1.1-fold increase in E I and CBH I accumulation respectively was observed in HO crosses when compared with the T5 generation controls. Other interesting germplasms are being tested."
(a) Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR 72401 (b) Applied Biotechnology Institute, California Polytechnic State University, San Luis Obispo, CA 93407

P10035 EST Analysis of Oil-producing Green Algae *Botryococcus braunii*
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"*Botryococcus brunii* is a fresh-water green alga known to produce high levels of oils (more than 50% of its dry weight). The oil produced by *B. braunii* is of high purity and chemically resembles fossil fuels. In fact, the oil can be directly used to make biofuels, cosmetics, and plastics using conventional methods. The compositions of oils and related metabolites produced by *B. braunii* have been well characterized since 1980's. However, the identities of enzymes and genes associated with oil metabolism in *B. braunii* still remain unknown. In order to gain such fundamental insight, we obtained expressed sequence tag (EST) data by constructing a full-length cDNA library from log-phase oil-producing cells and sequencing 11,904 randomly chosen cDNA clones. From these sequence data, we retrieved 2,897 independent genes. The genes were classified into 9 categories: signal transduction, photosynthesis, housekeeping metabolism, architectural proteins, oil metabolism, protein degradation, protein and RNA synthesis, channel like proteins, and unknown function. Each category contained 6.1%, 5%, 7.6%, 3.7%, 2.5%, 3.4%, 8%, 2.5%, and 61% of the 2,897 genes, respectively. Forty-nine genes were implicated in oil metabolism. Ongoing studies are focused on determining the time-course expression patterns of these genes via real-time PCR. The findings of this study will be useful for the future attempts to understand the oil metabolism in *B. braunii* and may allow us to genetically manipulate this organism for higher oil production. "
(a) National Institute for Environmental Studies (b) University of Tsukuba

P10036 Functional Genomics of *Miscanthus*
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"*Miscanthus*, a genus of the Poaceae, is one of the most important biofuel plants with characteristics of fast growth and high biomass. Species of *Miscanthus* in Taiwan possess extremely high genetic diversity. *M. sinensis* Anderss. is composed of morphologically distinct intraspecific taxa that are distributed along an altitudinal gradient and respond to various environmental stresses. The purposes of this study are to construct the transcriptome of &*M.* &*sinensis* and and improve the biofuel research of &*Miscanthus*. Cytogenetic evidence indicates that C-value is &3&.&5&-5.06-6.4& in &*M.* &*sinensis* (2n= 38). Transcriptome of a whole young plant of &*M.* &*sinensis* was constructed by using high-throughput sequencing techniques of Solexa and 454. Over 7G bases and 51M bases were obtained from Solexa and 454, respectively. Analyzing 5,216 contigs from Solexa and 454 assemblage was conducted by Gene Ontology. Via blasting the most recent UNIPROT protein database, 1/2 contigs belong to molecular functions, 1/3 contigs are involved in biological process, while 1/5 contigs are related to cellular components. De-novo transcriptome analysis of non-model organisms is very challenging. In the study, in addition to the discovery of common house-keeping genes, finding and identification of rare expressed genes indicate high efficiency of this approach. "
(a) Department of Life Sciences, National Cheng Kung University (b) Department of Biomedical Resources, National Institute of Biomedical Innovation

P10037 Cellulosic ethanol
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"Optional search for global fuel demand has led the pollution free cellulosic ethanol as the fuel of future. Cellulose is condensed form of glucose polymer present abundant in nature as plant cell wall. The crystallinity of cellulose makes it less vulnerable for degradation and expensive for the production of final product. Current cellulosic bioenergy research is mainly focused in reducing the production cost. The cellulases and pretreatment process involved are very expensive. We have invented popping pretreatment method to enable economical ethanol production by enzyme treatment. Popping method is very fast, cost effective and dose not require chemicals. The enzyme cost can be reduced by molecular farming cellulolytic enzymes and hemicellulase to lower the need for pretreatment process through lignin modification, together with the strategy of genetically increased biomass. Heterologous expression of cellulase in subcellular compartments and targeting the same enzyme to several compartments in the same plant will increase the level of enzyme production. Autodigestable plants can be produced by targeting different enzymes in one plant will also be necessary for efficient implementation this technology for biofuel production. Finally, ethanol can not be transported through pipelines due to its hydrophilic nature. Another important issue is usage of lands for biofuel crops. These scientific and economical challenges are need to be addressed in future for the affordable cellulosic ethanol. "
(a) Department of Wood Science and Technology, BK21program, Chonnam National University (b) Bio-energy Research Institute, Chonnam National University

P10038 Synthesis of phenylpropanoid-esters and -amides in Arabidopsis thaliana to engineer a cleavable lignin
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"The plant cell wall is a complex structure composed of cellulose microfibrils embedded in a matrix of hemicelluloses, proteins and lignin. Second-generation biofuels produced from this lignocellulosic biomass represent a renewable alternative to fossil fuels. This strategy consists in the breakdown of cell wall polysaccharides into simple sugars, and the subsequent conversion of these sugars to biofuels. The degradation of cell wall polysaccharides is however impeded by the presence of lignin, a recalcitrant phenolic polymer of hydroxycinnamoyl alcohols. Rerouting the lignin pathway towards the synthesis of phenylpropanoid-derived molecules such as hydroxycinnamic acid amides, sinapate esters, rosmarinic acid, and vanillate is here considered. Such approach would be used to partially replace conventional lignin monomers in the cell wall by these 'easily cleavable' phenylpropanoid-esters and -amides (phenolic dimers). Biosynthetic pathways for the production of these phenylpropanoid-derived compounds are presented, as well as the strategies used for their de novo synthesis in Arabidopsis. "
(a) Joint BioEnergy Institute, Lawrence Berkeley National Laboratory

P10039 Development and Application of Microsatellite Markers for Genomic Analysis of Sugarcane
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"The high cellulose content of sugarcane (*Saccharum sp.*) makes it an ideal candidate for cellulosic ethanol production. However, since it has a 7.5 Gbp complex octoploid genome, a large number of DNA markers are needed for genetic mapping and genome assembly. Microsatellites or simple sequence repeats (SSRs) are highly polymorphic, abundant, and widely dispersed in the genomes of eukaryotes. Our objectives of this study were to develop SSR markers for construction of a saturated genetic map and to characterize the frequency and distribution of SSRs in a polyploidy genome. SSR markers were mined from expressed sequence tag (EST), genomic, and bacterial artificial chromosome (BAC) sequences. These SSRs were surveyed using an agarose gel system. A total of 3141 SSR markers were surveyed in a segregating population. The overall successful amplification and polymorphic rates were 87.2% and 19.7%, respectively. Of the polymorphic markers, 419 were mapped, generating 791 scored markers. The di- and trinucleotide repeat motifs were most abundant with tri- and hexanucleotide motifs being the most abundant for the ESTs. BAC and genomic SSRs were mostly AT rich while the ESTs were relatively GC rich due to codon bias. Survey sequencing of sugarcane genomes is underway and additional SSRs will be mined and analyzed."
(a) Engery Bioscience Institute (b) University Of Illinois Department of Plant Biology (c) University of Illinois Department of Crop Science

P10040 Identifying genes controlling feruloylation in grass cell walls.
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"In the cell walls of grasses, ferulic acid residues are ester linked to arabinoxylans and have the ability to form ferulate dimers functioning in cell wall cross-linking. They are also proposed to act as nucleation sites for the formation of lignin and for the linkage of lignin to the xylan/cellulose network. Such coupling reactions,which occur predominantly in grasses,work as a barrier against efficient utilization of cell walls as a source of biomass for bioenergy production. We have shown previously that the expression of ferulic acid esterase FAE in different grass species resulted in a substantial reduction in cell-wall-esterified ferulates and diferulates. FAE was shown to impact cell wall hydrolysis, resulting in increased digestibility. Controlling the level of total feruloylation should have a direct impact on the level of cross-linking and thereby on cell wall degradation. Currently, the genes underlying arabinoxylans feruloylation have not been identified and the isolation of such genes could be of great importance in manipulating ferulates accretion to the wall. Accordingly, we have targeted the feruloylation pathway and to identify these genes we are taking a forward genetic approach combined with a spectroscopic screen followed by detailed genetic and phenotype analyses. We have chosen *Brachypodium distachyon* as our model grass system for mutagenesis.Mutation of the feruloyl transferase gene(s) should lead to less ferulates secreted to the cell wall and reduced ferulate cross-linked. If successful, the identification of these genes in *Brachypodium* can work as a handle for gene discovery in other important grass crop

species because of conservation of genome organization and gene order in grasses. "
(a) Penn State University

P10041 "Plant expression of fiber-degrading enzymes in maize leaf, kernel, and stem"
DeBrecht, Andrew A-presenter Andrew.DeBrecht@Syngenta.com(a) Arellano, Sergio (a) Azhakanandam, Kasi (a) Betts, Scott (a) Caffall, Kerry (a) Miles, Stacy (a) Wade, Nateefa (a) Winslow, Stephanie (a)
"Enzymes play a key role in many industrial processes that utilize plant parts as the starting material or feedstock. In the US, for example, corn grain is the major feedstock used to generate glucose for ethanol via enzymatic hydrolysis of starch. The biomass substrates for next-generation biofuels may come from corn seed fiber, corn stover, sugar cane bagasse, wood chips, or other plant feedstocks. Currently, conversion of these plant materials involves the exogenous application of microbially-expressed enzymes or enzyme cocktails onto the feedstock. Syngenta is developing plant-based expression systems to produce the large quantities of fiber-degrading enzymes that will be needed for conversion of biomass substrates to fermentable sugars. Here we report the stable expression of multiple enzymes in maize leaf, kernel, and stem including exocellulase, endoglucanase and xylanase."
(a) Syngenta Biotechnology Inc.

P10042 Identifying biological and genetic factors affecting protein accumulation in transgenic maize embryos
Teoh, Keat H-presenter tom.teoh@gmail.com(a) Flory, Ashley R (a) Hood, Elizabeth A (a)
"We have previously observed a phenomenon in maize that shows when transgenes are over-expressed in maize seed up to 100-fold increase can be achieved through breeding to elite germplasm and selection for recovery of the recombinant proteins. However, the mechanism for this increased protein accumulation is not understood. Our proposed work is to understand the mechanism of this empirical observation through combining biological experimental analysis and bioinformatics analysis. Microarray experiments will first be performed to assess genes influencing increased protein accumulation in maize near-isogenic lines that exhibit high and low accumulation of a fungal cellulase enzyme expressed from a transgene in seed. Models of the gene networks associated with high accumulation of cellulase in transgenic maize seeds will then be constructed from the microarray data to help identify genetic factors affecting protein accumulation in transgenic maize embryos. The information gained will be used to develop strategies to improve transgene expression and protein accumulation for plant bioproduction"
(a) Arkansas Biosciences Institute

P10043 Isolation and identification of lignin biosynthesis genes in Miscanthus and maize
Kim, Hyoung Seok-presenter hkim58@illinois.edu(a) Widholm, Jack M. (b) Juvik, John A. (a)
"Lignin is a complex polyphenolic compound that serves in the cross-linkage of cell wall polysaccharides. Plant genetic modification to reduce or modify lignin content and composition has been suggested as an approach to improve plant biomass conversion to cellulosic ethanol. Four lignin biosynthesis genes, p-*coumarate* 3-*hydroxylase* (*C3H*), *cinnamate* 4-*hydroxylase* (*C4H*), 4-*coumarate*: *coenzyme A ligase* (*4CL*) and *caffeic acid*/5-*hydroxyferulic acid O-methyltransferase* (*COMT*) were isolated from sorghum (*Sorghum bicolor* L.) and *Miscanthus* x *giganteus* by RT-PCR using maize othologous sequence primer sets. These genes averaged 90% sequence similarity with the corresponding maize genes and even greater sequence similarity between sorghum and *Miscanthus* (93%). Southern hybridization analysis showed variation in gene copy number between maize, *M.* x *giganteus* and two other *Miscanthus* species (*M. sinensis* and *M. sacchariflorus*, putative parents of the allotriploid *M.* x *giganteus*). Transcript expression levels of these genes was analyzed in different tissues (leaves and stems) of *M.* x *giganteus* and maize at three developmental stages (seedling, vegetative stage and reproductive stage) by Northern blots. To determine correlations between metabolite profiling and gene expression data, lignin contents were assayed from the same tissues. RNAi and antisense constructs have been developed for transformation studies to analyze the function of these lignin biosynthesis genes in maize. Several putative transgenic lines have been generated, confirmed by genomic PCR, and will be analyzed by Northern analysis and lignin content. "
(a) Department of NRES, University of Illinois (b) Department of Crop Sciences, University of Illinois

P10044 Engineering transitory starch breakdown for yield of biofuels
Sharkey, Thomas D.-presenter tsharkey@msu.edu(a) Weise, Sean E. (a) Jarou, Zachary J. (a)
"Starch is easily fermented to ethanol or other biofuels and leaves can accumulate up to 50% starch by dry weight. Unfortunately, starch-accumulating mutant plants grow slow and have poor yields. We have devised a method for delaying starch accumulation until later in the life cycle enabling plants to grow vigorously in the early exponential phase of growth and then switch to accumulate large amounts of starch at the end of their life cycle. Proof-of-concept experiments with Arabidopsis have shown that this approach can work. Growth analyses of plants in which starch degradation genes are turned off by RNAi will be presented."
(a) Michigan State University

P10045 Genetic Modification of Switchgrass for Improved Ethanol Production
Fu, Chunxiang-presenter cfu@noble.org(a) Xirong, Xiao (a) Yajun, Xi (a) Yaxin, Ge (a) Fang, Chen (b) Joseph, Bouton H. (a) Richard , Dixon A. (b) Zeng-yu, Wang (a)
"Switchgrass (*Panicum virgatum* L.), a perennial C4 warm-season grass native throughout North America, has been developed into an herbaceous bioenergy crop. The bioconversion of carbohydrates into ethanol is negatively affected by lignin in the biomass. Our project aims at producing low-lignin switchgrass by RNAi-induced down-regulation of key lignin biosynthetic enzymes, such as caffeic acid O-methyltransferase (COMT) and cinnamyl alcohol dehydrogenase (CAD). An *Agrobacterium tumefaciens*-mediated transformation protocol has been developed based on the use of embryogenic calluses derived from caryopses or inflorescences. Analyses of the transgenic switchgrass plants by RT-PCR and real time PCR showed that expression levels of the endogenous genes were down-regulated. Reduced enzyme activity, decreased lignin content and alerted ratios of S/G were found in these transgenic lines. The down-regulation of lignin biosynthesis resulted in improved sugar recovery. A strong negative correlation between lignin content and saccharification efficiency was found in the regenerated switchgrass lines, while S/G ratio did not correlate with the efficiency of sugar release from untreated or pretreated cell wall residues. Bioethanol fermentation analysis of the plants is being carried out."
(a) Forage Improvement Division,The Samuel Roberts Noble Foundation. (b) Plant Biology Division, The Samuel Roberts Noble Foundation.

P10046 A Geothermal Approach to Open Pond Production of Halophytic Microalgae for Biofuels
Lemos, Mark S-presenter mark.s.lemos@gmail.com(a) Hernandez-Gomez, Leyla T. (a) Albion, Rebecca L. (a) Shintani, David K. (a) Harper, Jeff F (a) Cushman, John C. (a)
"The global crude oil energy market has experienced a roller coaster of crude prices that reached a record peak in the United States on July 11, 2008 at $147.27 barrel crude and began a downward decline to sub $50.00 barrel crude in the first quarter of 2009. The volatility of the fossil petroleum

market and rising cost of biodiesel feedstocks affects the economic viability of the biofuel sector. The limiting factor for biodiesel production is the availability of cost effective feedstocks. Halophytic microalgae have a number of advantages over terrestrial, food-stuff, biofuel feedstocks. Microalgae can be up to 30-times more productive than terrestrial oilseed crop feedstocks and can be grown on marginal lands using waste-brackish- or saline water unsuitable for traditional agriculture. Furthermore, geothermal and solar resources can be leveraged to provide year-round, non-seasonal microalgae cultivation with the advantage of recycling CO2 and NOx emitted from natural gas- or coal-fired power plants. The goal of this research is to demonstrate the use of simulated geothermal heating as a means of boosting production of halophytic microalgae in open, raceway pond for biofuels at both pilot (2,000 L) and demonstration (20,000 L) scales. Previous studies in which twenty different species and strains were surveyed at laboratory scale revealed large variations in starch and lipid content. From this work, candidate strains, which produce up to 50% lipid content based on dry weight, were selected for large-scale cultivation. Results on the outdoor cultivation of *Dunaliella* at our demonstration scale will be reported."
(a) University of Nevada Reno

SESSION P11 – CELL CYCLE & DIVISION

P11001 Redox homeostasis and regulation in the cell cycle
Foyer, Christine H-presenter christine.foyer@ncl.ac.uk(a) Pellny, Till K (b) Locato, Vittoria (b,c) Diaz Vivancos, Pedro (a) Markovic, Jelena (d) Pallardo, Federico V (d) De Gara, Laura (c)
"Cellular redox homeostasis plays an important role in the regulation of the plant cell cycle. However, little information is available on the precise functions of ascorbate, glutathione and pyridine nucleotides in this process. We therefore examined the the changes in these redox pools during the exponential growth of cultured Arabidopsis cells in relation to various cell cycle markers. In contrast to ascorbate and glutathione, which were present largely in the reduced forms, the pyridine nucleotide pools were highly oxidised over the period of exponential growth and only became more reduced once growth had ceased. The glutathione pool increased in parallel with poly (ADP-ribose) polymerase (PARP) activities and with the abundance of PARP1 and PARP2 mRNAs, at a time of high cell cycle activity, as indicated by transcriptome information. Marked changes in the intracellular partitioning of GSH between the cytoplasm and nucleus were also observed. Intracellular redox state was modulated during the growth cycle but redox homeostasis was maintained by interplay of the major redox pyridine nucleotides, glutathione and ascorbate pools. The correlation between PARP expression and activity and GSH accumulation and the finding that GSH can be recruited to the nucleus suggest a relationship between redox regulation and nuclear enzyme activity."
(a) University of Newcastle Upon Tyne (b) Rothamsted Research (c) CIR Universita Campus Bio-Medico, Rome (d) Depto. de Fisiologia, University of Valencia

P11002 The molecular basis of organ growth
Inze, Dirk G-presenter dirk.inze@psb.ugent.be(a) Gonzalez, Nathalie (a)
http://www.psb.ugent.be
"Many genes have been described in *Arabidopsis thaliana* that, when mutated or ectopically expressed, form larger structures, such as leaves or roots. These 'intrinsic yield genes' (IYGs) are involved in various processes whose interrelationship in mostly unknown (Gonzalez et al., 2009). Furthermore, all experiments carried out worldwide to measure the effects of IYGs on growth were performed under different conditions and using different *Arabidopsis* ecotypes, making comparisons virtually impossible. To this end, we have recently initiated a large-scale project 'Yield Booster' to compare the effects of IYGs in the same genetic background (Columbia 0) and to analyze the cellular and molecular bases underpinning the increased growth and biomass production. Kinematic analysis revealed that enhanced cell proliferation (and not cell expansion) is the main driving force of increased leaf growth, underpinning the central role of cell cycle. Various 'omics' technologies are being used to decipher the molecular networks orchestrating the observed growth effects. Genetic analysis demonstrated that several growth enhancing pathways are operational. The long-term goal is to develop computational models describing the molecular basis of plant organ growth and to use these models to improve crop productivity. Gonzalez, N., Beemster, G. T.S. and Inze, D. (2009). David and Goliath: What can the tiny weed Arabidopsis teach us to improve biomass production in crops? Curr. Opin. Plant Biol. In press "
(a) VIB Department of Plant Systems Biology, UGent

P11003 The WD40 repeat protein NEDD1 plays a role in microtubule organization during mitotic cell division in *Arabidopsis thaliana*
Lee, Yuh-Ru Julie-presenter yjlee@ucdavis.edu(a) Zeng, Cui Jing Tracy (a) Liu, Bo (a)
"Microtubule (MT) organization depends on the evolutionarily conserved γ-tubulin complex. In plant cells, it is unclear how the activity of γ-tubulin-dependent MT organization is regulated spatiotemporally during the cell cycle. An *A. thaliana* WD40 repeat protein, AtNEDD1, is homologous to the animal NEDD1/GCP-WD proteins which interact with the γ-tubulin complex. Using immunofluorescence microscopy, we have determined that AtNEDD1 decorated spindle MTs preferentially toward the spindle poles at metaphase in root meristematic cells. The protein appeared at phragmoplast MTs toward their distal minus ends at telophase. The *AtNEDD1* gene was essential, and the T-DNA insertional *nedd1* allele was only found in a heterozygous mutant state. Genetic analyses revealed that the *nedd1* allele severely affected fertility. Anti-tubulin staining showed that approximately half of the dividing microspores from the heterozygous mutant plant exhibited aberrant MT organization. In the dividing mutant microspores, spindles were no longer restricted to the cell periphery and underwent abnormal elongation at metaphase. The phragmoplast array was also affected as demonstrated by MT aggregation between reforming nuclei and the absence of a bipolar configuration. Consequently, defective microspores failed to form a continuous cell plate, and the generative cell was not produced. Our results suggest that AtNEDD1 plays a critical role in MT organization during mitosis, and its function is likely linked to that of the γ-tubulin complex."
(a) University of California, Davis

P11004 Dual Functions of *Nicotiana benthamiana* Rae1 in Interphase and Mitosis
Pai, Hyun-Sook-presenter hspai@yonsei.ac.kr(a)
"The nuclear pore complex protein Rae1 performs multiple functions in animal systems, acting in interphase as an mRNA export factor and during mitosis as mitotic checkpoint and spindle assembly regulators. In this study, we characterized multiple functions of Rae1 in plants. Virus-induced gene silencing of *Nicotiana benthamiana* Rae1, *NbRae1*, which encodes a protein with four WD40 repeats, resulted in growth arrest and abnormal leaf development. NbRae1 was mainly associated with the nuclear envelope during interphase, and NbRae1 deficiency caused accumulation of poly(A) RNA in the nuclei of leaf cells, suggesting defective mRNA export. In the shoot apex, depletion of NbRae1 led to reduced mitotic activities, accompanied by reduced CDK activity and decreased expression of cyclin B1, CDKB1-1, and histones H3 and H4. The secondary growth of stem vasculature was also inhibited, indicating reduced cambial activities. Differentiated leaf cells of *NbRae1*-silenced plants exhibited elevated ploidy

levels. Immunolabeling in BY-2 cells showed that NbRae1 protein localized to mitotic microtubules and the cell plate-forming zone during mitosis, and recombinant NbRae1 directly bound to microtubules in vitro. Inhibition of NbRae1 expression in BY-2 cells using a β-estradiol-inducible RNAi system resulted in severe defects in spindle organization and chromosome alignment and segregation, which correlated with delays in cell cycle progression. Together, these results suggest that NbRae1 plays a dual role in mRNA export in interphase and in spindle assembly in mitosis."
(a) Yonsei University

P11005 "Genome-wide Identification of Meiotic Genes, Novel Transcripts and Transcript Variants in *Arabidopsis thaliana*"
Chen, Changbin-presenter chenx481@umn.edu(a) Farmer, Andrew (b) May, Greg D (b) Langley, Ray (b) Smith, Alan G (a) Huntley, James (b,c) Miller, Neil (b) Mudge, Joann (b) Retzel, Ernest F (b)
"Using the Capillary Collection of Meiocytes technique, we are able to deeply sequence RNA isolated directly from Arabidopsis meiocytes. We have sequenced both meiocyte and anther transcriptome twice with seedling control once by Illumina sequencing-by-synthesis technology. The Arabidopsis candidate meiotic genes, transcript variants can be identified by differential gene expression profile. Analyzing these datasets demonstrated that 1,483 genes have 3-fold or greater reads from meiocytes vs. anthers and 708 genes are preferentially expressed in anthers. Interestingly, there were two genomic blocks identified. One is on chromosome 4, where 10 genes that were expressed in meiocytes with 2-fold or greater read vs. anthers. The 10 detected genes encode regulatory proteins including transcription factors and receptor-like kinases. Another genomic block is on chromosome 2 , which is the pericentromeric region and is reported as mitochondrial genome insertion (MGI). All 34 detected genes on this block showed 4-fold or greater reads in meiocytes vs anthers. In addition, an average of 80% of meiocyte reads aligned to genome and only 30% of the reads aligned to transcriptome. These results suggest a great number of novel transcripts or transcript variants presented in meiocytes. Analysis of these datasets resulted in the discovery of 2,473 candidate novel transcripts or transcript variants in meiocytes. 1,307 candidate novel transcripts are only seen in meiocytes, 898 candidate genes presented in both meiocytes and anthers with significant differences, which include 717 genes up-regulated and 181 genes down-regulated in meiocytes versus anthers. In addition, mutants of 7 newly identified meiocyte preferentially expressed genes exhibit meiotic phenotypes. "
(a) University of Minnesota (b) National Center for Genome Resources (c) Illumina, INC

P11006 Searching for functional *Arabidopsis* CENP-E homologues
Burgos-Rivera, Brunilis-presenter brunilis@uga.edu(a) Dawe, R. Kelly (a,b)
"The kinesin superfamily in *Arabidopsis* has ~62 members that group phylogenetically into fourteen families based on structure and function. Kinesins share a common structure: a motor domain, a short neck, and a coiled-coil stalk. The motor domain, along with the neck, is responsible for microtubule binding and movement, while the stalk associates with cargo. All kinesin motor domains are highly conserved across kingdoms regardless of the family they belong to. The stalks are conserved in structure but not in sequence. The motor domain of human CENP-E is known to associate with mitotic spindle microtubules and its stalk has been shown to bind kinetochores. Previous studies have used the motor domain of human CENP-E to search for plant homologues. However, using this domain alone has failed to identify the closest candidates. Additionally, the few candidates previously identified have not been shown to be functional in plants. Thus, we have to find and characterize the true CENP-E homologues in *Arabidopsis*. We have taken the complete human CENP-E protein sequence, blasted against the *Arabidopsis* database and identified seven possible candidates. MEGA4 was used to generate phylogenies with these candidates and other previously characterized kinesins in *Arabidopsis*, human, and yeast. Phylogenetic analysis revealed that these seven candidates are more closely related to human CENP-E than to other kinesins in the *Arabidopsis* genome. Two of the seven candidates group in a single clade with human CENP-E, making them excellent candidates for the functional *Arabidopsis* CENP-E. We are currently cloning the coding sequence of the best candidates in order to determine if they localize to the kinetochore."
(a) University Of Georgia, Department of Genetics (b) University of Georgia, Department of Plant Biology

P11007 Flower development is Cyclin B-regulated and ploidy-dependent in *Phalaenopsis*
Yu-Lin, Kao-presenter yulinkao@nuk.edu.tw(a,b) Jia-Wen , Wu (b) Yuan-Chen, Weng (c) Pei-Fung, Wu (c) Yen-Ting , Wang (d) Ching-Yan, Tang (a) Wen-Huei, Chen (a,b)
"Endopolyploidy, the replication of DNA without complete cell division, is observed in *Phalaenopsis* orchids, concomitant with developmental processes. In sepals and petals of diploid *Phalaenopsis aphrodite* subsp. *formosana*, results of flow cytometry were showed a progressively increasing percentage of polyploid cells (including 4C and 8C nuclei), and also appeared a greatly decreasing pattern on diploid cells at the transition from unopened flower buds to blossoming. B1-type cyclin, *Phaap;CycB1*, were expressed strongly in both of sepals and petals, however, its transcripts were declined according to flowering process. The data suggested that endoreduplication cell cycle controls endopolyploidization which occurs in response to developmental cues. Their physiological significance has also been discussed. To assess *Phaap;CycB1* function, the full-length gene was over-expressed in sepals of *P. aphrodite*. Agrobacterium-mediated transformation with a *Phaap;CycB1* over-expression construct induced even higher endopolyploidy and might disturb flowering process. We conclude that *Phaap;CycB1* is involved in endoploidization and regulation of flower development. "
(a) Department of Life Sciences, National University of Kaohsiung, Kaohsiung, Taiwan (b) Institute of Biotechnology, National University of Kaohsiung, Kaohsiung, Taiwan (c) Department of Kinesiology, Health, and Leisure Studies, National University of Kaohsiung, Kaohsiung, Taiwan (d) Department of Medical Research and Education, Cheng Hsin General Hospital, Taipei, Taiwan

P11008 Gibberelin affects mopho-anatomical development and gene expression involved in cell cycle in sugarcane seedling
Brandao, Andrea D-presenter brandao.a.d@bol.com.br(a) Lembke, Carolina G (c) Leite, Debora (b) Souza, Glaucia M (c) Kerbauy, Gilberto B (b) Ceccantini, Gregorio T (b) Buckeridge, Marcos S (b)
"Sugarcane is a current target in biotechnology as it is one of the main sources of bioethanol production in the planet. Thus, biotechnological studies aimed at rising productivity of sugar and ethanol in industry is of great importance for the bioenergy market in the world. Gibberellins (GAs) are a large family of tetracyclic, diterpenoid plant hormones that induce a wide range of plant growth responses through increase in activity in the stem parenchymatic region, which in the case of sugarcane is where sucrose is stored. The present work aimed at understanding the morpho-anatomical development of seedlings of sugarcane and genes expression involved in cell cycle. The sugarcane seedlings (SP87425 x SP88813) were grown in vitro (MS culture medium) in the presence of gibberellic acid (3µ;M) at 25oC and a 12 hour photoperiod. Seedlings were collected after 21 days, the development was followed by measurements of length of seedling parts and anatomical sections were examined by light microscopy. Total RNA was isolated, treated with DNase and used to synthesize cDNA . The expression pattern of housekeeping genes (UB, UBE2, GAPDH, PUB, ACT, 60S, 25S RNA) and genes involved in the cycle cell (cycle cell, nucleic acid metabolism), including one transcription factor (NAM), was analysed by real-time reverse transcriptase polymerase chain reaction (qRT-PCR). Plants treated with GA significantly increased size of the epicotyl, vacuolization, cell division as well as expression of genes related to cell division and nucleic acid metabolism. These effects were reverted by paclobutrazol, an inhibitor of GA synthesis, indicating that its synthesis is related to the control several important steps of cell development in sugarcane seedlings. Scholarship:

CNPq "
(a) Campinas State University, Institute of Biology (b) Sao Paulo University, Institute of Biosciences (c) Sao Paulo University, Institute of Chemistry

SESSION P12 – CELL-TO-CELL & LONG-DISTANCE SIGNALING

P12001 Wound-Induced Systemic Synthesis of Bioactive Jasmonates in Arabidopsis
Koo, Abraham J.K.-presenter koojeon1@msu.edu(a) Gao, Xiaoli (b) Jones, A. Daniel (b) Howe, Gregg A. (a,b)
"The plant hormone jasmonoyl-L-isoleucine (JA-Ile) and its likely receptor, COI1, regulate wound-induced systemic changes in gene expression by promoting the degradation of JAsmonate ZIM-domain (JAZ) repressors. In Arabidopsis thaliana, it is not known whether wounding activates the synthesis of bioactive jasmonates (JAs) in systemic undamaged leaves or whether these signals are synthesized at the site of wounding and subsequently transported to systemic tissues. To address this question, we a developed liquid chromatography-tandem mass spectrometry procedure to measure 18 different JA derivatives, including the JA precursor 12-oxophytodienoic acid (OPDA), jasmonic acid, and various JA-amino acid conjugates. Systemic increases in the level of JA-Ile were detected within 5 min of mechanical wounding and were accompanied by a decrease in OPDA. Results from experiments conducted with a transgenic line in which the capacity for JA synthesis can be spatially manipulated with a dexamethasone-inducible promoter showed that the systemic JA-Ile burst requires JA production in systemic unwounded leaves but not in damaged leaves. Petiole excision experiments showed that the wound signal responsible for systemic JA-Ile synthesis exits the wounded leaf within 2 min of tissue damage. Based on these results, we suggest that wound-induced systemic responses in Arabidopsis are mediated by a rapid long-distance signal that activates de novo JA-Ile synthesis in undamaged leaves. "
(a) Michigan State University, DOE-Plant Research Laboratory (b) Michigan State University, Department of Biochemistry and Molecular Biology

P12002 New insights into the CLAVATA signal transduction pathway
Han , Linqu -presenter hanl@umich.edu(a) Clark , Steven
"In Arabidopsis, almost all aerial organs are developed from the shoot apical meristem (SAM). A functional SAM is maintained through a delicate balance between the restriction of stem cell proliferation and the promotion of organ primordia differentiation. In Arabidopsis, the stem cell population in the SAM is specified by a negative feedback loop which consists of WUSHEL (WUS) and CLAVATA genes. The CLAVATA (CLV) genes including CLV1, CLV2 and CLV3 are signaling proteins which are shown to function together in the stem cells to restrict its proliferation by repressing WUSHEL expression. It has been proposed that stem cells in the SAM secrete the putative CLV3 signaling ligand into extracellular space where it is perceived by a receptor complex compromising a receptor-like kinase CLV1 and a receptor-like protein CLV2. The CLV3 ligand has been shown to bind the ectodomain of CLV1 in vitro while the mechanism by which the CLV3 ligand activates CLV1 remains unknown. Recent genetic studies have identified multiple receptor-like kinases including CORYNE (CRN), BAM1 and BAM2, which genetically interact with the CLV1 or CLV2 to specify stem cell population. The BAM1 and BAM2 share a similar protein structure with CLV1 while the CRN is a membrane-associated kinase with a very short extracellular domain. Despite of the extensive genetic interactions demonstrated among these receptors, detailed biochemical analysis of these receptor proteins have been made difficult due to their limited expression in small number of cells. Here we took advantage of a heterologous protein expression system and conducted a preliminary biochemical study of these receptors. "
(a) The University of Michigan

P12003 "Dof transcription factors: to move or not to move, that is the question"
Kim, Ja-Yean-presenter kimjaeyean@gmail.com(a) Rim, Yeonggil (a) Lucas, William J. (b) Munawar , Ahmad (a) Cho, Won Kyong (a) Chu, Hyosub (a) Jo, Yeonhwa (a) Zhao, Xuping (a) Jeon, Che Ok (a) Kim, Hye-Jin (a) Hong, Jong-Chan (a)
"Plant cells developed a sophisticated signaling pathway through intercellular symplasmic channels termed plasmodesmata. Over the past decade, intercellular trafficking of transcriptional factors (TFs) has emerged as a novel mechanism of cell-to-cell communication in plant development. This movement through plasmodesmata occurs either via a selective pathway or a gated, non-selective pathway. To identify non-cell-autonomous transcription factors (NCATFs), we performed a genome-wide screen using the GAL4-UAS activation system in Arabidopsis. For this purpose, we used the CS9094 GAL4 enhancer trap line which drives the expression of GFPer in the cortex and endodermal layers of root tip. Among about 300 transgenic lines carrying a UAS-TF-mCherry construct, 75 TF T1 lines showed detectable mCherry fluorescent signal, and mCherry fluorescent signal in 27 lines was detected outside of the expression domain. Hence, approx. 37% of the tested TFs, including 19 members of 64 TF families, showed potential intercellular TF trafficking. As a model to study the trafficking mechanism and function of NCATFs, we selected the Dof family, a plant specific class of TFs. This family has a highly conserved Dof domain which recognizes an AAAG motif as the essential sequence element within the target DNA-binding site. The Dof domain performs the dual function of DNA binding and protein."
(a) Gyeongsang National University (b) University of California

P12004 Confronting our Racist Past: the Forgotten Genius of Sir Jagadis Bose
Minorsky, Peter V.-presenter pminorsky@mercy.edu(a)
"The Bengali scientist Sir Jagadis Bose (1858-1937) was one the most famous scientists of the early 20th century. Prior to becoming a plant physiologist, Bose made major pioneering contributions in physics in relation to wireless telegraphy, semi-conduction and cybernetics. As a plant biologist, Bose captivated both the popular and scientific worlds with his demonstrations that all plants coordinate their movements and environmental response by means of electrical signaling. Bose was an international celebrity in his own lifetime and numerous honors and prizes were bestowed upon him. Although many of his discoveries and conclusions remain topical today, Bose's contributions to plant physiology are barely acknowedged in the West today. So, why was Bose seemingly airbrushed out of the historical record of plant physiology? The proximate answer is not difficult to discern: Bose's detractors in academe accused him of virtually every scientific malfeasance, including plagiarism, mysticism, vitalism, fabrication, incompetence, and even insanity. I argue that none of these aspersions is valid; in fact, many of them are preposterous. I have uncovered many examples in the literature of Bose bring treated deplorably by his Western contemporaries. Clearly, scientists of the current generation are not responsible for the racist sins of the past but in those cases where the xenophobia and cultural ignorance of our forebears effectively erased, marred or diminished the legacy of a great scholar, it is morally imperative that we, as scholars, not be implicit in perpetuating a gross, historical injustice. Bose was one of the most brilliant minds to ever contemplate plant function. It is high time that we in the West recognize and celebrate his many contributions to plant physiology. "
(a) Mercy College

P12005 Regulation of KNOTTED1 cell-to-cell trafficking by a chaperonin protein.
Jackson, David p-presenter jacksond@cshl.edu(a) Xu, Morgan (a) Wang, Jing (a) Benitez Alfonso, Yoselin (a)

"Cell-to-cell communication plays critical roles in specifying cell fate and coordinating development in multi-cellular organisms. A new paradigm for such communication in plants is the selective trafficking of transcription factors through plasmodesmata (PDs), channels that traverse the cell wall and connect all plant cells. We have taken an unbiased genetic strategy to dissect the mechanism of PD trafficking. The maize KNOTTED1 (KN1) homeodomain protein was the first plant protein found to selectively traffic through PD, and its trafficking appears to be important for its function in stem cell maintenance. A gain-of-function trafficking assay in Arabidopsis was developed to demonstrate that the C-terminal region of KN1 is necessary and sufficient for trafficking in vivo. This system provides a simple and tractable model to understand how proteins traffic and to isolate mutants defective in trafficking. As a proof of concept for our strategy, a mutant with attenuated KN1 trafficking has been identified as a chaperonin gene. This chaperonin appears essential for PD trafficking of some but all non-cell-autonomous proteins, and biochemical evidence suggests a physical association between chaperonin and KN1. Proteins are thought to undergo partial unfolding during PD translocation, which makes the discovery of this chaperonin particularly exciting. A functional characterization of chaperonins, the first ever factor so far known to be critical for KN1 PD trafficking will further our understanding of developmental regulation and mechanisms of selective cell-to-cell trafficking. In addition, it may give mechanistic insights into this elaborate protein folding machinery, which is not well understood in any system at a molecular level. "
(a) Cold Spring Harbor Laboratory

P12006 Function of EKIP1 Proteins in *Arabidopsis*
Deng, Lihan-presenter ldeng@utk.edu(a) Rebecca , Wilson (a) Elena, Shpak (a)
" Plant organ size is determined by the size of organ primordia and by cell growth and proliferation during subsequent organ development. To understand regulation of organ growth, we are studying the ERECTA family of receptor-like kinases which regulate cell proliferation and differentiation in aboveground organ primordia. A yeast-two-hybrid screen with the ERECTA kinase domain as a bait identified an ERECTA Kinase Interacting Protein (EKIP1:1). EKIP1:1 contains a VWA domain and a RING-finger domain, and functions as an E3 ligase in an ubiquitination assay. It belongs to a family of 4 proteins all sharing similar structure and interacting with ERECTA in a yeast-two-hybrid assay. An analysis of *EKIP1:1* promoter activity by a β-glucuronidaze (GUS) reporter system suggested that *EKIP1:1* is expressed in a pattern similar to that of the *ERECTA* family genes. In young leaf primordia the *EKIP1:1* promoter is active throughout the epidermis, but later on its activity is restricted to stomatal-lineage cells. Other *EKIP1* family members are expressed in a very similar pattern. To study *EKIP1* gene family function we analyzed two available T-DNA insertion lines: *ekip1:1-2* and *ekip1:2-1*. RT-PCR suggests that both lines are knockdowns. The lines were outcrossed to wild type and an *ekip1:1-2 ekip1:2-1* double mutant was created in the wild type and in the *erecta-105* backgrounds. However, no obvious phenotype was detected. One of the possible explanations is that EKIP1:1 and other family members are acting redundantly. Currently we are creating *Arabidopsis* plants expressing inducible amiRNAs to knock down *EKIP1:1* as well as other members of *EKIP1* family. "
(a) University of Tennessee Knoxville BCMB Department

P12007 The signaling pathway in germination regulation in Arabidopsis
Liu, Yinggao -presenter 06459005@hkbu.edu.hk(a) Zhang , Jinahua (a)
"Seed germination is a complex process. Germination incorporates those events that commence with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryonic axis. It is well known that ABA and GA plays important role in seed dormancy and germination, ABA catabolism and GA biosynthesis are all required for seed germination. In our study we found that both H2O2 and NO involved in the regulation of ABA catabolism and GA biosynthesis during imbibition. Our results clarify a signaling pathway about ABA catabolism and GA biosynthesis during imbibition. Nitric oxide-induced rapid decrease of ABA is essential for the seed dormancy break in Arabidopsis. H2O2 acting as signaling molecule regulates seed dormancy by trigging both ABA catabolism and GA biosynthesis. The upregulation of ABA catabolism by H2O2 works through NO. High concentration of ABA also inhibits the GA biosynthesis but a balance of these two hormones jointly controls the dormancy and germination of Arabidopsis seeds. After analyzed all 10 MAPKKs and 20 MAPKs mutants we found that some of them involved in ABA and GA regulated seed germination. Our results indicated that some MAPKKs and MAPKs participated in ABA and GA regulated gene expression during germination in Arabidopsis. "
(a) HongKong Baptist University

SESSION P13 – CELLULAR GROWTH

P13001 Eukaryotic release factor 1-2 is involved in GA signaling pathway and regulates cell elongation in petioles
Zhou, Xiangjun (a,b) Cooke, Peter (c) Li , Li-presenter ll37@cornell.edu(a,b)
"Eukaryotic release factor 1 (eRF1) is responsible for recognition of the stop codons in mRNAs during protein synthesis. Accumulating evidence indicates that eRF1 functions in other processes in addition to translation termination. The physiological role of eRF1-2, a member of eRF1 family, was examined in *Arabidopsis* and cauliflower. The eRF1-2 was found to localize in both cytoplasm and nuclear, which is consistent with its role in protein translation termination. Overexpression and knockout of *eRF1-2* in *Arabidopsis* reversely regulated the plant responses to paclobutrazol, an inhibitor of GA biosynthesis. The *eRF1-2* overexpressing transgenic lines showed enhanced sensitivity to paclobutrazol and exogenous GA restored their normal growth. In contrast, the loss-of-function *erf1-2* mutant exhibited resistance to paclobutrazol, suggesting that eRF1-2 is a negative regulator in the GA signaling pathway. Analysis of transcript levels of GA biosynthetic and signaling component genes indicated that eRF1-2 probably participates in the GA signaling pathway through regulating the expression of *GA3ox2* and affecting bioactive GA synthesis. Furthermore, alteration of the *eRF1-2* expression also affected flowering time and shoot meristem tissue development in *Arabidopsis*. Transgenic cauliflower plants containing reduced transcript levels of *erf1-2* were generated by RNA interference. The cauliflower transgenic lines displayed longer petioles, low levels of free sugars, and enhanced accumulation of anthocyanins. Cytological study of petiole epidermal cells revealed that the longer petioles were attributed to increased cell elongation. Taken together, these data provide evidence in supporting a novel role of eRF1-2 in GA signaling pathway in modulating plant growth and development."
(a) Robert W. Holley Center for Agriculture and Health, USDA-ARS (b) Department of Plant Breeding and Genetics, Cornell University, Ithaca, NY 14853 (c) Microscopic Imaging, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038

P13002 Cytoskeletal control of allometric cellular growth
Geitmann, Anja-presenter anja.geitmann@umontreal.ca(a) Bou Daher, Firas (a)
http://www.geitmannlab.org
"Plant cells come in all sizes and shapes that must be generated by non-uniform growth from roughly spherical meristematic cells. While overall anisotropic growth is linked to the orientation of microtubules and cellulose microfibrils, the generation of spatially localized (allometric) growth events is largely controlled by the actin cytoskeleton through the precise spatial control of exocytosis. The pollen tube is a rapidly elongating cell in which the growth activity is spatially confined to a very small area on the cellular surface. Intriguingly, and critical for the success of this cell, is its

ability to change the precise localization of growth activity in order to change the direction of elongation. This enables the pollen tube to respond to external signals and to target the female gametophyte deeply embedded within in the complex tissues of the pistil. We investigate the role of the cytoskeleton (actin and microtubules) in the spatial and temporal control of growth activity in the pollen tube. To this end we have devised a galvano-assay that allows us to expose in vitro growing pollen tubes to directional signals at precisely defined times and under different conditions such as the presence of pharmacological agents. In the presence of microtubule-depolymerizing concentrations of oryzalin, the pollen tube's capacity to respond to a directional signal is unaltered. Low concentrations of latrunculin B, however, while ineffective in reducing cell elongation, delay cellular response to a galvano-signal and reduce the number of tubes that do respond at all. We discuss these results in the context of our recent findings that describe the role of the actin cytoskeleton in the targeted movement of secretory vesicles in pollen tubes. "
(a) University of Montreal, Department of biological sciences

P13003 "*NOECK*, a gene related to *MIXTA* of snapdragon, controls cell size and shape in Arabidopsis trichomes in unexpected ways."
Gilding, Edward K-presenter gildi002@umn.edu(a) Marks, Michael D (a)
"The Arabidopsis gene *NOECK* (*NOK*), encodes an R2R3-MYB protein that is structurally related to the *Antirrhinum majus* protein *MIXTA*. The implication of *NOK* in trichome development was discovered through a reverse genetics screen with *nok* mutants yielding an extra branched trichome phenotype. We describe *NOK* function via developmental, qPCR and microarray expression analyses in genetic backgrounds representing the loss, increase, and normal levels of expression. Observations of growing trichomes indicate that *NOK* stimulates progression through development possibly by moderating cell size and is expressed alongside the epidermal patterning gene *GL1*. Intriguingly, when *NOK* function is abolished in the *gl3-sst* background, the double mutant phenotype consists of much reduced trichomes that superficially resemble glands and is a phenotype contrary to the single *nok* mutants. Data from *gl3-sst* indicates that it is a heterochronic mutant and we conclude the effect of *NOK* is heavily dependent upon developmental context. Comparisons of *gl3-sst*, *nok*, and *gl3-sst/nok* trichome cell microarray data revealed an antagonistic relationship to *GL1* in the *gl3-sst* background and the potential implication of *NOK* as part of a feedback loop responsible for sensing and modulating cell expansion during trichome development. Working with the tools available in Arabidopsis, we produce data complimentary to published genetic studies further defining the functional scope of *MIXTA*-class R2R3-MYBs."
(a) University of Minnesota

P13004 *In vivo* analysis of myosin XI function in polarized plant cell growth
Vidali, Luis-presenter lvidali@bio.umass.edu(a) Burkart, Graham (a) Bezanilla, Magdalena (a)
http://www.bio.umass.edu/vidali/
"Myosin XIs are plant-specific and most similar to myosin Vs from animals and fungi. In plants, myosin XIs are responsible for cytoplasmic streaming, but their role in polarized growth is not well understood. Because of the large number of myosin XI genes in angiosperms, it has been difficult to determine their precise role. In contrast, in the moss *Physcomitrella patens*, there are only two myosin XI genes, which encode proteins that are 94% identical. To determine the role of myosin XI in polarized growth, we simultaneously silenced the expression of both myosin XIs by RNA interference (RNAi). Loss of myosin XI function results in a dramatic loss of polarized growth; plants are stunted and composed of small rounded cells. Interestingly, this phenotype is very similar to that caused by silencing proteins involved in regulating actin dynamics, suggesting that myosin XI may regulate actin turnover. We have also determined that the two myosin XI genes are functionally redundant by using specific RNAi constructs from the 5' untranslated regions. These constructs do not produce a phenotype, demonstrating that a single copy of myosin XI is sufficient for polarized growth. Importantly, expression of a construct generated by combining the 5' untranslated regions from each myosin XI phenocopies the coding sequence construct. In addition, this construct enables transient complementation studies, which are critical for the elucidation of the mechanism of myosin function in cell. Using this complementation assay, we show that GFP-myosin XIa completely rescues the myosin XI RNAi phenotype. To further dissect myosin XI mechanism of action during polarized growth, we are also analyzing the specific effect of myosin XI loss-of-function in intracellular motility."
(a) Biology Department, University of Massachusetts, Amherst

P13005 Ectoapyrase and extracellular nucleotides can modulate cotton fiber elongation in cultured ovules
Clark, Gregory Bland-presenter gbclark@uts.cc.utexas.edu(a) Torrez, Jonathan (a) Chen, Z. Jeffery (a) Roux, Stanley J (a)
"Ectoapyrase enzymes remove the terminal phosphate from extracellular nucleoside tri- and diphosphates. In Arabidopsis, two ectoapyrases, AtAPY1 and AtAPY2, have been implicated as key modulators of growth, and in cotton fibers, GhAPY1, a predicted ectoapyrase has a high sequence similarity to AtAPY1 and 2. In a cotton ovule culture system, fibers release ATP as they grow, and when their ectoapyrase activity is blocked by the addition of polyclonal anti-apyrase antibodies or chemical apyrase inhibitors, the ATP concentration in the media increases and fiber growth is suppressed. High concentrations of the poorly hydrolysable nucleotides ATPγS and ADPβS applied to the medium also inhibit fiber growth, while low concentrations of them stimulate growth, but treatment with AMPS has no affect on fiber growth rate. Both the inhibition and stimulation of growth by applied nucleotides can be blocked by PPADS, an antagonist that blocks purinoceptors in animal cells, and by the feedback inhibitor, adenosine. Aminovinylglycine, an ethylene antagonist, blocks promotion of fiber growth induced by low levels of ATPγS at a concentration that by itself has no affect on fiber growth. These data and others we will present indicate that ectoapyrases and extracellular nucleotides play a significant role in regulating cotton fiber growth."
(a) University Of Texas

P13006 The Arabidopsis *GRF-INTERACTING FACTOR* gene family performs an overlapping function in determining organ size as well as multiple developmental properties
Kim, Jeong Hoe-presenter kimjeon4@knu.ac.kr(a) Lee, Byung Ha (a) Jeon, Jae OK (a)
"Previously, the *GRF-INTERACTING FACTOR1* (*GIF1*)/*ANGUSTIFOLIA3* (*AN3*) transcription coactivator gene was characterized as a positive regulator of cell proliferation in lateral organs, such as leaves and flowers, of *Arabidopsis thaliana*. As yet it remains unclear how the *GIF1*/*AN3* affects cell proliferation process. Here in this study, we demonstrate that the other members of the *GIF* family, *GIF2* and *GIF3*, are also required for cell proliferation, as *gif1*, *gif2*, and *gif3* mutations synergistically cause a reduction in cell numbers. Kinematic analysis on leaf growth revealed that *gif* triple mutant as well as other strong *gif* mutants exhibited early cessation of cell proliferation, which was accompanied by low expression of cell-cycle-regulating genes, indicating that *GIF* genes acted upstream of cell cycle regulators. *gif* double and triple mutants clarified previously undescribed phenotypes of *gif1*/*an3*: *gif* mutants had small size of the shoot apical meristem (SAM), which was correlated with development of small leaf primordia and short plastochron. *gif* triple mutants also displayed defective structure of floral organs. These results suggest that the *GIF* gene family plays important roles in the control of cell proliferation and other developmental properties that are associated with the SAM function"
(a) Department of Biology

P13007 Culture and transformation of the single-cell C_4 *Bienertia sinuspersici* to study a unique C_4 system
Rosnow, Josh J-presenter jrosnow@wsu.edu(a) Offermann, Sascha (a) Park , Joonho (c) Okita, Tom (b) Dhingra, Amit (d) Tarlyn, Nathan (e) Edwards, Gerry (a)
"*Bienertia sinuspersici* is a halophytic species being developed as a model system for studying single-cell C_4 photosynthesis. Mature chlorenchyma cells have dimorphic chloroplasts in two separate cytoplasmic domains which are peripherally and centrally located, analogous to mesophyll and bundle sheath cells of Kranz anatomy. The selective positioning of chloroplasts, peroxisomes and mitochondria allows increased carbon acquisition and decreased photorespiration through C_4 function. A procedure for plant regeneration which is essential for future genetic investigations has not been established. Shoot primordial from mature plant material has been used to investigate the necessary media composition to maintain shoot growth by placing explant (stems or leaves) material on 2,4-D plates, and inducing red nodular structures (RNS) to develop. Subsequent culture of RNS on cytokinin media promotes shoot development and greening of leaves. To gain insight into the differential accumulation of nuclear encoded enzymes in the dimorphic chloroplasts, a biolistic transformation approch is being used to investigate the possibilites of selective targeting and/or selective degradation of mRNA and proteins as well as selective import of pre-proteins. Transit peptide constructs currently being used are: a GFP positive control with expression throughout the cytosol, the N terminus sequence of the Rubisco small subunit, the N terminus sequence of PPDK, and the N terminus of BADH (betaine aldehyde dehydrogenase, which is localized in both types of chloroplasts) fused to GFP. The results will provide insight into the selective targeting of nuclear encoded proteins to organelles. "
(a) School of Biological Sciences, Washington State University (b) Institute of Biological Chemistry, Washington State University (c) Department of Biochemistry, Queen's University Kingston Ontario, Canada (d) Horticulture Department, Washington State University (e) Plant Transformation Center, Washington State University

P13008 Major changes in gene expression accompany the suppression of growth induced by knocking down ectoapyrase expression in Arabidopsis
Wu, Jian (a) Yao, Jianchao (a) Clark, Greg B (a) Roux, Stanley J.-presenter sroux@uts.cc.utexas.edu(a)
"Ectoapyrases are enzymes that remove the terminal phosphate from extracellular nucleoside triphosphates (e.g., ATP) and nucleoside diphosphates. A recent study in our lab revealed that two ectoapyrase genes, AtAPY1 and AtAPY2, play important roles in the control of plant growth in Arabidopsis. Specifically, the suppression of these apyrases in an inducible RNAi system results in plants with a dwarf phenotype and disrupted auxin distribution. To better understand the implications of these findings, a thorough analysis of the underlying gene expression changes associated with apyrase gene suppression was carried out. We used an inducible RNAi construct to suppress APY1 in plants homozygous for the apy2 knockout mutation. When the RNAi lines are grown in the presence of the estradiol inducer, the growth differences between these plants and equivalent plants not exposed to estradiol become evident at 3 d. We compared gene expression differences between uninduced plants and plants grown continuously in the inducer for 3.5 d (dark grown) or 6 d (light-grown) using the NimbleGen Arabidopsis thaliana 4-Plex microarray. We compared the two sets of large-scale expression data and identified genes whose expression significantly changed after ectoapyrase suppression in light- and dark-grown plants, respectively. Major changes in numerous transcription factors and in hormone-regulated genes were observed, and these and other changes are being independently verified by RT-PCR and deep sequencing. Data analysis should yield a better understanding of the molecular bases underlying the relationship of ectoapyrase expression to growth. "
(a) University Of Texas

SESSION P14 – CELLULAR IMAGING TECHNOLOGIES

P14001 A new method to investigate the cell wall of living cells by high-resolution scanning electron microscopy
Mullendore, Daniel L.-presenter mullendore@wsu.edu(a) Knoblauch, Michael (a)
"Most three-dimensional investigations of cell wall structure using SEM and AFM deal with non-living woody tissue; where the plant clears cytoplasmic debris from cell walls during programmed cell death. However, investigation of living cells requires cytoplasm removal to expose the cell wall. Common methods to clear the cytoplasm include hypochloride and triton X-100. Due to the action of free radicals, fragile, thin cell walls often collapse before the cytoplasm is cleared. We have developed a method employing a progressive enzymatic digest of the cytoplasm to expose the thin cell wall of living cells (e.g. parenchyma, cambium etc.) while maintaining ultrastructure. Cell wall structures like plasmodesmata and cellulose fibrils are easily visualized. We have investigated the three dimensional structure of sieve plates and occlusion of sieve plate pores by callose in response to injury. It has been proposed that callose can occlude pores in a matter of seconds. Our data indicate that callose is deposited in the sieve pores at a rate of 16nm per minute. This may be sufficient to occlude smaller sized sieve pores and plasmodesmata within minutes. However, many plants contain sieve plate pores with diameters of 1 µ;m or more. These large sieve pores require additional occlusion mechanisms to prevent excess loss of assimilates in an injury event. Our data have a major impact on the understanding of phloem physiology and plant-insect interactions. Furthermore, this method allows for high-resolution investigations of cell walls in a number of other cell types."
(a) Washington State University, School of Biological Sciences

P14002 Development of high-throughput in-situ hybridization system for plant gene expression analysis
Ma, Junying-presenter jma@noble.org(a,b) Thaller, Christina (c) Tang, Yuhong (a,b)
"In-situ hybridization (ISH) is a well established technique that facilitates the understanding of gene function by revealing gene expression patterns within tissues. However, ISH has been difficult to use for high throughput analysis of gene expression patterns because it is a complicated and lengthy procedure that involves labor intensive steps. In plants this problem becomes even more pronounced because gene expression signals tend to be masked by background from the cell wall. Here we present a semi-automated ISH system for high throughout analysis of gene expression in plant tissues. This protocol utilizes the Tecan Freedom EVO150 platform to perform ISH on paraffin sections to detect mRNA with a digoxigenin (DIG)-labeled probe on plant tissues. This system can perform all pre-and post-hybridization steps (post-fixing, washing and staining) as well as automating the hybridization procedure itself in a tightly temperature controlled environment. The result is a truly automated ISH system that can process 48 slides in 30 hours and provide consistent results by exerting accurate control of critical experimental conditions such as temperature, pipetting volume and incubation time. Using this system, expression patterns of different genes in diverse tissue type such as flowers, stems, roots and the floral meristem have been tested. "
(a) The Samuel Roberts Noble Foundation (b) The BioEnergy Science Center of U.S. Department of Energy (c) Gene Expression Core Services Department of Biochemistry & Molecular Biology, Baylor College of Medicine

P14003 The utilization of Arabidopsis genetic variants and infrared spectroscopy to understand cell wall structure
Smith, Andreia M.-presenter asmith@lbl.gov(a) Christiansen, Katy M. (a) Loque, Dominique (a) Heazlewood, Joshua L. (a)
"A better understanding of plant cell wall components and its inherent structure is necessary in the development of specialty plant biomass for bio-

energy purposes.Linking these morphological and compositional aspects to a complex series of biochemical processes and finally to specific gene functions have proven problematic due to genetic redundancies and undetectable changes.Common techniques have used mutant collections in genetic screens to directly target and disrupt genes of interest.Since such techniques are reliant on phenotypic discrimination to asses gene function,the absence of measurable difference often results in little useful information.Another approach utilizes genetic differences in naturally occurring variants to provide important information about gene function and genetic diversity.Spectroscopic platforms can be essential methods to characterize and reveal biochemical compositional, structural, and conformational differences between wild-type and variants.Accessions have previously been shown to have differences in Ara-Rha ratios in cell wall extracts.This genetic variant information and the utilization of recombinant inbred lines will be used to map QTLs identified in these and other Arabidopsis accessions.To further characterize these variants, Infrared Spectroscopy and multi-variant analysis can be used to classify and thus investigate plant cell wall composition and structure.In particular, Fourier Transform Infrared Spectroscopy (FTIR)gives information in the spectral fingerprint region where chemical bonds associated with plant cell wall components can be easily assigned and therefore compared.Taken together, these classifications and chemical signatures can be used to identify loci that contribute to functional differences in plant cell walls. "
(a) Joint Bioenergy Institute, LBL

SESSION P15 – CELL WALLS

P15001 In search of novel recalcitrant gene targets: genomic dissection of vascular system in switchgrass (*Panicum virgatum* L. Alamo)
Srivastava, Avinash C-presenter acsrivastava@noble.org(a,b) Palanichelvam, Karuppaiah (a) Ma, Junying (a,b) Steele, Jarrod (a) Tang, Yuhong (a,b) Blancaflor, Elison B (a,b)
"Lignin is a phenolic polymer that is an integral component of secondary plant cell walls. It interferes with the conversion of cellulose to fermentable sugars by preventing chemical access to cellulose. Thus, one strategy for the efficient production of cellulosic ethanol is the down-regulation of lignin content in plants. To achieve this goal, it is important to understand the molecular processes involved in lignification and secondary wall synthesis. Using a targeted approach, transcripts of genes involved in lignification and secondary cell wall synthesis in switchgrass were enriched by laser-capture microdissection (LCM) of vascular bundles. In addition to an on-going 454 run, 5716 ESTs were sequenced from the cDNA libraries derived from laser dissected cells. These Sanger sequences converged into 2865 unigenes with an average length of 665 bp. More than 90% of the assembled consensus sequences aligned with maize or rice genes at an e-value ranging from 0 to $1X10^{-7}$. Gene ontology of the unigenes indicated 1% and 2% of the sequences were lignin and cell wall biosynthesis related genes respectively. *In situ* localization of few representative genes confirmed expression of *brittle stalk*, *dehydrin* and *dirigent* in vascular bundles. These results validated our LCM experimental approach for identifying novel targets for modifying functional expression of lignin and cell wall biosynthesis related genes. Genes that show preferential expression in lignified versus non-lignified tissues are being used as candidate genes for reducing recalcitrance. We have identified 32 genes related to lignification and secondary cell wall formation for genetic modifications and efficient cellulosic ethanol production."
(a) The Samuel Roberts Noble Foundation, Ardmore, OK 73401, USA (b) BESC - The BioEnergy Science Center of U.S. Department of Energy

P15002 Roles of NAC transcription factors in secondary wall biosynthesis
Zhou, Jianli-presenter jzhou@plantbio.uga.edu(a) Ye, Zheng-Hua (a)
"Biosynthesis of the three major secondary cell wall components, cellulose, xylan and lignin, is controlled by a transcriptional cascade in *Arabidopsis*. It was previously shown that SECONDARY WALL-ASSOCIATED NAC DOMAIN PROTEIN1 (SND1) and NAC SECONDARY WALL THICKENING PROMOTING FACTOR1 (NST1) are transcription factors activating the secondary cell wall biosynthesis in fiber cells. We have been investigating the functions of these NAC domain proteins in regulating secondary wall biosynthesis. NACs were shown to regulate a number of downstream transcription factors that are essential for secondary wall biosynthesis. Further study of the functions of these transcription factors will help understand the transcriptional network regulating secondary wall biosynthesis. "
(a) Plant Biology Department, University of Georgia

P15003 Changes in cell wall pectin through the culture cycle of wild type *Arabidopsis thaliana*; analysis of chelator extracted pectin
Qouta, Lolita A-presenter lqouta@philadelphia.edu.jo(a) Abdel-Massih, Roula M (b) Nimmo, Hugh (c) Brett, Chris (c) Baydoun, Elias (d)
"Pectins, one of the main components of the extracellular matrix, are complex polysaccharides containing homogalacturonans (HG), rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II) and xylogalacturonan (XGA) domains. They play a distinct role in cell wall porosity, expansion and cell-cell adhesion that in turn determines the mechanical properties of the cell wall. The results obtained indicated that the phosphate/EDTA extracts from Arabidopsis cells were rich in HG, RG-I, and possibly xylogalacturonan fractions. The degree of methyl esterification of HG increased from 36% to 60% as the cells progressed during the culture cycle and decreased as the cells were entering the stationary phase. The HPAEC-PAD analysis of the neutral fraction of trifluoroacetic acid (TFA) hydrolysates of the extracted pectin showed that the major neutral sugars detected were characteristic of pectins and included galactose, arabinose, glucose, and rhamnose, whereas xylose, mannose and fucose represented the minor sugars. The results suggest that both galactan and arabinan showed opposing changes during the division phase between days 3 to 7. The same period showed an increase in the detected rhamnose while there was no detectable change in the proportions of glucose, xylose and mannose. The immunochemical analysis of the extracted pectin and the in situ labelling of resin embedded cells supported the biochemical data and indicated the presence of JIM7 reactive highly methylated pectins, and LM6 detected arabinan in the primary cell walls, middle lamellae and intercellular junctions across the growth cycle. "
(a) Philadelphia University, Jordan (b) University of Balamand, Lebanon (c) University of Glasgow, UK (d) American University of Beirut, Lebanon

P15004 The substitution pattern of plant cell wall cross-linking glycans is determined by apoplastic glycosidases
Pauly, Markus-presenter paulymar@msu.edu(a) Gunl, Markus (a) Souza, Amancio (a) Neumetzler, Lutz (b) Florian, Kraemer (a)
"Among plant cell wall polymers cross-linking glycans play a major role in maintaining the structural integrity of the wall by their tight association with cellulose microfibrils. More importantly, their metabolism is thought to be essential resulting in cell elongation and thus plant growth. Unlike cellulose, crosslinking glycans (also known as hemicelluloses) contain sidechains of various length making them water soluble and thus enzyme accessible. Although many glycosyltransferases and glycansynthases have been characterized in recent years, it is not known what determines the heterogeneity of the sidechain structures. Our data presented here provides strong evidence that this heterogeneity is determined by apoplastic glycosidases and not by the biosynthetic machinery in the Golgi-apparatus. We used a semi-automated forward genetic approach facilitating oligosaccharide mass profiling (OLIMP) to identify Arabidopsis mutants with altered cross-linking glycan structures, specifically xyloglucan, the most dominant crosslinking glycan in the primary walls of dicots. So far, 36 mutants have been identified containing xyloglucans with different degrees of carbohydrate

substitutions including patterns of O-acetylation as well as novel hitherto unknown structures. Positional cloning of two of the mutants indicated a mutation in two different apoplastic glycosidases acting on specific xyloglucan substituents. The characterisation of those mutants in terms of cell wall polysaccharide structure but also functional aspects on cross-linking glycan metabolism as well as effects on plant growth and development will be presented. "
(a) DOE-Plant Research Laboratory (b) Max-Planck Institute for molecular Plant Physiology

P15005 "An Expansin, VfEXPA1, Is Involved in Regulation of Stomatal Movement in *Vicia faba* L"
Wang, Xue-Chen-presenter xcwang@cau.edu.cn(a) Chen, Su (a) Wei, Pengcheng (a) Zhang, Xiuqing (a) Zhao, Ping (a) Xiong, Yanmei (a) Chen, Jia (a)
"Expansins are cell wall proteins that facilitate the in vitro extension of isolated plant cell wall; they play important roles in the regulation of a variety of plant processes. *VfEXPA1* is an expansin gene cloned from *Vicia faba* epidermal strips. Expression of *VfEXPA1* is regulated by darkness and submergence, and is not affected by light and ABA. The results of *in situ* hybridizations show that *VfEXPA1* is primarily expressed in the guard cells of mature leaves. Transformation of *VfEXPA1* into tobacco enhances light-induced stomatal opening, and overexpression of *VfEXPA1* increases both transpiration and photosynthesis rates under favorable growth conditions. Further results show that exogenous expansins purified from mature leaves of *Vicia faba* are able to facilitate stomatal opening, meanwhile expansin antiserum decelerates this process. Our results suggest VfEXPA1 play an important role in promoting stomatal opening through regulating guard cell wall loosening."
(a) College of Biological Sciences,China Agricultural University

P15006 Development of microarray-base assays for the high-throughput analysis of glycosyltransferase activities
Egelund, Jack-presenter hop@dsr.life.ku.dk(a) Pedersen, Henriette L (a) Sorensen, Iben (a) Willats, William G.T. (a)
"The industrial and therapeutic applications of complex carbohydrates are becoming increasingly important. Efforts to produce energy-efficient crops for bio-fuel production are also underpinned by a detailed knowledge of cell wall biosynthesis, and especially the activities of glycosyltransferase (GT) enzymes. One approach for identifying and characterizing the in planta activities of GTs is to produce knock-out plants. However, screening large populations of mutants is time consuming and complex because knocking out a single GT often has multiple pleotropic effects. Biochemical characterization of GTs involves heterologous expression of the enzymes followed by in vitro activity assays. This process is complicated by the fact that the appropriate acceptors and donors substrates are often unknown. In the work presented, we will describe work towards the development of microarray-based high-throughput assays for multiplex analysis of GT activities."
(a) Dept. of Mol. Bio,, University Of Copenhagen, Denmark

P15007 A comprehensive bioinformatics/biochemical approach to identify key cell wall glycosyltransferases.
Oikawa, Ai-presenter AOikawa@lbl.gov(a) Geshi, Naomi (b) Scheller, Henrik Vibe (a)
http://www.jbei.org/
" Plant cell walls are composed mainly of polysaccharides, which not only have important roles for plant growth and development but also constitute the most abundant biomass component on the earth. The key enzymes for polysaccharide biosynthesis are glycosyltransferases (GTs). Approximately 450 GT genes in Arabidopsis thaliana have been identified based on their sequence, but the biochemical activity is unknown for most of the cell wall related GTs. To determine the function of a putative GTs it is essential to demonstrate activity of heterologously expressed protein. Unfortunately, common expression systems based on yeast or animal cell cultures have often been found to be inefficient for expression of cell wall related GTs. To identify novel GT activities, we used Agrobacterium-mediated transient expression in Nicotiana benthamiana. To determine GT activities in a high throughput manner, we used a 96-well format to quantify incorporation of radiolabel from NDP-14C-sugars into endogenous acceptors. We are exploring the possibility of using the 96-well format for use with exogenous acceptors as well. As for the target genes, about 200 GTs in Gateway vectors have been prepared. To select genes of highest priority, we used available expression data to identify GT genes that are highly expressed in tissues that are important for biomass such as dicot woody tissue. In addition, we selected GTs where a rice homolog was highly expressed. This gene selection, using both the rice GT database and the transcriptional data sets regulated by known transcription factors for woody tissue formation, picked up a total of 30 GT genes. In this study, we will present the methodology for high throughput screening of key cell wall GTs important for biomass accumulation. "
(a) Joint BioEnergy Institute (b) University of Copenhagen

P15008 Sucrose synthase : a crucial enzyme in secondary cell wall synthesis?
Furbank, Robert T-presenter robert.furbank@csiro.au(a) Brill, Elizabeth (a) White, Rosemary (a) Fallahi, Hossein (a) Ruan, Yong-Ling (b)
"Sucrose synthase is the principal enzyme of sucrose metabolism in many plant tissues. In developing cotton fibres and other crop species, it is believed to provide UDP-glucose to cellulose synthase for cellulose synthesis. However, recent evidence using gene knock outs in Arabidopsis cast doubt on the indispensible nature of this enzyme in cellulose biosynthesis. Here we compare the sucrose synthase gene family in cotton with that in Arabidopsis and present cell biological and reverse genetics evidence for the role of these sucrose synthase isoforms in sucrose metabolism in these two species."
(a) CSIRO Plant Industry (b) University of Newcastle

P15009 The cell wall polymers of the Charophycean Green Algae: snapshots of the land plant ancestors.
Domozych, David S.-presenter ddomoz@skidmore.edu(a) Sorensen, Iben (b) Kiemle, Sarah N. (c) Willats, William G. T. (b) Gretz, Michael R. (c)
"The Charophycean Green Algae or CGA represent a diverse assemblage of organisms that are ancestral to land plants. Their extracellular matrices include cell walls that are structurally and biochemically diverse yet remain poorly understood. We have recently completed a detailed study of the cell walls of ten taxa of five recognized orders of the CGA. Our analyses included immunocytochemical and biochemical approaches and the Comprehensive Microarray Polymer Profiling (CoMPP) technique that combines monoclonal antibodies with carbohydrate microarrays. Homogalacturonans (HGs) were found in all taxa of the advanced orders (e.g. Zygnematales, Coleochaetales, Charales) but not in the more primitive groups (Chlorokybales, Klebsormidiales). HGs were associated with either the primary cell walls or constituted one of the dominant polymers of the entire wall complex. α(1→5)-arabinan was identified in Chara corallina of the Charales, suggesting the presence of Rhamnogalacturonan-I. Xyloglucans and xylans were found in various taxa of the advanced orders, whereas and β(1→3)-glucans were found in both advanced and primitive taxa. Mixed linkage β(1→3)(1→4)-glucan (MLG) was abundant in several of the placoderm desmids, and MLG from some species was digestible by the lichenase enzyme that is used to detect MLG in higher plants. Arabinogalactan proteins (AGPs) were identified in the cell walls of Chlorokybus and taxa of the most advanced orders. In the Zygnematales, AGPs were associated with adhesion structures critical to entry into biofilm communities.

The results of this study demonstrate that many of the cell wall polymers found in higher plants are distributed in the CGA and most likely evolved prior to the emergence of plants onto land."
(a) Department of Biology, Skidmore College (b) Department of Biology, Copenhagen Biocentre, University of Copenhagen (c) Department of Biological Sciences and Biotechnology Research Center, Michigan Technological University

P15010 Understanding plant cell wall acylesterification and its biological functions
Liu, Chang-Jun -presenter cliu@bnl.gov(a) Yu, Xiao-Hong (a) Gou, Jin-Ying (a)
http://www.biology.bnl.gov/plantbio/liu.html
"Cell wall acylesterification is a common modification, widely occurred in non-cellulosic components, such as pectin, hemilcellulose, and lignin. Different types of acyl moieties, including acetyl-, feruloyl-, *p*-coumaroyl were found esterified at the specific positions of those cell wall components. Acylesterification potentially affects cell wall structural property, therefore, affects its degradability. Our studies revealed that cell wall acylestrification was developmentally changed, implicating a complicated biological process involved in this type of modification, in which it may require at least two types of enzymes, the acyltransferases and acylesterases, to add or remove the acylesters from the cell wall polymers. The enzymatic *O*-acylation and de-acylation reactions also occur in the biosynthesis of a variety of non-structural primary and secondary metabolites, such as phenolics, suberine, and volatile alcohols that are required for disease resistance and/or the heart wood formation in trees. By employing biochemical genomics and reverse genetics approaches, we characterized several novel acyl-CoA dependent acyltransferases, and acylesterases in relation to the cell wall biogenesis and modification. Manipulating the expression of two particular acyltransferase and acylesterase respectively *in planta* disturbed the acyl content of polysaccharide pectin and resulted in the severe effects on plant growth and development. The progresses in characterizing pectin and lignin acetylesterification will be discussed. "
(a) Biology Department, Brookhaven National Laboratory

P15011 Identification and characterization of hydroxyproline β-galactosyltransferase activity involved in arabinogalactan-protein biosynthesis in tobacco and Arabidopsis
Liang, Yan (a) Faik, Ahmed (a) Tan, Li (b) Kieliszewski, Marcia K (b) Showalter, Allan M-presenter showalte@ohio.edu(a)
http://www.plantbio.ohiou.edu/epb/faculty/faculty/ams.htm
"Arabinogalactan-proteins (AGPs) are involved in a wide range of processes in plants, including plant growth and development, cellular morphology, programmed cell death and plant-microbe interactions. The sugar side chains of AGPs are attached to hydroxyproline (Hyp) residues in the protein backbone through *O*-glycosidic bonds and account for more than 90% of the molecular mass of these glycoproteins. This study seeks to understand the biochemistry and molecular genetic basis of AGP glycosylation in order to elucidate the biosynthetic pathway and function of AGPs. We previously expressed a synthetic gene encoding an AGP core protein motif of fifty-one [Ala-Pro] repeat units in tobacco BY-2 cells and obtained the $[Ala\text{-}Hyp]_{51}$ peptide glycosylated with arabinogalactan side chains, indicating that tobacco BY-2 cells have a complete AGP glycosylation system. An AGP galactosyltransferase (GalT) assay was developed using microsomal membranes from tobacco BY-2 cells as the enzyme source and HF-deglycosylated $[Ala\text{-}Hyp]_{51}$ peptide ($[AO]_{51}$) as the substrate acceptor, in the presence of UDP-[^{14}C]Galactose as the sugar donor. In addition, chemically synthesized $[Ala\text{-}Hyp]_7$ peptide ($[AO]_7$) was also used as the substrate acceptor in place of $[AO]_{51}$. Hyp: β-GalT activity was detected in the assay with both peptide substrate acceptors and was verified by structural analysis of the products using reverse phase-high performance liquid chromatography analysis, sugar analysis and linkage analysis. Similar Hyp: β-GalT activity was also detected in microsomal membranes from Arabidopsis suspension cells. Purification of the Hyp: β-GalT enzyme from Arabidopsis cells is currently underway to allow for proteomic analysis and identification/testing of candidate Hyp: β-GalT genes."
(a) Ohio University, Department of Environmental & Plant Biology, Molecular & Cellular Biology Program (b) Ohio University, Department of Chemistry and Biochemistry, Molecular & Cellular Biology Program

P15012 Signalling Mechanisms involved in the Plant Cell Wall Integrity Maintenance System
Carraca, Luis-presenter karrace@hotmail.com(a) Chairam, Issarya (a) Deness, Lucinda (a) Kjar, Lars (a) Madhou, Priyadharshini (a) Wormit, Alexandra (a) Hamann, Thorsten (a)
"Plant cell walls consist of polysaccharides that are the source of energy of lignocellulosic biomass derived biofuels. Their composition/structure influences the efficiency with which biofuels can be produced. The responsive nature of the cell wall to external stimuli suggests the existence of a complex system for sensing, signalling and feedback responsible for adaptations to the changing environment (Humphrey *et al*, 2007). We are interested in understanding this mechanism in order to optimise cell wall composition / structure for increased efficiency of biofuels production. Cellulose biosynthesis inhibition (CBI) is a highly specific tool to create cell wall stress (CWS), allowing to characterise changes in cell wall composition / structure. After 4h of CBI, lignin biosynthetic, pathogen signalling and mechano-stress responsive genes are activated (Hamann *et al*, 2008). Lignin deposition is detectable in the primary root of A. thaliana after 5h of CBI. Providing osmotic support suppresses this response suggesting that CWS perception may occur through mechano-perception. Global expression profiling has identified 5 genes that are transiently activated during the first 12h of CBI suggesting a specific role in early signalling mechanisms during CWS response. A functional characterisation of these genes is currently under way and we are determining whether homologues exist in bioenergy crops like miscanthus, poplar or willow. If so, their biological function in the bioenergy crops will be determined by altering their gene expression levels and their phenotype characterised, being the ultimate goal the optimisation of biofuel production from these crops."
(a) Imperial College London

P15013 Functions of rhamnogalacturonan-I in plant growth
McCann, Maureen C.-presenter mmccann@purdue.edu(a) Zhou, Shaohua (a) Penning, Bryan (a) Carpita, Nicholas C. (a)
"The cell wall constrains the sizes and shapes that plant cells achieve. The synthesis of cellulose microfibrils is essential for plant growth and the orientations in which microfibrils are deposited determine the directions in which cells can expand. However, we have obtained evidence that another cell wall polysaccharide, rhamnogalacturonan-I (RG-I), has crucial functions in plant and organ growth. Seven genes (MYST genes) in Arabidopsis have sequence similarity to microbial rhamnogalacturonan lyases (RG-lyases), secreted enzymes that cleave RG-I in plant cell walls. For one gene family member, AtMYST6, we have confirmed that the gene encodes a functional RG-lyase by heterologous expression in E. coli. A genetic functional analysis of AtMYST6 shows that the MYST6 protein is required for root hair development and over-expression of AtMYST6 increases root growth and alters root system architecture, overcoming constraints on normal root growth. These constraints may be biophysical as the substrate for RG-lyase, RG-I, may act to constrain root cell expansion, or physiological as the reaction products may act as signal molecules. Expression of a second MYST family member, MYST4, is required for normal plant stature: knockdown of MYST4 transcription results in severe dwarfing of the aerial parts of the plant. The key challenge is to reconcile the activity of RG-lyase in the cleavage of RG-I molecules with the dramatic growth phenotypes in our gain-of-function and loss-of-function lines. Structural variants of RG-I with different side-chains are developmentally regulated but their functions in cell wall architecture and in plant growth are not known. We hypothesize that endogeneous RG-lyases regulate biophysical properties of cell walls by

modulating RG-I structure. "
(a) Purdue University

P15014 Decipher the molecular pathways involved in intensive transfer cell wall formation and modification
Wang, Hong Li -presenter hxwang@ualr.edu(a)
"Transfer cells are highly specialized plant cells in which cell wall ingrowths increase their plasma membrane surface extensively. Their wall ingrowths are a specialized form of secondary cell walls that are deposited on the inner face of an initially ordinary primary cell wall. The major components of the wall ingrowths are similar to those of plant primary cell walls and contain cellulose, esterified pectins and hemicelluloses. Thus, elucidation of the cellular processes and genes involved in the development of transfer cell wall ingrowths will provide knowledge on the formation and modification of plant cell walls. Our structural studies on the wheat nucellar projection transfer cells (NPTR) revealed that the NPTRs are sequentially undergoing three stages of formation: Stage I, NP cells initiate in papillate form from the primary cell walls and begin to branch into antler-shaped ingrowths; Stage II, the antler-shaped wall ingrowths continue to increase their branches and exhibit anastomising labyrinths; Stage III, transfer cells have developed massive flange-shaped wall ingrowths formed by the fusion of a complex labyrinth. In the stage I and II, numerous paramural and multivesicular bodies are found closed to and associated with wall ingrowths, suggesting vesicle trafficking may be involved in the intensive cell wall formation and modification. Through proteomic and microarray analysis using the mRNAs and proteins extracted from pure wheat NPTRs, we have identified 14 transfer cell specific genes involved in the protein translation and vesicle trafficking pathways. Now we are analyzing the contributions of these identified genes to the cell wall formation using gene silencing in wheat transfer cells and functional complementation in *Dictyostelium*. "
(a) Department of Biology, University of Arkansas-LR

P15015 'Lignified' seaweeds: mechanical consequences of cell wall elaboration in a red alga
Martone, Patrick T-presenter pmartone@interchange.ubc.ca(a) Estevez, Jose M (b)
http://www.botany.ubc.ca/martone/
"The recent discovery of secondary cell walls and lignin in the intertidal red alga *Calliarthron cheilosporioides* has raised many questions about the adaptive significance and evolutionary history of these traits. In land plants, lignified cell walls mechanically stabilize upright growth and facilitate hydraulic transport. In *Calliarthron*, thick lignified cell walls strengthen tissues, helping fronds resist breaking under waves crashing on the shore. In this study, we performed standard material testing techniques to further explore the mechanical properties of this unique algal tissue. Engineering stress-strain analyses reveal that *Calliarthron* tissue is stronger and stiffer than other algal tissues, but not as stiff as terrestrial plant tissues. *Calliarthron* tissues are also highly extensible, able to stretch more than twice their original length, unlike lignified terrestrial tissues which generally cannot stretch more than 1-3%. The addition of secondary walls makes *Calliarthron* tissues stronger and tougher, absorbing more than ten-times the energy per volume as most woody or algal tissues before breaking. This mechanical augmentation coincides with a doubling of cellulose content within the walls and may be unrelated to lignin content. Surprisingly, as *Calliarthron* cell walls get thicker, they also get weaker per unit area, suggesting that either primary walls deteriorate over time or that secondary walls are made of weaker materials than primary walls."
(a) Department of Botany, University of British Columbia (b) Laboratorio de Fisiologia y Biologia Molecular, IFIByNE (CONICET), University of Buenos Aires

P15016 The structure of rhamnogalacturonan I (RG I) and its subtending neutral side-chains is developmentally regulated during tomato fruit ripening
Datsenka, Tatsiana-presenter datsenkt@purdue.edu(a) McCann, Maureen C. (a) Carpita, Nicholas C. (a) Kotte, Karsten (b) Ozminkowski,Jr., Richard (b) Handa, Avtar K. (a)
"The plant cell wall is a major determinant of texture of fruits and vegetables, and changes in the matrix pectins and other non-cellulosic polysaccharide structure and architecture are implicated in softening that occurs during ripening and impacting many desired quality traits in tomato products. We have analyzed the composition and linkage structure of the fruit cell walls of four tomato cultivars varying in temporal textural changes from Grass Green through Red Ripe stages of development, and subsequently in Red Ripe fruits stored post-harvest for 4, 8 and 13 days. The cultivars varied in the ripening behavior during the time course, but in all cultivars except one, processed tomato fruit juice samples showed a marked loss of galactose followed by loss of arabinose during the ripening. This cultivar also exhibited altered textural changes during fruit ripening. Linkage analyses showed that the loss of arabinose and galactose is due to the depolymerization of (1->5)-α-L-arabinan and (1->4)-β-D-galactan side-chains of RG I, with a subsequent decrease in the degree of branching of the RG I backbone. FTIR spectroscopy confirmed that the changes in wall composition are consistent with arabinan and galactan content. We hypothesize that RG I and its subtending neutral side-chains function is an architectural scaffold whose modifications are responsible for changes in texture during ripening."
(a) Purdue University (b) HJ Heinz Co

P15017 Genomic dissection of cell wall biosynthesis in yellow-poplar *(Liriodendron tulipifera L.)*
Xu, Yi-presenter yix@clemson.edu(a) Liang, Haiying (a)
"As an integral component of sustainable agriculture, woody plants are essential for lumber production and serve as an important reservior of lignocellulosic materials. Given current energy concerns high yielding biomass production systems are in greater demand than ever. Research into the genetic mechanisms underlying cell wall formation is of great importance. Our study aims to reveal the functions of two lignin (an integral component of cell wall) biosynthesis pathway genes, cinnamyl/sinapyl alcohol dehydrogenase (CAD/SAD), and their promoters from yellow-poplar (Liriodendron tulipifera L.). Yellow-poplar is an important timber tree species with potentials to be a feedstock resource for biofuel production. As a basal angiosperm species, yellow-poplar is also a bench mark species in studies on plant evolution. However, information on gene structure and function in yellow-poplar is very limited. Information obtained through this project will help improve yellow-poplar and other woody species as feedstock resources for renewable energy production by provide the missing information on CAD/SAD in basal angiosperm species, shed light into the evolution of CAD and SAD genes, and bridge the currently available information from gymnosperm, monocots and eudicots."
(a) Clemson University

P15018 Molecular dissection of a poplar mutant with a more flexible and digestible cell wall
Liang, Haiying-presenter hliang@clemson.edu(a) Chen, Chin-Fu (a) Xu, Yi (a) Frost, Chris (b) Gong, Fang (b) Carlson, John (b) Tien, Ming (b)
"Lignocellulosic materials are a common source of feed for livestock, a raw material for pulping, and also a promising source for biofuel production via cellulosic ethanol. Given current energy concerns, there is a momentum for research towards development of high yielding biomass production systems and bioconversion technologies. Mutants, both natural with low lignin content and genetically engineered woody plants with reduced lignin content or modified lignin monomer composition, have provided opportunities to study the functions of important genes involved in secondary cell

wall formation. These mutants have demonstrated that lignin content and type can be altered by transgenic methods. However, questions concerning fitness of such transgenic plants, such as growth, productivity, and resistance to environmental stresses, still remain to be evaluated over the course of rotations. Here we investigated another kind of mutant whose cell wall is both more flexible and digestible, while maintaining normal overall lignin, cellulose, and hemicellulose contents. We previously generated this mutant by introducing a tyrosine-rich (TYR) cell wall peptide gene into the hybrid poplar clone Ogy. These transgenic lines present a unique opportunity to study the molecular basis of how cell wall proteins affect lignin flexibility and digestibility. A microarray study will be conducted and expression profiles between wildtype and transgenic stems will be compared. Genes that are up-regulated or down-regulated in the transgenic poplar lines carrying a tyrosine-rich peptide gene will be identified. After functionally characterized, these genes will provide clues to the genetic mechanisms leading to the more flexible and digestible cell walls in such transgenic lines."
(a) Clemson University (b) Penn State University

P15019 Disruption of maize Cellulose Synthase-Like D1 results in reduced growth and warty-like phenotype.
Hunter, Charles T-presenter ibe@ufl.edu(a) McCarty, Don R (a) Koch, Karen E (a)
"Cellulose Synthase-Like D (CslD) genes remain one of the few classes of genes from the Cellulose Synthase Superfamily without a known function. They contain domains characteristic of enzymes responsible for synthesizing β-linked cell-wall polysaccharides. These polysaccharides might include cellulose, hemicellulose, or some other cell wall component. To test this hypothesis, we identified Mu transposon insertions in ZmCslD1 through reverse genetics utilizing the UnifromMu and TUSC maize populations. Plants homozygous for transposon insertion develop an abaxial epidermal phenotype similar to maize warty mutants. Individual cells and groups of cells balloon out of the cell surface as leaves mature, resulting in a rough texture. Individual cells expand up to 25 times their usual size. This phenomenon progressively extends to neighboring cells, resulting in large areas that take on a distinctive warty appearance in mature leaves. Mutant plants also show reduced growth, with mature plant heights of approximately 60% that of wildtype, and leaf width being roughly half that of wildtype leaves. Transcript profiling using qRT-PCR identified ZmCslD1 expression only in developing leaves, with the highest levels in immature leaves prior to leaf emergence from the whorl. Transcript abundance drops to undetectable levels by the time the epidermal warty-like phenotype is apparent. "
(a) University of Florida

P15020 Functional study of two cinnamoyl CoA reductases with different biochemical properties in *Medicago truncatula*
Zhou, Rui-presenter rzhou@noble.org(a) Chen, Fang (a) Jackson, Lisa (a) Shadle, Gail (a) Dixon, Richard A (a)
"Cinnamoyl CoA reductase (CCR) catalyzes the first committed step in monolignol biosynthesis. Two distinct CCRs, MtrCCR1 and MtrCCR2, were identified from *Medicago truncatula* and their biochemical properties were studied with the purified recombinant proteins expressed in *E. coli.* MtrCCR1 preferred feruloyl CoA as the substrate while MtrCCR2 showed preference for caffeoyl and coumaroyl CoAs. *M. truncatula* mutant lines harboring retrotransposon insertions in these two genes showed different phenotypes. The MtrCCR1 mutant had retarded growth and reduced lignin content, while the growth of MtrCCR2 mutants did not change significantly but the lignin concentration was reduced. We propose that MtrCCR2 plays an important role in an alternative pathway for monolignol biosynthesis. "
(a) The Samuel Roberts Noble Foundation

P15021 Modifications in cell wall during the development of the papaya fruit.
Cavalari Corete, Aline A.-presenter alinecavalari@gmail.com(a) Buckeridge, Marcos (b)
"The plant cell wall is a unique component of plant tissues and its polysaccharide composition is essential to understand food texture and its changes during post-harvesting, especially of climateric fruits, as is the case of papaya. The changes in polymers and their proportions in these domains are a result of enzyme action, which in the case of fleshy fruits lead to the softening of the pulp. Hydrolysis of hemicelluloses such as xyloglucan can play important functions in cell expansion, cell growth and cell wall degradation. Therefore, studying the modifications in xyloglucan by looking at is fine structure may be an important way to understand polysaccharide change during fruit development. The present work aimed at understanding the modifications in cell wall during the development of the papaya fruit. Fruits of Carica papaya L. Cv.Sunrise solo, were collected in Caliman, SA, Linhares, Espirito Santo, Brazil. Samples of fruits were harvested at intervals between 30 and 150 days after anthesis. Our results showed that there were drastic changes in the cell wall of the mesocarp in relation to other compounds, such as soluble sugars. We observed that the main soluble sugar found in fruits is sucrose, this being probably the principal source of energy for development of the organ. In general, the proportion of less branched xyloglucan oligosaccharides decreased at 120 daa, whereas the OXG branched with fucose increased constantly during development up to the same stage. These results suggest that xyloglucans (or part of their molecules) that are poorely brached with fucose are retrieved from th cell wall. This seems to lead to enrichment of fucosylation of xyloglucan. As these OXG turn xyloglucan more interactive with itself and with cellulose, it is possible that these would be the principal effects that the cell walls provoke in the fruit. On the basis of these results, we suggest that the cell walls of papaya fruits undertake structural changes untill 120daa after which the wall becomes more accessible to hydrolases denoting the preparation of the papaya fruit for ripening."
(a) unicamp (b) usp

P15022 Functional Characterization of Arabidopsis Glycosyltransferases
Suttangkakul, Anongpat-presenter ASuttangkakul@lbl.gov(a) Oikawa, Ai (a) Truong, Michelle (a) Manabe, Yuzuki (a) Scheller, Henrik V. (a)
"Polysaccharides are the major components of plant cell wall. Their composition affects plant in a variety of ways as well as how efficiently sugar can be extracted from plant biomass for biofuel production. Glycosyltransferases (GT) are families of enzymes catalyzing the transfer of sugar from nucleotide sugars onto acceptors; monosaccharides, oligosaccharides, or even proteins. Various plant GTs have been predicted based on sequence homology to other previously identified GTs. However, the activity and the specificity regarding the nucleotide sugar donors and the acceptors of most of plant GTs are not known. This is particularly true for membrane bound GTs likely to involved in cell wall biosynthesis. In an effort to better understand the functions of plant GTs, we test in vitro activities of heterologously expressed Arabidopsis GTs. Previous studies of membrane bound GTs involved in cell wall biosynthesis have made use of eukaryotic expression hosts. However, we have investigated the possibility of using *E.coli* for expression and compared the observed activitiy with the alternative transient expression in *Nicotiana benthamiana*. "
(a) Joint BioEnergy Institute

P15023 "Expression profiling and knock-out anayses of the maize cellulose synthase (*CesA*) gene family at the protoplast, cellular, and tissue-specific Levels."
O'Brien, Brent a-presenter bob2373@ufl.edu(a) Avigne, Wayne T (a) McCarty, Donald R (a) Settles, A M (a) Hannah, L C (a) Vermerris, Wilfred E (a) Koch, Karen E (a)
" The plant cell wall is a composite of diverse carbohydrate-based compounds. Understanding the regulation of genes that control how these

components are synthesized and integrated will be invaluable to our efforts to better utilize maize for grain, fiber, and renewable energy. Here, we profile expression of the cellulose synthase (*CesA*) gene family at cellular and tissue-specific levels. Tissue-specific expression was analyzed during three phases of development; seedling emergence, vegetative growth, and reproduction. Some of the *CesA* family members show similar expression patterns, however their mRNA levels do not appear to be coordinately regulated. Expression profiles also changed throughout development, as did family members that group together based on mRNA levels. A protoplast system was used to examine expression of *CesAs* throughout cell-wall regeneration. Results show a 3-to 35-fold upregulation of *CesA* family members until after 60hrs of wall regeneration. Upregulation was not evident until after 12hrs of incubation. Reductions in sucrose levels of media during cell wall regeneration shifted the timing, scale, and identity of *CesA* upregulation. In addition,we characterized knockout mutations in three cellulose synthases (*CesA7*, *CesA8*, and a new *CesA7* paralog) generated in the transposon-mutagenic Uniform Mu population. Thus far, single-knockout mutants have not had visible phenotypes, however double and triple mutants are currently being generated. Collectively, the profiles indicate that expression of the *CesA* gene family is dynamic at both the cellular and tissue-specific levels. This is consistent with previous hypotheses that different CESA's may function in diverse hetero-hexameric complexes and are highly regulated."
(a) University if Florida

P15024 Cell Wall Changes During Papaya Fruit Development
Cavalari-Corete, Aline A.-presenter alinecavalari@gmail.com(a) Buckeridge S., Marcos (b)
" Understanding plant cell wall changes is thought to be essential to access food texture. Changes in polymers and their proportions in cell wall domains are a result of enzyme action and hydrolysis of hemicelluloses such as xyloglucan can play important functions in cell expansion, cell growth and cell wall degradation. The present work aimed at understanding the modifications in cell walls during development of the papaya fruit. Fruits of Carica papaya L. Cv.Sunrise solo, were collected from crops cultivated by Caliman, SA, Linhares, Espirito Santo, Brazil. Samples of fruits were harvested at intervals between 30 and 150 days after anthesis. Our results showed that there were drastic changes in the cell wall of the mesocarp in relation to other compounds, such as soluble sugars. We observed that the main soluble sugar found in fruits is sucrose, this being probably the principal source of energy for development of the organ. In general, the proportion of less branched xyloglucan oligosaccharides (OXG) decreased at 120 daa, whereas the fucose containing OXG increased constantly during development up to the same stage. These results suggest that xyloglucans (or part of their molecules) that are poorely brached with fucose are retrieved from the cell wall. This seems to lead to enrichment of fucosylation of xyloglucan. As these OXG turn xyloglucan more interactive with itself and with cellulose, it is possible that these would be the principal effects that the cell walls provoke in the fruit. On the basis of these results, we suggest that the cell walls of papaya fruits undertake structural changes untill 120daa after which the wall becomes more accessible to hydrolases denoting a possible preparation of the papaya fruit for ripening."
(a) unicamp (b) usp

P15025 Cell Wall Changes During Papaya Fruit Development
Cavalari-Corete, Aline A.-presenter alinecavalari@gmail.com(a) Buckeridge , Marcos S (b)
" Understanding plant cell wall changes is thought to be essential to access food texture. Changes in polymers and their proportions in cell wall domains are a result of enzyme action and hydrolysis of hemicelluloses such as xyloglucan can play important functions in cell expansion, cell growth and cell wall degradation. The present work aimed at understanding the modifications in cell walls during development of the papaya fruit. Fruits of Carica papaya L. Cv.Sunrise solo, were collected from crops cultivated by Caliman, SA, Linhares, Espirito Santo, Brazil. Samples of fruits were harvested at intervals between 30 and 150 days after anthesis. Our results showed that there were drastic changes in the cell wall of the mesocarp in relation to other compounds, such as soluble sugars. We observed that the main soluble sugar found in fruits is sucrose, this being probably the principal source of energy for development of the organ. In general, the proportion of less branched xyloglucan oligosaccharides (OXG) decreased at 120 daa, whereas the fucose containing OXG increased constantly during development up to the same stage. These results suggest that xyloglucans (or part of their molecules) that are poorely brached with fucose are retrieved from the cell wall. This seems to lead to enrichment of fucosylation of xyloglucan. As these OXG turn xyloglucan more interactive with itself and with cellulose, it is possible that these would be the principal effects that the cell walls provoke in the fruit. On the basis of these results, we suggest that the cell walls of papaya fruits undertake structural changes untill 120 daa after which the wall becomes more accessible to hydrolases denoting a possible preparation of the papaya fruit for ripening."
(a) Unicamp (b) Usp

P15026 Biochemical characterisation of Arabidopsis enzymes involved in plant cell wall metabolism
Chairam, Issariya-presenter ic207@ic.ac.uk(a) Carraca, Luis (a) Deness, Lucinda (a) Kjaer, Lars (a) Madhou, Priyadharshini (a) Wormit, Alexandra (a) Hamann, Thorsten (a)
"Plant cell walls provide structural support to plants and form the bulk of the plant derived biomass. The walls provide protective barriers which perceive environmental stress signals allowing specific cell responses. Changes in the composition and structure of cell walls affect plant growth and development. To understand plant cell wall biosynthesis, many studies have been performed using Arabidopsis as a model species due to the sequenced genome, the genetic, molecular and bioinformatic tools available. However, the mechanisms regulating cell wall biosynthesis and remodelling are still unclear as many enzymes involved in these metabolism have not yet been characterised. This study aims to characterise the biochemical properties of Arabidopsis enzymes involved in plant cell wall formation from 5 different families including UDP-glycosyltransferase, pectin esterase, pectate lyase and glycoside hydrolase family 9 and 16. Additionally, the biological functions of the candidate genes will be determined by characterising Arabidopsis knock-out lines. In parallel, to gain additional insight into cell wall metabolism, a reporter-based forward genetic screen has been designed to isolate positive regulators for the expression of a putative xylose epimerase (*UXE4*) gene. We will present the results of our work on the cell wall biosynthetic enzymes and the pilot screen to identify transcriptional regulator."
(a) Imperial College London

P15027 The *Mn1*-encoded cell wall invertase is a secreted protein that is required for normal growth and development of wall-in-growths and of basal endosperm transfer cell in the maize kernel
Xiong, Yuqing-presenter yqxiong@ufl.edu(a) Kang, Byung-Ho (a,c) Chourey, Prem S. (b,d)
"The maize *Mn1* locus encodes a cell wall invertase, INCW2, that localizes exclusively to the basal endosperm transfer layer (BETL) of developing seeds. A common feature of all transfer cells is the cell wall-in-growth (WIG) that may confer greater solute transport and/or nutrient uptake capacity to these cells. *mn1-1* is a loss-of-function mutant of *Mn1* and the mutant maize produces stunted but fertile seeds. To better understand WIG formation in the basal endosperm transfer cells (BETCs) and roles of INCW2 in BETC maturation, we carried out electron microscopy analysis of kernels from the two genotypes at 7, 12, 17 days after pollination (DAP). In *Mn1* seeds, WIGs developed uniformly along the BETL from 7 DAP to 17 DAP and Golgi/trans-Golgi network (TGN) complexes and multivesicular bodies (MVBs) proliferated in the BETCs during the period. Mitochondria accumulated to the basal cell wall where WIGs are most elaborated and this accumulation began before any WIG was detected. In the *mn1-1* BETCs, WIGs were stunted, the endoplasmic reticulum (ER) was swollen, and density of Golgi stacks was 51% of the *Mn1* BETC Golgi density. However, the

polar distribution of mitochondria was not affected. INCW2-specific immuno-gold particles were associated with WIGs, the ER, Golgi stacks and the TGN. Additionally, we examined INCW2 localization in the *empty pericarp4* (*emp4*) kernels in which WIG sizes are heterogeneous among BETCs. The density of INCW2 Immunogold particles was ~4 times higher in the thicker WIGs than in the undersized WIGs. These results indicate that polarized secretion in the BETC is activated during WIG formation and that INCW2 is an apoplastic protein required for normal WIG development but its deposition is dependent of sustained WIG growth."
(a) Department of Microbiology and Cell Science, University Of Florida (b) Department of Plant Pathology, University of Florida (c) Interdisciplinary Center for Biotechnology Research, University of Florida (d) US Department of Agriculture, Agricultural Research Service

P15028 Binding of maize pollen β-expansins (group-1 allergens) to plant cell walls
Tabuchi, Akira (a) Wagner, Edward (a) Cosgrove, Daniel-presenter dcosgrove@psu.edu(a)
http://www.bio.psu.edu/expansins/
"Grass pollen characteristically produce copious amounts of β-expansins, known in the immunological world as group-1 grass pollen allergens. Named 'Zea m1' in maize, these glycosylated wall-loosening proteins are encoded by two groups of genes (Class A, B). We purified 4 β-expansin isoforms (Zea m1a-d) from maize pollen and studied their binding to plant cell walls by construction of binding isotherms using depletion analysis and by labeling cell walls and tissues with fluorescently-tagged isoforms. Zea m1d (Class B) binds with 5X higher affinity and binding capacity to maize cell walls, as compared with Zea m1a (Class A). Zea m1d also shows a pronounced positive cooperativity (a region of positive slope) in Scatchard plots, whereas cooperativity is lacking in Zea 1a. We are testing the possibility that Zea m1d unveils masked binding sites in the wall, thereby generating the appearance of positive cooperativity. With confocal microscopy we find that fluorescently-tagged Zea m1 binds in a general pattern to most cell walls of both grasses and dicots, but does not bind to lignified walls. 'Hot spots' of fluorescence appear to be localised callose deposits. Binding is also found on the inner surface of the cuticle. In summary, binding of these pollen β-expansins varies greatly by isoform class, suggesting functional differentiation at the level of protein-wall interactions. Supported by DOE. "
(a) Penn State University

P15029 Development of a VIGS system for cotton
Tuttle, John R. (a) Roberts, April D. (a) Haigler, Candace (a) Robertson, Niki-presenter niki_robertson@ncsu.edu(a)
"We developed a Virus-induced gene silencing system (VIGS) for use in cotton (Gossypium sp.). The A component of the bipartite Geminivirus Cotton Leaf Crumple Virus was used to deliver gene fragments of up to 750-bp for silencing endogenous genes. Using this system we have demonstrated the silencing of genes involved in chlorophyll-biosynthesis (ChlI, PDS), cell-cycle regulation (RbR), sugar metabolism (SS3) and terpene aldehyde biosynthesis (CDN). The system is temperature sensitive, with lower temperatures (22 degrees C) enhancing both silencing of the target gene and vector accumulation. When a 747-bp full-length GFP was inserted into the vector, expression was found in the companion cells and vascular parenchyma of the phloem. Expression of GFP was also present in the outer seed coat of the developing cotton ovule, which is in close proximity to the fiber initials. Given the mobility of the silencing signal, and the location of expression from the vector, we hypothesized that the system would be useful in the analysis of gene function in the economically important cotton fiber. This hypothesis was tested by silencing Sucrose Synthase 3 (SS3), which is highly expressed in fibers and thought to play a role in cell wall biosynthesis and fiber elongation. A significant down-regulation of SS3 in developing cotton fibers was shown by semi-quantitative RT-PCR. However, the phenotype of SS3-silenced fibers was much less severe than when the same fragment was used for RNAi (Ruan et al. 2003, Plant Cell 15:952). Surprisingly, silencing SS3 in cotton produced daytime accumulation of starch in the spongy mesophyll of source leaves that could be visualized by staining with iodine. Supported by Cotton Incorporated. "
(a) North Carolina State University

P15030 "Understanding the functional role of the CBM family 49 in endo β-1, 4 glucanases AtGH9C1 and AtGH9C2 from *Arabidopsis thaliana*"
Gaddam, Sivacharan -presenter siva.gaddam@gmail.com(a) Vergara, Armando (a) del Campillo, Elena (a)
"Many plant growth and development processes require dynamic changes on all faces of the plant cell wall. However, in some instances like root hair initiation and elongation, cell wall changes appear to be cell wall-side and site specific. The mechanism involving the unidirectional expansion and loosening of the cell wall is not completely understood. Plant endo-β-1,4- glucanases are hydrolases that catalyze β-1,4 glucan bonds, and can potentially mediate cell wall loosening and expansion. Although there is no evidence that these hydrolases breakdown crystalline cellulose, the Solanum lycopersicum Cel8 enzyme (SlCel9C1) showed binding to crystalline cellulose (Urbanowicz et al. 2007) through a unique cellulose binding module (CBM family 49). Arabidopsis thaliana has 3 endo-β-1,4 glucanases that possess a CBM family 49 at the C-terminal end of unknown function. Two of these, AtGH9C1 and AtGH9C2 are expressed while AtGH9C3 is a duplicon of AtGH9C2 and has very low expression. Both proteins contain a predicted signal peptide directing the protein to the cell wall. Transgenic plants carrying an AtGH9C1 promoter-GUS fusion showed AtGH9C1 expression prior, during and after the emergence of the root hair. The staining pattern was alternative along the longitudinal axis indicating that AtGH9C1 was expressed only in the H-type epidermal cells (root hair forming). The objective of this project is to analyze the function of the CBM in AtGH9C1 and AtGH9C2 by overproducing the proteins with and without the CBM and determining the protein localization via AtGH9C1::GFP and AtGH9C2::GFP fusion proteins in transgenic plants. These studies will help to determine the role of the CBM in site and side specific cell wall changes."
(a) Dept. of Cell biology and Molecular Genetics, University of Maryland, College Park

P15031 *Physcomitrella patens* as a heterologous expression system for investigating the functions of CESA-like gene products
Stein, Alexis I-presenter aeigense@emich.edu(a) Liepman, Aaron (a) Roberts, Alison (b)
"Plant cell walls are dynamic structures composed mainly of carbohydrates, including cellulose, hemicelluloses, and pectins. They provide structural support and are barriers against pathogens and mechanical injury, and cell walls hold great promise as a source of renewable biomass. *Cellulose synthase-like (CSL)* genes are proposed to encode glycan synthases that polymerize the backbones of non-cellulosic cell wall polysaccharides. This hypothesis has been supported in several studies: *AtCSLA* genes encode mannan synthases, *OsCSLF* and *HvCSLH* genes have been implicated in mixed-linkage β-glucan synthesis, and an *AtCSLC* gene likely encodes the enzyme that polymerizes the backbone of xyloglucan. Members of the *AtCSLB, AtCSLE*, and *AtCSLG* families have not yet been functionally characterized. This study investigates the functions of members from the *AtCSLB, AtCSLE, AtCSLG , OsCSLH* and *OsCSLF* gene families by using heterologous expression in the moss *Physcomitrella patens*. *P. patens* was chosen as a heterologous expression system because its genome lacks representatives from these gene families. The expression of *AtCSLB3, AtCSLE1, OsCSLF2* and *OsCSLH1* proteins has been detected by immunoblot analysis and immunofluorescence. Results of analyses of the transgenic moss lines will be presented. "
(a) Eastern Michigan University (b) University of Rhode Island

P15032 Plant single cells with intact walls: A tool for cell based manipulation and applications
Ponsamuel, Jayakumar-presenter psjayakumar@dow.com(a) Samboju, Narasimha C (a) Schmitzer, Paul PR (a) Gonzalez, Delkin DO (a) Webb, Steven R (a) Burroughs, Frank FG (a)
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"Plant cells have a unique structural feature, a rigid cell wall envelope when compared to mammalian or other eukaryotic cell counterparts. These plant cell walls restrict the ability of scientist to investigate cellular processes on isolated, clonal plant cells because intact cell walls lead to the formation of cell aggregates. The ability to produce plant cells with intact cell walls represents a valuable research tool for genetic and metabolic engineering options. We present a novel method of producing plant single cells with intact cell walls that will be useful for biotechnological manipulations and for production of various secondary metabolites and chemicals using these cells as chemical factories. In the present study we show that treating plant cells with 4-Chlor-1,5-diphenyl-1H-pyrazol-3-yloxy)-acetic acid ethyl ester produces a culture consisting of single cells with intact cell wall components. The single cells show all the characteristic features of a normal plant cell at the cellular and sub-cellular compartment levels. We present our findings on cell characterization from live imaging, FITC, AFLP, gene expression, and molecular analyses. We also show that these single cells could be genetically/plastomically transformed and thus would be very useful tool for basic and applied plant biological application. "
(a) Dow AgroSciences LLC

SESSION P16 – CHROMATIN

P16002 Signal integration on chromatin: The histone language of photosynthetic gene expression in maize
Peterhansel, Christoph-presenter cp@botanik.uni-hannover.de(a) Horst, Ina (a) Dreesen, Bjoern (c) Offermann, Sascha (b)
http://www.botanik.uni-hannover.de
"Photosynthetic gene expression is regulated by a wealth of stimuli that are integrated into a single promoter response. We use transcriptional control of genes controlling C4-metabolism in maize as a model to study information storage and read-out on chromatin. Histones, the building blocks of the nucleosome, can be modified in various ways. Many of these modifications have been associated with gene activity or repression, respectively. Our analyses reveal that positional and environmental stimuli control specific promoter modifications independent of whether the gene is finally activated: Three acetylation sites are modified on the core promoter upon illumination whereas most others are constitutively modified. This effect is regulated by light-dependent inactivation of a histone deacetylase [1,2]. Trimethylation of histone H3 lysine 4 is controlled by developmental information and set in a tissue-specific manner long before genes are activated [3]. This code is restricted to the proximal promoter region, whereas histone modifications on distal promoters follow a more simple charge neutralization model [2]. Pseudogene copies are repressed by a DNA methylation signal and dimethylation of histone H3 lysine 9. Both markers are precisely limited to a short region closely behind the transcription start site. We currently extend our studies to circadian control of gene regulation and genome-wide analyses of stimulus-dependent histone modifications. These results will allow new insights into the vocabulary of the histone language in plants. [1] Offermann et al (2006) Plant Physiology 141: 1078-1088, [2] Offermann et al (2008) Genetics 179: 1891-1901 [3] Danker et al (2007) Plant Journal 53: 465-474"
(a) Leibniz University Hannover (b) Washington State University (c) RWTH Aachen University

SESSION P17 – CLIMATE CHANGE BIOLOGY

P17001 Elevated carbon dioxide and ozone concentrations alter soybean antioxidant metabolism
Gillespie, Kelly M-presenter kramig@life.uiuc.edu(a) Xu, Fangxiu (a) Rogers, Alistair (b) Leakey, Andrew DB (a) Ort, Donald R (a) Ainsworth, Elizabeth A (c)
http://www.life.uiuc.edu/ainsworth
"One important mechanism by which plants sense and respond to their environment is through redox control. Oxidative damage at the cellular level can feed forward to decrease leaf photosynthesis and therefore canopy and ecosystem productivity. How rising atmospheric carbon dioxide ($[CO_2]$) and tropospheric ozone ($[O_3]$) will alter oxidative stress and resultant antioxidant metabolism in the future is largely unknown. Our goal is to understand and integrate the molecular, biochemical and physiological responses of soybeans to those climate change factors, using the Soybean Free-Air gas Concentration Enrichment (SoyFACE) site. SoyFACE enriches the $[CO_2]$ and $[O_3]$ to levels predicted for 2050 under fully open-air conditions without disturbing the microclimate. We investigated antioxidant metabolism at the genomic and biochemical scales in upper canopy soybean leaves throughout two growing seasons using Affymetrix soybean microarrays and high-throughput assays of ascorbate, phenolic content, total antioxidant capacity, lipid peroxidation and enzyme activity of six antioxidant enzymes. One challenge of this experiment is interpreting the results in a biologically meaningful way. In order to meet this challenge, we have adapted the Mapman visualization software, originally written for *A. thaliana*, for use with soybean. Our results indicate total antioxidant capacity was lower in soybeans grown at elevated $[CO_2]$ and higher in soybeans grown at elevated $[O_3]$, which was mirrored in leaf total phenolic content. Elevated $[CO_2]$ also improved the redox potential of the ascorbate pool. We are integrating these results with changes in antioxidant transcripts and enzymes to provide a mechanistic analysis of the response of the soybean antioxidant system to two factors of global change."
(a) Institute for Genomic Biology , University of Illinois at Urbana Champaign (b) Environmental Sciences Department, Brookhaven National Laboratory (c) USDA Photosynthesis Research Unit, Univeristy of Illinois at Urbana Champaign

P17002 Increased cell-wall extensibility in elevated $[CO_2]$ and $[O_3]$ indicates modification of leaf cell-wall structure.
McGrath, Justin M-presenter jmcgrath@illinois.edu(a) Taylor, Gail (c) Ainsworth, Elizabeth A (b)
"Soybean leaf size is increased by growth in elevated $[CO_2]$ and decreased in elevated $[O_3]$. The mechanism likely involves changes in cell biophysical properties. Cell growth rate is a function of cell-wall extensibility, a measure of how easily the wall expands in response to turgor. Modification of cell-wall structure through cleavage of cellulose cross links or change in chemical composition can modify extensibility, factors which may change with growth in elevated $[CO_2]$ or $[O_3]$. However, extensibility has not been examined in response to elevated $[CO_2]$ and $[O_3]$ in combination in a field setting. Free-Air Concentration Enrichment (FACE) experiments are able to elevate gas concentration in the field with minimal disturbance to the microclimate. We measured stress-strain curves of FACE-grown soybean leaf tissue from growing and mature leaves to determine if elevated $[CO_2]$ and $[O_3]$ affect cell-wall extensibility. Elevated $[CO_2]$ and $[O_3]$ singly and in combination increased cell-wall extensibility of leaf tips on average by 11%. Extensibility at the base of the leaf was unchanged, indicating a possible spatial difference in growth rates, a characteristic not usually seen in soybean leaves. These data implicate possible changes in cell-wall structure and correlate with other studies that show an increase in the activity and expression of cell-wall loosening enzymes in elevated $[CO_2]$ and $[O_3]$."
(a) Plant Biology Department, The University of Illinois (b) United States Department of Agriculture, Agricultural Research Service (c) Plants and Environment Lab , University of Southampton

P17003 Arabidopsis mutants and how they decompose: legacies of defense and shade avoidance
Austin, Amy T-presenter austin@ifeva.edu.ar(a,b) Martinez, M Laura (a,b) Ballare, Carlos L (a,b)
"Decomposition of plant material in terrestrial ecosystems is critical for ecosystem functioning, and is a crucial link in both carbon and nutrient cycling. The chemical and physical quality of senescent plant material, including the proportion of recalcitrant compounds and nutrient content, are major determinants of litter decomposition. Litter quality is ultimately determined by the genotype and the history of morphological and functional responses that plants activate while they are still living. We explored the relative importance of defense and shade-avoidance pathways on subsequent litter decomposition using a range of Arabidopsis mutants that displayed altered phytochrome responses (*phyB*), jasmonate-mediated defense induction (*jar-1*) or lignin biosynthesis (*irx-4*). All Arabidopsis plants were grown under similar greenhouse light conditions with optimal water and nutrients, and senescent plant material was collected from rosette leaves over the life cycle of the plants. Arabidopsis plants that constitutively expressed the characteristic shade-avoidance phenotype (*phyB* mutants) or were impaired in their ability to mount defenses against herbivores (*jar-1* mutants) produced litter which decomposed more quickly than wild-type counterparts. At the same time, litter from *irx-4* lignin mutants did not demonstrate differences in mass loss. These results suggest that the previously unconnected legacies of herbivore attack or competition with neighboring plants could have large effects on litter decomposition. The genetic tools available in Arabidopsis provide an opportunity to dissect the mechanisms whereby plant responses to biotic and environmental stresses which impact carbon turnover in terrestrial ecosystems. "
(a) University of Buenos Aires (b) IFEVA and CONICET

P17004 Temperature and precipitation interactions eliminate benefits of free-air CO_2 enrichment to soybean water relations in two out of five years
Gray, Sharon B-presenter sbgray@illinois.edu(a) McGrath, Justin M (a) Dermody, Orla (b) Ainsworth, Elizabeth A (a,c) Leakey, Andrew DB (a)
"A key assumption in projections of future food supply and ecosystem function is that elevated $[CO_2]$, through reduced stomatal conductance (g_s), results in lower water use, conservation of soil moisture and amelioration of losses in productivity due to drought stress. A 5-year dataset from the soybean Free Air CO_2 Enrichment (soyFACE) facility in the Midwest U.S. demonstrates that soybean did not benefit from this mechanism in 2 out of 5 years. Interannual variability in the effects of elevated $[CO_2]$ (550 ppm) on photosynthesis (*A*) and leaf area index (LAI) drives this variation in soil moisture and this interannual variability was a function of precipitation and temperature. Soil moisture in elevated $[CO_2]$ was greater in 2004 (up to 60%), 2005 (up to 20%), and 2007(up to 25%). On average, this was associated with a ~27% reduction in midday g_s, a ~21% stimulation in midday *A*, and a ~5% stimulation in peak LAI in elevated $[CO_2]$. In 2006 and 2008 however, elevated $[CO_2]$ did not significantly affect soil moisture, despite reducing g_s~25%. In 2006, high rainfall throughout the season resulted in soil moisture at or near field capacity, eliminating any benefits of decreased g_s in elevated $[CO_2]$. In 2008, an unusually wet spring delayed planting by one month, resulting in soybeans developing under higher temperatures than usual. This late planting and higher temperatures amplified the stimulation of LAI by elevated $[CO_2]$, offsetting reductions in g_s and eliminating any improvement in soil moisture at elevated $[CO_2]$. Consequently, during a drought in August, there was no stimulation of *A* at elevated $[CO_2]$. This suggests that increasing temperature and drought will diminish the benefits of elevated $[CO_2]$ to plant productivity in the future more than currently predicted."
(a) University of Illinois at Urbana-Champaign (b) Pioneer Hi-Bred Switzerland S.A. (c) USDA/ARS, Photosynthesis Research Unit

P17005 "Biochemical, Physiological and Yield Variation in Soybean Cultivar Responses to Chronic Elevated Ozone Concentration"
Betzelberger, Amy M-presenter betzelbe@illinois.edu(a) Gillespie, Kelly M (b) McGrath, Justin M (a) Koester, Robert P (a,e) Nelson, Randall L (c,d) Ainsworth, Elizabeth A. (a,e)
"Tropospheric ozone (O_3) is currently the most damaging air pollutant to plants, with crop losses in the United States costing $1-3 billion and expected to increase with the predicted increase of O_3 levels by the year 2050. This challenge to crop production provides an opportunity to breed for O_3 tolerance in sensitive crop species, like soybean. This study aimed to test that there is significant cultivar variation in the response of soybean seed yield to growth at elevated [O_3] and to identify possible physiological and biochemical markers for O_3 tolerance in soybean. Ten different genotypes of soybean were grown at elevated [O_3] from germination through maturity at the Soybean Free Air Concentration Enrichment (SoyFACE) facility. Photosynthetic gas exchange, leaf area index (LAI), leaf chlorophyll content and leaf antioxidant capacity of ten soybean cultivars were monitored throughout the growing season in order to determine if changes in these physiological and biochemical parameters could be used to predict the sensitivity of seed yield to elevated [O_3]. Cultivar differences in response to O_3 were observed in seed yield, net carbon assimilation (A), stomatal conductance to water vapor (g_s), LAI, leaf chlorophyll content, and, during reproductive growth, total antioxidant capacity. Although significant intraspecific variability of yield response among soybean cultivars has already been demonstrated, we identified a number of potential predictive traits correlated with variation in seed yield. This indicates there is potential to use these traits to screen germplasm for O_3 tolerance."
(a) Department of Plant Biology, University of Illinois, Urbana-Champaign (b) Program in Physiological and Molecular Plant Biology, University of Illinois, Urbana-Champaign (c) Department of Crop Sciences, University of Illinois, Urbana-Champaign (d) USDA ARS Soybean/Maize Germplasm, Pathology, and Genetics Research Unit, Urbana, IL (e) USDA ARS Photosynthesis Research Unit, Urbana, IL

P17006 HIGH TEMPERATURE TRIGGERS SIGNIFICANT EPIGENETIC RESPONSES IN *Arabidopsis thaliana*
Zhai, Hongli (a) Liao, Will (b,c) Zhang, Michael Q (c) Liu, Qiong A-presenter qliu@bnl.gov(a)
" Previous studies have demonstrated that a few degree increase of temperature can induce early flowering and lengthened hypocotyls in *Arabidopsis*. Flower Locus C (FLC) and Flower Locus M loci have been reported to be involved in regulating the early flowering, and plant hormone, auxin and the bHLH transcription factor, PIF4 in the regulation of lengthened hypocotyls. The study in vernalization has shown that FLC expression were regulated by epigenetic mechanism such as histone modification and DNA methylation, and cold stress may lead to changes of small RNA population and expression in *Arabidopsis*. In this study, we have identified additional phenotypes in *Arabidopsis* plants grown at 26°C and 28°C. In addition, deep sequencing of small RNA libraries generated from *Arabidopsis* plants grown under high temperature conditions led to identification of novel and small RNAs with altered expression levels. We have also discovered significant genomic DNA methylation changes in *Arabidopsis* resulting from elevated temperatures. Our results demonstrated that elevated temperature altered small RNA population and expression and induced changes in genomic DNA methylation in *Arabidopsis.*"
(a) Medical Department, Life Science, Brookhaven National Lab (b) The Department of Applied Mathematics and Statistics, Stony Brook University (c) Cold Spring Harbor Laboratory

P17007 Higher temperatures rather than elevated CO_2 concentration enhance growth and biomass allocation during establishment of late-successional specie *Hymenaea courbaril* L seedlings.
Yepes, Adriana-presenter adyepes@usp.br(a) Buckeridge, Marcos (a)
"One of the climate-change scenarios for 2050, as proposed by the IPCC, forecasts that CO_2 atmospheric concentration will rise, leading to an approximate 3°C increase in temperature. As a consequence, it is possible that due to these factors, photosynthetic rates will grow. Several studies have demonstrated that late-successional species revealed lower responses to elevated CO_2 than early and mid-successional ones. In the present study, we measured the growth and biomass of seedlings and young plants of *H. courbaril*, when grown in Open Top Chambers (OTC) with both ambient and elevated CO_2 concentrations (760 ppm) in combination with ambient temperature and elevated temperature (+ 3°C) conditions. Measurements were taken up to 240 days after imbibition. The results showed that increased growth and biomass allocation are correlated with elevated temperatures and only slightly correlated to elevated CO_2 concentrations. A rise in assimilation rates in elevated CO_2 treatments and higher dark respiration in ambient temperature treatments were observed. However, little effect was observed in biomass under elevated CO_2 conditions. From previous work, more related to development, we concluded that the effects of CO_2 are very subtle in changing the stomatal index and carbohydrate metabolism, for example. On the other hand, a much stronger effect on growth was observed under higher temperature conditions. Furthermore, there was no apparent interaction between the two factors when acting together. Financed by FAPESP. "
(a) University of Sao Paulo

P17008 Photosynthesis and growth of maize and sorghum under double-ambient CO_2 and soil water deficit
Vu, Joseph C-presenter Joseph.Vu@ars.usda.gov(a,b) Kakani, Gopal V (b,c) Boote, Kenneth J (b,b) Allen, Jr., Leon H (a,b)
"Maize and grain sorghum were grown for 39 d in sunlit environment-controlled chambers at 360 (ambient) and 720 (double-ambient, high) µ;mol mol^{-1} [CO_2]. The most enhancement by high [CO_2] on canopy photosynthesis (P_g), measured at 1,200 µ;mol m^{-2} s^{-1} solar PPFD, occurred at early plant growth stage, 17-22 d after seed planting (DAP), when increases in P_g by high [CO_2] were 5-22% for maize and 27-95% for sorghum. Drought stress, which was imposed at 26 DAP and became evident at 28-34 DAP for maize and 30-36 DAP for sorghum, caused P_g declines of 54-84% and 29-46% at ambient [CO_2] and 17-31% and 1-20% at high [CO_2] for the stressed maize and sorghum, respectively. The water-use efficiency (expressed as mmol [CO_2] mol^{-1} H_2O) during the drought-evident periods averaged 5.4, 3.4, 6.9, and 11.2 for maize, compared with 5.5, 6.6, 7.5 and 19.8 for sorghum, for the ambient-[CO_2] well-watered, ambient-[CO_2] stressed, high-[CO_2] well-watered and high-[CO_2] stressed plants, respectively. Although growth analyses of the young developing plants, performed at 34 DAP for maize and 39 DAP for sorghum, did not reveal a clear-cut difference in biomass between the ambient-[CO_2] and high-[CO_2] well-watered plants, above-ground dry weights of the ambient-[CO_2] stressed plants were 70% for maize and 80% for sorghum, when compared with the high-[CO_2] stressed plants. Thus, maize and sorghum used water more efficiently at high [CO_2], and both plants grew better at high [CO_2] than their counterparts at ambient [CO_2] in the presence of drought."
(a) Chemistry Research Unit, USDA-ARS-CMAVE, Gainesville, FL 32608 (b) Agronomy Department, Univ. of Florida, Gainesville, FL 32611 (c) Plant & Soil Sciences, Oklahoma State Univ., Stillwater, OK 74078

P17009 How will limiting N availability alter the response of C_4 photosynthesis in maize to elevated [CO_2] and drought under open-air field conditions?
Markelz, R.J. Cody-presenter markelz@illinois.edu(a,b) Strellner, Reid S. (a,b) Leakey, Andrew D.B. (a,b)
"Photosynthetic CO_2 uptake (A) of maize (*Zea mays*), a C_4 crop, is not directly stimulated by elevated [CO_2] under the normal production conditions of the U.S. Corn Belt. However, A can be enhanced indirectly by elevated [CO_2], as a result of lower water use and amelioration of stress during periods of drought. Unlike the heavily fertilized U.S. Corn Belt, maize production in many regions of the world is limited by low availability of nitrogen (N). This study tested the predictions that limiting N availability will enhance the sensitivity of A in maize to elevated [CO_2] by: (1) reducing enzyme activities such that the initial slope of the A response to intercellular [CO_2] (A/c_i) curve is decreased; and (2) increasing drought stress. To test this

hypothesis the effects of ambient [CO_2] (385 ppm) and elevated [CO_2] (550 ppm) on maize grown with either normal N fertilization (168 kg N ha^{-1}) or no N fertilization were assessed at the SOYbean Free-Air CO_2 Enrichment (SOYFACE) facility in Urbana, Illinois. In 2008, the early growing season was wet, but drought conditions developed in August. Counter to our first prediction, in the absence of drought stress there were no significant effects of [CO_2] on *A* of the youngest fully expanded leaf at either level of N supply. During periods of drought, elevated [CO_2] ameliorated the inhibition of *A*, while limiting N availability exacerbated stress. However, counter to our second prediction, there was no interaction effect and the benefit from elevated [CO_2] during drought was not greater under limiting N supply. These results suggest that across a broad range of growth conditions C_4 crops such as maize will benefit less from elevated [CO_2] in the future than is currently assumed in projections of future food supply."
(a) Institute for Genomic Biology, University of Illinois Urbana-Champaign (b) Department of Plant Biology, University of Illinois Urbana-Champaign

P17010 Thermal plasticity of photosynthesis: implications for forest response to warming
Gunderson, Carla A.-presenter gundersonca@ornl.gov(a)
"Climate change may alter forest ecosystem function and structure if rising air temperatures exceed those optimal for carbon gain. Survival of sensitive species or life stages may be threatened; local extinctions, range migrations, and unique species assemblages have been predicted. This study investigated photosynthetic sensitivity to warming and the potential for acclimation in five species of deciduous trees, *Liquidambar styraciflua, Quercus rubra, Q. falcata, Betula alleghaniensis,* and *Populus grandidentata.* Open-top field chambers supplied seedlings with three levels of warming (0, 2, or 4 degrees C above prevailing ambient temperatures) over three growing seasons. Responses were compared with seasonal changes in mature trees. Optimal temperature for net CO_2 assimilation (*A*) was strongly correlated with daytime temperature, but rates of *A* at the optima were not affected, except in autumn. Acclimation to daytime temperatures occurred in all species, whether temperatures varied with season or treatment, and regardless of climate in the species range or provenance. Temperature optima adjusted from 17 to 34 degrees C and acclimation potentials ranged from 0.55 to 1.07 degrees C per degree change in daytime temperature. Responses to the temperature manipulation were not different from seasonal acclimation in mature trees. Optima were determined largely by non-stomatal factors. Results suggest that modeling photosynthetic responses with static temperature functions will underestimate *A* and NPP. Direct impacts of climatic warming on forest productivity, species survival, and altered range limits may be less than predicted by current models."
(a) Oak Ridge National Laboratory

SESSION P18 – COMPARATIVE GENOMICS

P18001 TrichOME: A Comparative Omics Database for Plant Trichomes
Dai, Xinbin (a) Wang, Guodong (a) Tang, Yuhong (a) Marks, David (b) Broun, Pierre (c) Sumner, Lloyd W (a) Dixon, Richard A (a) Zhao, Patrick Xuechun-presenter pzhao@noble.org(a)
http://bioinfo.noble.org
"Plant secretory trichomes have a unique capacity for chemical synthesis and secretion, and have been described as biofactories for the production of natural products. However, with a few exceptions, most of the metabolic pathways and involved genes in trichomes remain largely unknown; and, only a limited amount of plant trichome genomics information is available in scattered databases. We present an integrated omics database, TrichOME, for studying genes and metabolic pathways expressed in plant trichomes. TrichOME hosts comprehensive 'omics' data from trichome and non-trichome control tissues of multiple species. For example, the database hosts a large number of trichome-related EST sequences from various species, which is a major component of the database. The TrichOME also integrates microarray hybridization results from corresponding trichome and non-trichome control tissues as well as details of trichome-related genes curated from published literatures. The EST sequences were thoroughly cleaned and assembled. We further annotated the Unigenes on the basis of UniProtKB/Swiss-Prot, InterPro Scan, Gene Ontology, KEGG, TCDB and PlantTFDB databases. The microarray hybridization results were universally normalized across experiments. These trichome-related Unigenes, probe sets and curated genes were further mapped to enable comparative analysis. We also performed in-silicon gene expression analysis to mine trichome-specific Unigenes or probe set target sequences. These trichome 'omics' data and corresponding analysis functions are valuable resources to trichome biology research community, since the genes and metabolites expressed in trichomes may be under-represented in the non-tissue-targeted libraries. The TrichOME is freely available at http://trichome.noble.org/."
(a) The Samuel Roberts Noble Foundation (b) Department of Plant Biology, University of Minnesota (c) Nestle R & D Center Tours, Plant Science & Technology

P18003 Abundant novel small protein and non-coding RNA genes in the *Arabidopsis thaliana* genome
Lehti-Shiu, Melissa D-presenter lehtishi@msu.edu(a) Moghe, Gaurav (a) Yin, Shan (a) Chen, Yani (a) Boniface, Jordan R (a) Juntawong, Piyada (b) Bailey-Serres, Julia (b) Shiu, Shin-Han (a)
http://shiulab.plantbiology.msu.edu
"Transcription evidence indicates that there are likely thousands of novel genes that are presumed to be non-coding. However, their protein-coding potential is rarely verified experimentally. Using computational approaches we have identified thousands of potential small open reading frames (sORFs) in the *Arabidopsis thaliana* genome that are conserved across species and/or have evidence of transcription [Hanada et al. (2007) Genome Research 17:632]. To verify sORF translation on a large scale, we are sequencing mRNAs that are associated with polyribosome complexes and are therefore likely actively translated. We are also verifying the translation of several sORFs directly by monitoring the expression of sORF-YFP fusion proteins *in planta*. Together, these approaches as well as recently published proteomics studies have allowed us to demonstrate the translation of a class of proteins that, despite their potential importance as small signaling peptides, have been virtually overlooked. In a parallel line of work, we have identified >1600 novel non-coding RNA (ncRNA) genes in *A. thaliana*. These genes do not have similarity to known ncRNAs or protein-coding sequences; therefore, they represent a completely unstudied set of molecules. As a first step towards their functional characterization, we are verifying that these ncRNAs are in fact non-coding by looking for their absence in polyribosome complexes as well as testing their ability to be translated *in planta*. By rigorously verifying the protein-coding potential of putative ncRNAs, and of predicted sORFs, we will get insight into the properties that distinguish protein-coding from non-coding RNA genes. This information can be applied towards more accurate gene annotation."
(a) Michigan State University (b) University of California, Riverside

P18004 Investigating extreme lifestyles through mangrove transcriptomics
Dassanayake, Maheshi-presenter dassanay@illinois.edu(a) Haas, Jeff (a) Bohnert, Hans J (a) Cheeseman, John M (a)
http://mangrove.illinois.edu/
"Mangroves represent phylogenetically diverse taxa in tropical intertidal habitats and exemplify one of the strongest cases for convergent evolution in the plant kingdom. Mangroves are also an untapped resource for understanding and exploiting plant adaptations to extreme environments. We have used recent advances in the 454/Roche pyrosequencing technology together with bioinformatics tools to explore the transcription-level strategies

applied by two mangrove species -*Rhizophora mangle* and *Heritiera littoralis*- in surviving the multiple stresses inherent to their ecosystems, including light, salt, temperature, low nutrient, and oxidative stress. For maximal representation of conditional transcripts, mRNA was isolated from a wide range of developmental stages, tissues types, and habitats. Sequencing of a normalized cDNA library for each species yielded a combined 537K sequences that were assembled, de novo, and annotated as >17000 distinct gene models for each species. Forty five percent of these were annotated using previously reported sequences in GenBank. Through gene ontology (GO) and KEGG orthology annotations, we highlight remarkable similarities in the transcriptome profiles of the two mangroves, and their substantial differences from the model plants, Arabidopsis and poplar. These similarities support a model of convergent evolution at the transcriptome level not apparent in their phylogenetic relationships or the diverse physiological and life history strategies they use to thrive in the intertidal environment. To further investigate this model at the gene family level we compare all mangrove metallothionein protein sequences publicly available to date with metallothioneins in *Arabidopsis thaliana*, *Populus trichocarpa*, *Oryza sativa*, and *Physcomitrella patense*."
(a) University of Illinois

P18005 Comparative Analysis Of Repetitive DNA Sequences In Genus *Oryza*
Gill, Navdeep-presenter gilln@purdue.edu(a) San Miguel, Phillip (b,c) Wing, Rod A (d) Jackson, Scott A (a)
"Repetitive DNA sequences play a major architectonic role in higher order physical structuring of genomes. To be able to understand how these sequences shape plant genomes during evolution, BAC-end sequences representing ~10% of ten Oryza genomes were used to sample their repetitive fraction. 36-76% of these genomes was repetitive and was correlated to the genome size (r=0.96). Analysis of the largest and smallest diploid genomes indicated their role in determining genome size of a species. Repetitive sequences specific to each species were identified for potential use as molecular markers in practical breeding and cytogenetic studies. Comparison of orthologous regions identified genomic rearrangements and conserved/variable regions with implications for changes at these loci both pre and post-genome differentiation from a common ancestor. Fragments of transposable elements were also found associated with conserved noncoding sequences, some of which were mapped to the untranslated regions and introns of genes, implying possible roles in gene regulation. Thus, the data suggests an evolutionary as well as functional significance of transposable elements in the host genomes. These determinations will be used to improve our understanding of the processes that might have played a role in shaping Oryza genomes."
(a) Department of Agronomy, Purdue University, West Lafayette, Indiana 47907 (b) Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, Indiana 47907 (c) Genomics Core Facility, Purdue University, West Lafayette, Indiana 47907 (d) Arizona Genomics Institute, University of Arizona, Tucson, Arizona 85721

P18006 Assigning domain functions to the unkowneome using the Gestalt Domain Detection Algorithm.
Dowd, Peter-presenter dowd@wisc.edu(a) van Rossum, Damian (b) Patterson, Randen (b) Gilroy, Simon (a)
http://www.botany.wisc.edu/gilroy/Site/Welcome.html
"A fundamental limitation to understanding the roles of many genes identified as key to plant function is the large proportion of all proteomes that lack a functional annotation. This incomplete coverage places a serious constraint on our ability to fully utilize the information from sequenced genomes towards a broad-scale understanding of the molecular processes behind plant development and physiology. It also reduces the power inherent in comparative genomics. Standard domain analysis such as using rpsBLAST has a high mammalian bias in the domain profiles used to search within proteins and is insensitive to divergent domain structure showing low absolute pairwise amino acid identity. Such limitations in search algorithms lead to the failure to detect motifs that have diverged from the animal model, such as those in many plant proteins, but that retain ancestral function. We show that an advanced seeded domain analysis tool, the Gestalt Domain Detection Algorithm (GDDA) can reveal such divergent domain structure in plant proteomes that is undetected by standard rpsBLAST analysis. Validation of this approach was performed through analysis of proteins annotated as of unknown function in the Arabidopsis and rice proteomes for cryptic lipid binding domains. This analysis revealed that the DUF246 family likely encodes proteins with phospholipid binding activity. Empirical verification using recombinant protein confirmed phosphoinositide binding. These analyses suggest that there is a large body of potential functional annotation residing in current proteomes that is potentially accessible through GDDA analyses. Funded by USDA and NSF."
(a) University Of Wisconsin (b) The Pennsylvania State University

P18007 Sequence-level comparative analysis of the *Brassica napus* genome around a stearoyl-ACP desaturase locus
cho, kwangsoo-presenter kscho@rda.go.kr(a,d) kwon, soojin (a,b) yang, taejin (a,c) ONeill, Carmel (a,d) Smooker, Andrew (a,d) Bancroft, Ian (a,d)
"We conducted the first sequence-level comparative analyses, at the scale of complete BAC clones, between the genome of the most economically important Brassica species, B. napus (oilseed rape), and those of B. rapa, the genome of which is presently being sequenced, and Arabidopsis thaliana. We constructed a new B. napus BAC library and identified and sequenced a clone that contains a region of the genome including a stearoyl-ACP desaturase-encoding gene. We sequenced the orthologous region of the genome of B. rapa and conducted comparative analyses between the Brassica sequences and those of the orthologous region of the genome of A. thaliana. The B. napus genome segment contains collinear homoeologues of 24 of the 61 annotated genes present in the corresponding region of the genome of A. thaliana, as does the genome of B. rapa This represents a lower rate of gene conservation (39%) than has been reported previously between A. thaliana and Brassica (~66%). The gene models for 19 of these sets of conserved genes were used to determine the extent of nucleotide conservation of coding regions. This was found to be 86.3 +/- 3.3% between B. napus and A. thaliana, which is consistent with previous results for other Brassica species, and 98.6 +/- 1.2% between B. napus and B. rapa. This divergence from the B. rapa genes was greater than anticipated and indicates that the A genome ancestor of the B. napus cultivar studied was relatively distantly related to the cultivar of B. rapa selected for genome sequencing."
(a) Highland Agriculture Research Centrer (b) National Institute of Agricultural Science and Technology, Rural Development Administration (c) Department of Plant Science, Plant Genomics and Breeding Institute (d) Department of Crop Genetics, John Innes Centre

P18008 Linking a set of tomato exotic libraries and a potato mapping population with a framework of Conserved Ortholog Set II (COSII) markers
Tripodi, Pasquale (a) Maurer, Sandra (b) Grandillo, Silvana-presenter grandill@unina.it(a) Di Dato , Francesco (a) Alseekh, Saleh (c) Frusciante, Luigi (d) van Haaren , Mark (e) Mohammad, Ayed (c) Tanksley, Steven D (f) Zamir, Dani (g) Gebhardt , Christiane (b)
"Comparative mapping has shown extensive genome colinearity among plant species of the same family, and recent applications of genome mapping have shown that the genetic diversity stored in germplasm banks can be utilized with a much higher level of efficiency than previously imagined. In order to facilitate comparisons between function maps of tomato and potato and to enhance the rate of introgression breeding in tomato, a diploid mapping population of potato and four exotic libraries of tomato from a diverse selection of accessions (Solanum pennelii LA716, S. habrochaites LA1777, S. chmielewskii LA1840, S. neorickii LA2133) are being anchored to a common set of Conserved Ortholog Set II or COSII markers

(http://www.sgn.cornell.edu/markers/cosii_markers.pl). This work is performed within the framework of the EU-SOL project (http://www.eu-sol.net/). A large number of single genes and quantitative trait loci (QTL) for numerous traits of agronomic importance have been mapped in the potato/tomato genomes in different genetic backgrounds. The development of a common, PCR-based marker framework, which links the tomato and potato maps will facilitate QTL identification, additional mapping, cloning of the underlying genes and the use of the novel variation in marker-assisted breeding. Examples will be given on linking tomato and potato function maps for pathogen resistance. "
(a) CNR-IGV, Institute of Plant Genetics, Portici, Italy (b) Max-Planck Institute for Plant Breeding Research, Cologne, Germany (c) Hebron University, Faculty of Agriculture, Hebron, Palestine (d) Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples "Federico II", Portici, Italy (e) Keygene N.V., Wageningen, The Netherlands (f) Department of Plant Breeding & Genetics, Cornell University, Ithaca NY, USA (g) The Hebrew University of Jerusalem, Faculty of Agriculture, Institute of Plant Sciences, Rehovot, Israel

P18009 Comparative functional genomics of grapevine and Arabidopsis: Identification of differentially expressed genes during the early fruit development
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http://pgel.or.kr/
"Sexual reproduction in flowering plants begins with flowering and ends with formation of fruits and seeds. In most plants fruit set, initiation of fruit growth, requires fertilization. Fruit development results from coordinated action of plant hormones and is a highly regulated process in which activation of specific genes takes place. Although many of the molecular details of fruit ripening are now known in several plants, not many genes, except cell cycle-related ones, are fully identified to be regulated at the early stages of fruit development. To identify genes that are specifically or prominently expressed at the early stage of grape berry development, we performed differential display reverse transcription-polymerase chain reaction that includes annealing control primers (ACPs). Sixteen of these differentially expresses bands were identified and the corresponding genes were cloned. The isolated genes or expressed sequence tags (ESTs) all showed significant sequence homology with known grapevine genes or ESTs of other plant species. Therefore, the isolated ESTs were used for digital expression analysis throughout fruit development in several fruit species for comparative functional genomics. Comparative genomics have provided some corresponding Arabidopsis genes to be identified to be differentially expressed during silique development, suggesting some common regulatory elements in many types of fruits including climacteric and non-climacteric ones. Further analysis of the temporally regulated genes that we identified should provide better understanding the biological basis of fruit development. Recent progress in our lab on the characterization of the differentially expressed genes (DEGs) during the early fruit development will be discussed."
(a) Dankook University (b) BK21 Graduate Program for RNA Biology

P18010 Lignin biosynthesis genes in the papaya genome and comparative analysis with orthologs in *Arabidopsis* and poplar
Gschwend, Andrea R-presenter agschwe3@illinois.edu(a) Yu, Qingyi (b) Hou, Shaobin (c) Paull, Robert E (d) Alam, Maqsudul (c) Ming, Ray (a)
"Lignin is an important cell wall component in plants. The draft genome of *Carica papaya* allows for the identification of genes involved in the papaya lignin biosynthesis pathway and the comparison of papaya lignin biosynthesis genes to two other dicot model species, *Arabidopsis thaliana* and *Populus trichocarpa*. Sequence alignment analyses of *Arabidopsis* and poplar lignin genes to the papaya genome detected 32 lignin candidate genes that encode the 10 gene families involved in lignin biosynthesis. Gene expression analysis confirmed transcription for all but three candidate genes. Most lignin biosynthesis genes were expressed consistently at all tissue types and stages tested. *CpC4H1* showed increased expression as the plant develops and *CpC4H2* expression was not detected until week 8, suggesting that the *C4H* step in lignin biosynthesis is rate limiting, as the plant matures and requires a greater production of lignin. Phylogenetic analyses depict the evolutionary relationships of lignin gene families among the three dicot species, papaya, *Arabidopsis*, and poplar, dividing genes into classes based on sequence similarity and their corresponding functions. Along with sequence alignment and phylogenetic analyses, conserved domain sequences, expression analyses, and map positions were utilized to narrow down the papaya candidate genes to 17 genes with strong support for their involvement in the papaya lignin biosynthesis pathway. "
(a) Department of Plant Biology, University of Illinois at Urbana-Champaign (b) Hawaii Agriculture Research Center (c) Advanced Studies in Genomics, Proteomics and Bioinformatics, University of Hawaii (d) Department of Tropical Plant and Soil Sciences, University of Hawaii

P18011 1KP: Sequencing one thousand plant transcriptomes
Stewart, Neal-presenter nealstewart@utk.edu(a) Burris, Jason (a) Wong, Gane K-S (b,c) Consortium, 1KP (b)
http://www.extremophytes.com
"The University of Alberta and the Beijing Genomics Institute (Shenzhen), along with a multidisciplinary international consortium, will be sequencing 1000 plant transcriptomes, each at the level of 1 Gb or more. There are multiple goals to this study. First, there is a real need to understand evolutionary genomic relationships among various plant taxa: from algae to angiosperms. Second, while there has been considerable effort to acquire genomic and transcriptomic data from crops and models, many other plants have been excluded from the genomics revolution. These include medicinal plants and weedy and invasive plants. Therefore, 1000 plant transcriptomes should yield valuable data that can lead to rapid gene discovery useful not just for basic science but also for practical endeavors like manipulating biosynthetic pathways for medicinal metabolites. Finally, plants that have unusual traits, such as movement (e.g., Venus flytrap, sensitive plant and dancing plant), rapid growth, metal hyperaccumulation, or extreme habitats will also be sampled. Having transcriptomes of extremophytes should be valuable in its own right, but could also provide timely outreach and educational opportunities. See www.extremophytes.com for a complete list of extremophytes and other plants that will have their transcriptomes sequenced beginning summer 2009. Transcriptome data will be assembled and annotated, then made freely available in an open access forum. "
(a) University of Tennessee (b) University of Alberta (c) Beijing Genomics Institute

P18012 Comparative analysis of *Ddm*1 gene involved in Epigenetic regulation
Picelli, Eduardo C.M. (a) Carrer, Helaine-presenter hecarrer@esalq.usp.br(a)
"At the transcriptional level, two types of mechanisms are known to mediate silencing of transposable elements (TEs) in plants: DNA methylation and histone deacetylation. In *A. thaliana*, an important gene that appears to play a major role in the former mechanism is *Ddm1* (Decrease in DNA Methylation 1), which encodes a chromatin remodeling ATPase with homology to SWI2/SNF-2 proteins. Nevertheless, the available genetic data strongly implicate that this protein is pivotal in silencing of TEs in general. Sugarcane (*Saccharum* spp.) is a very important crop for biofuel production, however breeding programs is very laborious and takes long time due to the large genome which is highly polyploidy. Genetic transformation is an important alternative to the development of new varieties. This technology requires plant regeneration in vitro culture that is known to increase the activity of TEs as showed in Arabidopsis, tobacco and rice. Sugarcane ESTs project identified 68 TEs, where 57 were expressed in callus. The objective of this work was the in silico characterization of *Ddm*1 gene in different species providing information for sugarcane transgenic studies using *Ddm*1 gene. This gene may contribute to decrease TEs activity through epigenetic regulation mechanism. Using Ddm1 from

Arabidopsis as reference, sequences from members of the Poaceae family were aligned by Clustal W software and the conserved domain characterized and used on phylogenetic analysis (Neighbor-joining parameter) checked by bootstrap test. It was observed that sorghum, maize and sugarcane are in a clade separated from rice. Arabidopsis as a Dicot is in more distant clade. Although they are evolutionary distant, AtDDM1 and SoDDM1 have 63% amino acids similarities showing to be highly conserved."
(a) ESALQ-Universidade de Sao Paulo

P18013 Gramene: A Resource For Comparative Grass Genomics
Dharmawardhana, Palitha-presenter palitha@oregonstate.edu(a) Avraham, Shulamit (c) Stein, Lincoln (c) Thomason, Jim (c) Tung, Chih-Wei (b) Ware, Doreen (c) Wei, Sharon (c) Yap, Immanuel V (a) Youens-Clark, Ken (c) Jaiswal, Pankaj (a) Buckler, Edward S (b) Casstevens, Terry M (b) DeClerck, Genevieve (b) Liang, Chenzhi (c) McCouch, Susan S (b) Ni, Junjian (b) Ren, Liya (c) Spooner, Will (c)
http://www.gramene.org
"The **Gramene** database (**www.gramene.org**) is a comparative plant genomics platform. The database allows researchers to carryout online data analysis, hypothetical modeling or confirmation of lab based findings using both forward and reverse genetics approaches to find genes, proteins, phenotypes (mutants and QTLs), function(s), gene-gene interaction(s), metabolic pathways and polymorphisms in a genomic region of interest. It also allows researchers to make comparisons across genetic maps, genomes, genes and gene families and provides a mechanism to find candidate genes associated with phenotype and/or functional characteristics by way of whole genome alignments and synteny views. The database hosts annotated genomes for *Oryza sativa indica* and *japonica, O. glaberrima, O. rufipogon, Zea mays, Sorghum bicolor, Arabidopsis thaliana, Arabidopsis lyrata, Vitis vinifera*, and *Populus trichocarpa* genetic maps from over 30 intra- and/or inter-specific crosses. Storage of genetic markers and plant nucleotide sequences from GenBank along with alignments to the sequenced genomes provides easy access to over 66,000 species and allows users to target genomic regions of interest to find underlying genes, ESTs, FSTs, SNPs, gene orthologs, phenotypes and associated metabolic pathways."
(a) Oregon State University (b) Cornell University (c) Cold Spring Harbor Laboratory

SESSION P19 – CYTOSKELETON STRUCTURE & DYNAMICS

P19001 "Myosin functions in organelle trafficking, F-actin organization, and plant development"
Dolja, Valerian V.-presenter doljav@science.oregonstate.edu(a) Prokhnevsky, Alex I (a) Peremyslov, Valera V (a)
"To understand the biological significance and mechanisms of the myosin-dependent cell dynamics, a series of the single, double, and triple gene knockouts of the class XI myosins of Arabidopsis was generated and analyzed. It was found that the rapid trafficking of Golgi stacks and peroxisomes is empowered by the myosins XI-K, XI-1, and XI-2, whereas mitochondria are transported primarily by myosins XI-K and XI-1. In addition to ~5-fold reduction of the organelle velocities, simultaneous inactivation of the myosins XI-K and XI-2 resulted in a dramatic rearrangement of the F-actin bundles in the leaf epidermal cells indicating that the myosins shape the tracks they run on. The double and triple gene knockouts xi-k/1 and xi-k/1/2 exhibited progressive reduction of the plant growth and fecundity that correlated with the cumulative reduction in organelle trafficking. Finally, myosins XI-K, XI-2, and XI-B were implicated in polarized elongation of the root hairs. Taken together, these data suggested that the myosin-driven trafficking of organelles and, perhaps, vesicles is required for the general growth and polarized elongation of the cells, as well as for the plant development and reproduction."
(a) Oregon State University

P19002 Class II Formins are Required for Apical Actin Polymerization and Tip Growth
Vidali, Luis (a) vanGisbergen, Peter (a,c) Guerin, Christophe (b) Franco, Paula (a) Li, Ming (a) Burkart, Graham (a) Augustine, Robert C. (a) Blanchoin, Laurent (b) Bezanilla, Magdalena-presenter bezanilla@bio.umass.edu(a)
"Formins are essential for the creation of actin-based structures responsible for a diverse array of processes in eukaryotes. Plants contain large formin gene families. *Arabidopsis* has 21 formins that group into two classes. This large number of isoforms has made it difficult to establish the physiological role of formins in plants. Using RNAi, we analyzed the function of all formins in the moss *Physcomitrella patens*. Moss has 9 formins that group into three classes. Two of these classes (I and II) are conserved in seed plants. We show that plants lacking class II formins are severely stunted and composed of spherical cells, with disrupted actin organization, and lacking tip growth. In contrast, silencing of all other formins results in normal cell morphology and actin organization. We show that class II formins localize at the apex of growing cells and demonstrate that the N-terminal PTEN-like domain mediates this localization. The PTEN-like domain is followed by the formin signature domains, FH1 and FH2, which are known to promote actin filament formation. To determine if apical localization of any formin FH1-FH2 domain mediates tip growth, we performed domain swapping experiments coupled with quantitative complementation analyses. Only the class II FH1-FH2 domain rescues tip growth, since it cannot be replaced with a similar domain from class I formins. To dissect the functional differences between these FH1-FH2 domains, we used *in vitro* polymerization assays to characterize them. We found that class II formins mediate exceptionally rapid rates of actin filament elongation, compared to class I or any other known formin. Taken together our results demonstrate that rapid rates of actin elongation drive the formation of apical filamentous actin necessary for tip growth."
(a) University of Massachusetts Amherst (b) Institut de Recherches en Technologie et Sciences pour le Vivant (c) Wageningen University and Research Centre

P19003 Stochastic dynamics of actin filaments in the cortical array of Arabidopsis epidermal cells
Staiger, Chris-presenter staiger@purdue.edu(a) Sheahan, Michael (b) Khurana, Parul (a) Wang, Xia (a) McCurdy, David (b) Blanchoin, Laurent (c)
"Eukaryotic cells harness the power of actin dynamics to create cytoskeletal arrays that stimulate protrusions and drive intracellular organelle movements. In plants, the actin cytoskeleton is generally understood to participate in cell elongation and responses to biotic and abiotic stimuli; however, a detailed description and molecular mechanism(s) underpinning filament nucleation, growth and turnover are lacking. We have used variable-angle epifluorescence microscopy (VAEM) to examine the organization and dynamics of the cortical cytoskeleton in growing and non-growing epidermal cells from Arabidopsis hypocotyls. Collectively, actin filaments in the cortical array are randomly oriented and surprisingly dynamic. Single actin filaments grow at rates of 1.7 micron/s, but are mostly short lived. Instead of depolymerization at their ends, actin filaments are disassembled by prominent severing activity. Incessant remodeling of the cortical actin array also features filament buckling and straightening events. We consider several mechanisms for the control of actin dynamics, including rapid polymerization from a large pool of profilin-actin, specific severing and capping activities, and myosin-driven filament-filament interactions. Aspects of these models have been tested with pharmacological agents, and future work will use reverse-genetics to further dissect the molecular mechanisms underlying actin dynamics. Our observations, the first to describe single actin filament behavior in plant cells, indicate a mechanism inconsistent with treadmilling, instead resembling the stochastic dynamics of a recently

described biomimetic system for actin assembly in vitro."
(a) Dept of Biological Sciences, Purdue University (b) Plant Science Group, Newcastle University (c) iRTSV, CEA/CNRS/UJF, Grenoble

P19004 Reduction in guard cell microtubule stability correlates with stomatal closure in Arabidopsis
Eisinger, William R.-presenter weisinger@scu.edu(a) Briggs, Winslow (b)
"Microtubules (MTs) establish the pattern of the transversely ordered cellulose microfibrils that are responsible for the functional shape of guard cells. However, the role of cortical MTs in mature, functional guard cells is less clear. At night when stomates are normally closed, most guard cells show few organized MTs. By contrast, imaging of GFP-labeled tubulin with confocal microscopy revealed that during the day, guard cells with open stomates have large numbers of radially oriented cortical MTs. However, using GFP labeled-tubulin expressed in Arabidopsis we found that microtubule (MT) numbers decrease about 50% as guard cells close their stomates. Similar decreases in MT numbers were seen whether closure occurs as the result of darkness or treatment with ABA (10 µ;M), hydrogen peroxide (1.0 mM), or sodium hydrogen carbonate (1.2 mM). During stomatal closure we observed no changes in microtubule (MT) numbers in adjacent epidermal cells with any of the above treatments. Therefore, since MT numbers in guard cells, but not other epidermal cells, show a decline in response to these varied stimuli, we hypothesize a causal link between decreasing numbers of guard cell MTs and stomate closure. In addition we used GFP labeled-EB1 protein (a marker for MT growth) to investigated rates of MT assembly in guard cells and adjacent epidermal cells. The length and number of GFP:EB1 comets seen at the growing ends of MTs remain unchanged during stomate closure. Since EB1 showed no changes apparent MT assembly rates during stomate closure, we conclude that MT numbers in guard cells decrease because of reduced MT stability rather than a reduced rate of MT assembly. "
(a) Dept Biology, Santa Clara University (b) Dept Plant Biology, Carnegie Institution

P19005 BRK/SCAR/ARP2/3-dependent actin polymerization regulates growth response to light in *Arabidopsis*.
Dyachok, Julia-presenter jdyachok@noble.org(a) Blancaflor, Elison (a)
"The ARP2/3 complex, a highly conserved nucleator of F-actin polymerization, and its activator, the SCAR protein complex, play an important role in cell morphogenesis and growth in Arabidopsis. Putative SCAR complex subunits BRK1 and SCAR1 are localized to the plasma membrane at sites of cortical F-actin enrichments in expanding cells of leaves and roots. Mutations disrupting the SCAR or ARP2/3 complex reduce the growth rate of roots. However, the mechanisms in which SCAR complex and ARP2/3 -dependent actin polymerization regulate plant growth remain unclear. We demonstrate that the influence of the SCAR and ARP2/3 complex on root growth depends on light conditions. Mutations disrupting SCAR or the ARP2/3 complex reduce root growth under constant light, but they promote the growth of roots in the dark. Inhibition of growth in the light correlates with reduced number of dividing cells in mutant roots, while cell division is stimulated in mutant roots grown in the dark. Alterations in the mitotic cytoskeleton in mutants of the ARP2/3 or SCAR complex subunits are currently being assessed. BRK1 and SCAR1 subunits of SCAR1 complex are localized to the plasma membrane in cells of inner layers of light grown roots. In dark grown roots, SCAR1 is degraded and BRK1 diffuses from the plasma membrane into the cytosol, suggesting disassembly of SCAR complexes. Cortical actin is prominent in light grown roots but is depleted in the dark. Cortical actin is also depleted in roots of *brk1* mutants, both in the light and in the dark. Together, our results suggest that BRK/SCAR/ARP2/3-dependent actin polymerization promotes events that are important for cell division and such events are under tight control by light."
(a) The Samuel Roberts Noble Foundation

P19006 Intracellular trafficking of cargo by myosin motors affects plant growth and response to the environment
Sattarzadeh, Amir-presenter as825@cornell.edu(a) Owens, Thomas G. (b) Hanson, Maureen R. (a)
"Myosin motors move plant organelles on actin microfilaments, resulting in cytoplasmic streaming. Mobility and positioning of vesicles and organelles are essential for optimal plant growth and for responses to abiotic stress and pathogen attack. Members of the myosin VIII and myosin XI families of motors in plants are involved in the endocytic pathways for internalization or secretion of molecules and in intracellular trafficking of cargo. Complete genome sequences have revealed the presence of multiple myosin genes in Arabidopsis (17) and rice (14). A major challenge is to determine the functions, locations, and cargoes of different myosins. We have approached these questions by making YFP fusions with different portions of the tail domain of myosin XIs and observing co-localization with other compartments. By expressing different regions of the myosin tails, we have found that the particular region that is expressed is critically important for detection of interactions with various cargoes. We have observed new interactions with particular cargoes and have confirmed reports of localization of certain myosins on peroxisomes, Golgi, or mitochondria. We have identified YFP fusions that interact with these organelles and others that localize to endoplasmic reticulum, vesicles, or plastids. To investigate the role of myosin XIs in various plant functions, we performed virus-induced gene silencing to down-regulate myosin gene expression. We observed that myosin gene silencing can impair the usual responses to suboptimal light levels and low humidity and affect cell expansion and leaf growth. Our results expose the central roles played by myosin motors and the actin cytoskeleton in intracellular transport, cell and plant growth, and response to the environment."
(a) Cornell University, Department of Molecular Biology and Genetics (b) Cornell University, Department of Plant Biology

P19007 "A comparative study on the involvement of Arabidopsis 17 myosin family members in the motility of Golgi, mitochondria and peroxisomes"
Avisar, Dror (a) Abu-Abied, Mohamad (a) Belausov, Eduard (a) Sadot, Einat-presenter vhesadot@agri.gov.il(a)
"Gene families with multiple members are predicted to have individuals with overlapping roles and functions. We examined all members of the Arabidopsis myosin family for their involvement in the motility of several organelles in plant cells. Dominant negative mutants, lacking the head actin binding domain of all 17 annotated Arabidopsis myosins were fused to GFP and co-expressed with RFP markers of Golgi, mitochondria or peroxisomes in leaves of N. benthamiana. By tracking and calculating each orgenelle's velocity in the presence of each myosin we found different combinations of six preferable myosins from group XI affecting best the motility of these organelles. No co-localization was found between these myosins and organelles in our system suggesting the sequestration of an intermediate molecule. No actin disruption was observed in the presence of the mutant myosins. Evidence for cytoplasmic flows in the presence of the inhibitory myosins was obtained from monitoring the motility of GFP-myosins aggregates them selves as well as from monitoring the motility of RFP-FYVE labeled particles in the presence of these myosins, suggesting that no global arrest of cytoplasmic streaming occurred. Taken together, our data suggest that the six myosins are involved, directly or indirectly, in the movement of Golgi, mitochondria and peroxisomes in plant cells."
(a) The Institute of Plant Sciences

P19008 Myosin XI-K of *Arabidopsis thaliana* is required for efficient secretion during root hair growth
Park, Eunsook-presenter epark3@utk.edu(a) Nebenfuehr, Andreas (a)
"Root hairs and pollen tubes are highly polarized cells that grow only at their tips. This tip growth is accompanied by vigorous organelle movements along actin filaments in the shank of the cell, presumably driven by myosin motor proteins. It is generally assumed that these rapid movements are

necessary for delivery of secretory vesicles to maintain rapid elongation at the tip. To address this question, we have isolated mutants of all class XI myosins in *Arabidopsis thaliana*. One of these mutants, *xi-k*, resulted in significantly shorter root hairs due to both a reduced growth rate and premature cessation of growth. Several organelles (peroxisomes and Golgi stacks) showed altered movements in the mutant. In addition, we found that YFP-RabA4b labeled vesicles did not accumulate normally in the tip of mutant root hairs, which might explain their reduced growth rate. Interestingly, movement of YFP-RabA4b vesicles was not affected in *xi-k* mutants, suggesting that myosin XI-K acts indirectly on the accumulation of these vesicles at the tip. To determine its direct target, we have generated plants that express a tagged version (YFP-XI-K) under control of the native promoter. This construct was able to complement the mutant phenotype and showed a distinct accumulation near growing tips, similar to ER. It is conceivable that this distribution of XI-K represents a feedback loop that couples ER localization and actin organization. However, preliminary evidence suggests that overall actin filament organization is not altered in *xi-k* root hairs. We are now examining the dynamic organization of actin filaments as well as other markers in *xi-k* root hairs to test for subtle perturbations during maintenance of the tip growth apparatus. This work is supported by NSF."
(a) The university of Tennessee Knoxville Department of Biochemistry and Cellular and Molecular Biology Knoxville, TN 37996-0840, USA

P19009 Forisome ultrastructure
Froelich, Daniel R.-presenter DFroelich@gmail.com(a)
"Forisomes are contractile proteins in the phloem found in members of the *Fabaceae* family. Recently, they attracted interest as a proteinaceous smart material with potential applications in micro- and nanotechnology, since they do not require ATP as a chemical source of energy. In vivo, they are spindle-shaped, lie inside the phloem, and do not overly impede the mass flow in sieve elements. In response to injury, forisomes act as a wound response mechanism by undergoing a dramatic conformation change. They contract longitudinally and expand laterally, filling the inner diameter of the sieve element, thus sealing off the wound site. After the elevated calcium levels normalize, the forisome longitudinally re-expands, allowing flow to resume. Plant mediated calcium influx triggers this action, but no known calcium binding domains were found in the three known forisome proteins. The key to this conformation change lies in the interconnections of protein filaments, therefore, we studied forisomes by high resolution TEM and SEM. In the expanded conformation, cross-striations of 12nm are visible on TEM micrographs, however, while longitudinally contracted, the filaments disperse to a loose interweave with no recognizable pattern. A complete lack of organization is improbable since the forisomes are able to perform this action several thousand times *in vitro*. SEM images reveal protein bundles perpendicular to these cross-striations, running along the axis of the forisome. Our ultrastructural investigations lead to a new model to explain forisome action and leakage free sieve tube occlusion."
(a) Washington State University

P19010 The Role of Mitogen-Activated Protein Kinase Signaling in Regulation of Plant Microtubule Function
Walia, Ankit-presenter ankitwalia@hotmail.com(a,c) Lee, Jin Suk (a,c) Wasteneys, Geoffrey (b,c) Brian, Ellis (a,c)
"The plant cytoskeleton comprises a network of microtubules (MTs) and actin microfilaments that organizes the structures and activities of the cell. Mitogen-activated protein kinase (MAPK) signalling networks are important regulators of environmental responses and development processes in plants, and substantial evidence in mammalian systems indicates a potential role of MAPK based signaling in mediating microtubule function and dynamics. To understand the role of MAPK signalling modules in the regulation of plant microtubule functions, we searched for MAPKs that interact with the dual-specificity MAPK phosphatase, *PROPYZAMIDE HYPERSENSITIVE 1 (PHS1)*, which was previously reported to confer hypersensitivity to microtubule-disrupting drugs in Arabidopsis. Using yeast two-hybrid and BiFC assays, we identified a novel MAPK that specifically interacts with PHS1. The *PHS1-MAPK* dyad is widely expressed across Arabidopsis tissues and the proteins are primarily localized in the cytoplasm. To further understand the biological significance of MAPK signaling in the regulation of MT functions, we analysed loss-of-function alleles of the PHS1-interacting MAPK for defects in microtubule-related functions. Mutant seedling roots show altered sensitivity to low doses of microtubule-disrupting drugs in terms of root skewing and root length and have moderately stabilized microtubules. We propose a model where the PHS1-MAPK dyad is involved in a phosphorylation-dephosphorylation switch that regulates cortical microtubule functions. "
(a) Michael Smith Laboratories (b) Department of Botany (c) University of British Columbia

P19011 Multiple role of AtAurora1 kinase in acentrosomal plant cells
Petrovska, Beata-presenter petrovska@ueb.cas.cz(a) Pochylova, Zaneta (a) Cenklova, Vera (a) Binarova, Pavla (b,a)
"Aurora kinases with a crucial role in cell division belong to the family of serine/threonine kinases that are highly conserved from yeast to humans. In metazoan cells centrosomal AuroraA plays a major role in spindle pole organization and spindle maintenance, while AuroraB, a chromosomal passenger is essential for correct cytokinesis. Three Aurora kinases AtAurora1, 2 and 3 are present in Arabidopsis genome. As specific properties are expected for plant Aurora kinases due to acentrosomal nature of the plant cells, we analyzed function of AtAurora1 kinase in Arabidopsis. Higher transcript levels of AtAurora1 were found in dividing compare to differentiated cells. GFP-AtAuroral was localized in nuclei, with nuclear membrane and nuclear periphery, associated with preprophase band and spindle microtubules during metaphase, relocalized to midzone in anaphase and was observed with phragmoplast and forming cell plate in telophase. AtAurora1 T-DNA knockout mutants and RNAi plants showed similar developmental defects - disruption of root cell files, ectopic root hairs with disturbed anisotropic growth, ectopic satellite meristemoids and clustered stomata and disturbed trichome branching. Misaligned chromosomes, defects in spindle organization, and aberrant anaphase/telophase transition resulted in bi- or multinuclear cells and in polyploidy. We found that AtAUR1 kinase affects multiple mitotic events including histone H3 phoshorylation, chromosome segregation, acentrosomal mitotic spindle organization as well as cytokinesis and cell polarity and thus display characteristics of both Aurora A and B kinases of metazoan cells. This work is supported by grant GACR - 204/09/P155, GACR - 204/07/1169 and MSMT - LC06034. "
(a) Institute of Experimental Botany, AS CR (b) Institute of Microbiology, AS CR

P19012 Synergistic regulation of dimerization and cargo binding in myosin XI
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"Cytoplasmic streaming is a ubiquitous process in plant cells which is thought to be driven by the active movement of myosin XI motor proteins along actin filaments. We have previously found that during cytoplasmic streaming these myosin motors bind to organelles through their C-terminal globular tail domain. However, our and other studies also suggested a perplexing role of the central coiled-coil region during organelle binding. We have investigated the relationship between these two protein domains of the Arabidopsis myosin MYA1 and revealed that dimerization of the coiled-coil region stabilizes organelle binding of the globular tail. Surprisingly, yeast two-hybrid, bimolecular fluorescence complementation, Förster resonance energy transfer as well as in vitro pull-down experiments all demonstrated that dimerization of the 174-residue-long MYA1 coiled coils by themselves was unstable. Most of this weak dimerization was contributed by the first of the two major coiled-coil segments in MYA1. In contrast, dimerization of myosin tail constructs that included the organelle-binding globular tail was stable, whereas the globular tails by themselves did not interact with each other. We further found that it was the targeting of two globular tails to the single organelle surface that stabilized the coiled-coil dimerization. Thus, our data suggested an interdependent relationship between dimerization and organelle binding in myosin XI where each process synergistically stimulated the other. Our findings uncovered an interesting mechanism by which myosin XI coordinates dimerization and organelle

attachment to prevent non-processive (and hence non-functional) myosin monomers from stably occupying motor binding sites on the organelle surface. "
(a) Department of Biochemistry, Cellular and Molecular Biology, The University of Tennessee (b) Department of Molecular Biology, Massachusetts General Hospital

P19013 Partially redundant functions of class XI myosins in differentiating *Arabidopsis* trichomes and root hairs
Ojangu, Eve-Ly (a) Jarve, Kristel (a) Truve, Erkki-presenter erkki.truve@ttu.ee(a) Paves, Heiti (a)
"Myosins form a large superfamily of molecular motors that move along actin filaments. Class XI myosins are characterized by the large number of IQ domains and long C-terminal tail. In 2007, we described the first abnormal phenotype for loss-of-function mutant of class XI myosin - XIK. Homozygous *XIk* mutants were having short root hairs and stem and leaf trichomes with twisted shape and irregular size. Others have later reported that also *Arabidopsis* plants missing *mya2* gene have shorter root hairs and that *mya2/XIb* double knockouts have root hairs with the length of only 15% of the wild type value. In addition, it was demonstrated that the motility of Golgi stacks, peroxisomes and mitochondria is reduced in *XIk* as well as in *mya1* and *mya2* homozygous knockouts. Here we report that the root hair phenotype of *mya2* knockout plants is dependent on growing conditions. The root hairs of double knockout line *XIk/mya2* has stably short root hairs. Moreover, the stem and leaf trichomes of *XIk/mya2* as well as *XIk/mya1* plants are severely malformed whereas *mya1* single knockout lines do not reveal any detectable phenotypic changes. These data indicate that class XI myosins have partially redundant functions in the epidermal differentiation of *Arabidiopsis*. "
(a) Dept. of Gene Technology, Tallinn University of Technology

P19014 Live cell imaging of cortical microtubule nucleation events in Arabidopsis cells
Nakamura, Masayoshi-presenter mas-naka@bs.naist.jp(a) Ehrhardt, David W. (b) Hashimoto, Takashi (a)
"Plant cells initiate nascent cortical microtubules from gamma- tubulin-containing complexes dispersed on existing microtubules as branching patterns. We simultaneously visualized microtubules by mCherry-TUB6 and microtubule-nucleating complexes by gamma-tubulin-complex protein (GCP) 2-GFP or GCP3-GFP in transgenic Arabidopsis plants. In interphase epidermal cells, we found that the nucleation complexes transiently associate with cell cortex in a microtubule-independent manner, and a fraction of the complexes immediately nucleate nascent microtubules upon association with previously established mother microtubules. Daughter microtubules are sometimes nucleated parallel to the mother microtubules, thereby generating instantaneous bundles. The GCP2/3-containing complexes are anchored at the basis of branching points until the Katanin-dependent activity severs daughter microtubules at their minus ends or their dynamic plus ends completely depolymerizes. These observations suggest that the nucleation complexes are activated upon association with the microtubule lattices, and become destabilized when daughter microtubules are lost."
(a) Graduate School of Biological Sciences, NAIST (b) Carnegie Institution for Science, Stanford

P19015 Gamma-tubulin is essential for stomata patterning and cytokinesis in *Arabidopsis* plants
Cenklova, Vera-presenter cenklova@ueb.cas.cz(a) Doskocilova, Anna (b) Gallova, Barbora (b) Benada, Oldrich (b) Binarova, Pavla (b) Kofronova, Olga (b) Pochylova, Zaneta (a)
http://www.ueb.cas.cz/Olomouc1/OLD_WEB/www-ueb%20-%20110602/lcb/lcb.htm
"Gamma-tubulin is a conserved member of the tubulin family that is central to the nucleation of microtubules. Downregulation of γ-tubulin by RNAi in Arabidopsis seedlings revealed its essential role in microtubule nucleation from dispersed sites; strong morphogenetic effects of gamma-tubulin depletion by RNAi suggest functions for γ-tubulin other than microtubule nucleation. We found that RNAi seedlings showed a disturbed organization of root cell files resulting from misaligned cell divisions plane, binuclear cells were often present. Depletion of gamma-tubulin level by inducible RNAi in plants with GFP visualized microtubules revealed that since microtubules were still present, RNAi cells could progress through mitosis and cytokinesis, although the cell division plane was misaligned. Compared to leaves of control plants with lobbed pavement epidermal cells and stomata developed according to the one neighbor rule, leaves of γ-tubulin RNAi plants exhibited a blistered-like surface with large swollen epidermal cells intercalated with groups of smaller isodiametric cells with clustered and aberrantly divided stomata. For comparison, leaves of control plants developed under a treatment with low dose of the anti-microtubular drug amiprophosmethyl showed large swollen cells typically observed in cells with impaired microtubular function. We found that GFP visualized microtubules were preserved in clustered stomata as well as in aberrantly divided stomata of RNAi plants with reduced gamma-tublin levels. The phenotype that was typical only for RNAi seedlings suggests that functional γ-tubulin is essential for cytokinesis, cell specification and polarity. Supported by grants MSMT LC06034, GACR 204/07/1169, MSMT LC545, IAA 500200719 "
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P19016 Signaling to microtubule ordering in *Arabidopsis*
Fu, Ying-presenter yingfu@cau.edu.cn(a) Xu, Tongda (b) Yang, Zhenbiao (b)
"To regulate oriented cell division and shape formation during plant development, microtubules (MTs) organize into different types of structures with distinct functions. Interphase well-ordered transverse cortical MTs promote cell elongation and restrict radial expansion. However the molecular mechanism controlling MTs ordering is poorly understood. Using jigsaw puzzle-shaped pavement cell system, we demonstrate a ROP GTPase signaling pathway that regulates this ordering in *Arabidopsis*. In the indentation region of pavement cells, well-ordered MTs locally restrict cell expansion. Deleting ROP6, a Rho-family GTPase, randomized cortical MTs and released the localized restriction of cell expansion, whereas ROP6 overexpression enhanced MT ordering, leading to the cylindrical cell shape. ROP6 directly activates a MT-associated protein, RIC1, to achieve the MT ordering. The ROP6-RIC1 pathway also affects MT ordering of hypocotyl cells. This is the first demonstrated signaling pathway that promotes the ordering of cortical microtubules, and has a broad role in the spatial regulation of cell expansion."
(a) China Agricultural University (b) University of California, Riverside

P19017 Identification and localization of a cotton kinesin GhKCH2
Liu, Guoqin-presenter liu@cau.edu.cn(a) Xu, Tao (a) Zhe, Qu (a)
"Kinesin is an ATP-driven microtubule motor protein that plays important roles in controlling of microtubule dynamics, intracellular transport, cell division and signal transduction. Here, a plant-specific kinesin, GhKCH2, was identified by ATPase activity assay, microtubule-binding, transgenic analysis, and immunolocalization. The putative motor domain of GhKCH2, M396-734 corresponding to amino acids Q396-N734, has ATPase activity that could be stimulated 30-fold max by microtubules. M396-734 could bind to microtubules *in vitro*, but its GFP-fusion proteins were not colocalized with cytoskeleton filaments *in vivo*. Immunofluorescence labeling by using self-prepared antibody showed that the distribution of GhKCH2 was related to cell cycle. During the interphase, GhKCH2 mainly located in nucleolus. When the preprophase band formed, GhKCH2 distributed sporadically in whole cytoplasm; during the metaphase and anaphase, GhKCH2 moved to the central region of dividing cells; from telophase to cytokinesis, it was concentrated in the midzone of phragmoplasts; as the reassembly of nuclear materials in daughter cell after cytokinesis, GhKCH2 was re-localized in

nucleus. Cotton kinesin GhKCH2 might be involved in the formation or function of cotton root cell phragmoplasts."
(a) College of Biological Sciences, China Agricultural University

P19018 Regulation of secondary cell wall development by cytoskeleton in metaxylem vessel differentiation
Oda, Yoshihisa-presenter oda@biol.s.u-tokyo.ac.jp(a) Iida, Yuki (a) Yamaguchi, Masatoshi (b) Demura, Taku (b) Fukuda, Hiroo (a)
"Xylem vessel cells deposit secondary cell walls in appropriate patterns during plant development. To analyze the regulatory mechanism of the secondary wall patterns, we newly developed in vitro metaxylem vessel differentiation system using Arabidopsis suspension cells harboring inducible *VND6*, a master regulator of metaxylem vessel differentiation. In this culture system, suspension cells synchronously deposited metaxylem-like secondary cell wall at high frequency after induction of VND6. In this study, we focused on arrangement of cortical microtubules and actin microfilaments during differentiation. We found that the cortical microtubules were rearranged at random orientation and then disappeared at the future secondary wall pit sites. The cortical microtubules were preferentially disassembled at the secondary wall pit site by depolymerization and sliding. Disruption and stabilization of microtubules by drug treatment dramatically affected the secondary wall patterns. Disruption of actin microfilaments inhibited the microtubule rearrangement and affected secondary wall patterns. However, actin microfilaments did not show any significant co-localization with secondary walls and cortical microtubules. These results suggested that site-specific microtubule disassembly was required for metaxylem-like secondary wall pattern development and that the actin microfilaments were indirectly involved in the secondary wall pattern development. "
(a) Graduate School of Science, The University of Tokyo (b) RIKEN Plant Science Center

P19019 "AtFH8, an Actin Filament Nucleator and Bundler, Has a Relationship with Root Development in Arabidopsis"
Xue, Xiuhua (a) Guo, Chunqing (a) Lu, Quanlong (b) Zhang, Chuanmao (b) Ren, Haiyun-presenter hren@bnu.edu.cn(a)
"Formins have been paid much attention for their potent nucleating activity. In addition to nucleate actin filament assembly, some formins can bundle actin filaments. But, less is known about how its nucleating and bundling activity affects the physiological processes *in vivo*. In this study, we characterized the bundling activity of AtFH8 (*Arabidopsis thaliana* Formin Homologue 8) *in vitro*. Biochemical analysis showed that AtFH8(FH1FH2) could form dimers and bundle preformed actin filaments. However, during the polymerization processes, it not only bundled actin filaments but also induced stellar structures consisting lots of actin bundles. To investigate the localization and function of AtFH8 *in vivo*, full-length cDNA and truncated forms of AtFH8 were expressed as GFP fusion protein in Arabidopsis. It was found that AtFH8 localized to nuclear envelope in interphase and to the forming cell plate during cytokinesis, which was significantly dependent on its N-terminal transmembrane domain. The immunolocalization of AtFH8 confirmed the nuclear envelope and cell plate localization. Overexpression of AtFH8 promoted mitosis of root tip cells and increased the primary root growth rate in the young transgenic seedlings and vise verse in its N-terminal transgenic lines. The lateral root initation of T-DNA insertion mutant seedlings was inhibited by a F-actin-depolymerizing drug, latrunculin B, treatments for 8 days, and the wild-type AtFH8 transgene complements the lateral root phenotype, indicating that AtFH8 linked stabling of actin filament structures may associate with the initiation of lateral roots. Our results suggest that AtFH8 is a potent actin bundle organizer that contributes to primary root growth and lateral root initiation in Arabidopsis young seedlings."
(a) The Key Laboratory of Cell Proliferation and Regulation Biology of Ministry of Education, Beijing Normal University, China (b) The Key Laboratory of Cell Proliferation and Differentiation of Ministry of Education, Peking University, China

P19020 *Arabidopsis* NodG - a novel interactor of plant microtubular cytoskeleton
Plihal, Ondrej-presenter plihal@biomed.cas.cz(a) Volc, Jindrich (a) Doskocilova, Anna (a) Chumova, Jana (a) Halada, Petr (a) Binarova, Pavla (a)
"γ-Tubulin is a distant and highly conserved member of the tubulin superfamily present in cells with discrete nucleation sites such as yeast or animal somatic cells as well as in acentrosomal cells including higher plant cells. Large γ-tubulin ring complexes (γ-TuRCs) and small γ-tubulin complexes (γ-TuSCs) are well characterized γ-tubulin molecular forms while other γ-tubulin molecular assemblies reported in nuclei, with membranes and with microtubules are much less understood. We found that plant γ-tubulin similarly to its yeast or animal cells counterparts forms complexes with *Arabidopsis* homologues of γ-tubulin complex proteins (GCPs), AtGCP2 and AtGCP3, that are core components of γ-tubulin small complexes. Moreover, we also confirmed another γ-tubulin interaction with *Arabidopsis* GCP family protein, homologue of GCP4, the component of large γ-TuRCs. Among other proteins that were co-purified with *Arabidopsis* γ-tubulin we identified yet uncharacterized protein AthNodG. Its interaction with microtubular cytoskeleton was further supported by the data from two hybrid screen. Similarly, localization studies supported by biochemical data revealed that NodG was localized mostly in the cytoplasm, but a portion of the protein was associated with microtubular arrays. T-DNA mutants and seedling expressing dsRNA with reduced protein levels of AthNodG showed similar phenotype with aberrant lateral root formation. Further functional studies as well as biochemical characterization are under progress to elucidate the role of AthNodG in cytoskeleton organization and plant growth and development. Supported by The Grant Agency of the ASCR (KJB500200705, IAA 500200719) and Ministry of education (MSMT LC545)."
(a) Institute of Microbiology v.v.i.

P19021 ADF is critical for actin dynamics in tip-growing cells
Augustine, Robert C.-presenter raugusti@nsm.umass.edu(a) Pattavina, Kelli (a) Vidali, Luis (a) Bezanilla, Magdalena (a)
"Actin depolymerizing factor (ADF)/cofilin family proteins are small, conserved proteins that sever and disassemble actin filaments. Pollen tubes, root hairs, and other cells that require actin dynamics for growth utilize ADF as a major regulator of actin turnover, however the exact role of ADF in growth has remained elusive. The moss *Physcomitrella patens* serves as an excellent system to study ADFs physiological importance because it is encoded by a single, essential gene. We have generated a stable moss line expressing Lifeact-GFP, which allows live-cell visualization of actin organization without defects to cell growth. Control plants have an accumulation of actin at the apex, and extending rearward from that is a highly dynamic cortical actin network. In contrast, ADF-RNAi plants form star-shaped actin cables that emanate from multiple foci and exhibit dramatic reductions in formation and disassembly of filaments. This indicates that ADF is critical for regulating actin turnover, organization, and dynamics. We have worked to generate tools to facilitate further in vivo studies of ADF function. For example, we generated a C-terminal tetracysteine fusion to ADF that is functional based on its ability to rescue in a transient complementation analysis. This is significant because it will permit in vivo localization of a functional ADF. Furthermore, we have worked to generate temperature sensitive (TS) ADF mutants based on previously identified TS mutants from yeast. These tools will aid our understanding of ADFs contributions to actin architecture and dynamics as well as cell growth."
(a) University of Massachusetts at Amherst

P19022 Identifying the ADF kinase in the moss *Physcomitrella patens*
Pattavina, Kelli A.-presenter kpattavi@student.umass.edu(a) Augustine, Robert C. (a) Vidali, Luis (a) Bezanilla, Magdalena (a)
"Actin depolymerizing factor (ADF) is an essential protein in plants that is responsible for severing and depolymerizing actin filaments to promote

actin dynamics and polarized plant cell growth. Phosphorylation of ADF at a conserved serine residue inhibits its activity. While an ADF kinase has not yet been identified in plants, evidence suggests a role for calcium dependent protein kinases (CDPKs) in this process. There are 24 CDPKs in the moss *Physcomitrella patens* that group into four subfamilies (Groups I-IV). To identify the kinase, we designed RNAi constructs to target and inhibit each group. This process involved cloning and transforming the RNAi constructs into moss, and then imaging transformed plants using fluorescence microscopy. We looked for a phenotype that would mimic an unphosphorylatable ADF mutant plant (ADF S6A), which is significantly smaller than wildtype. Images of control and CDPK-RNAi plants were analyzed for area and morphological parameters, and compared with the ADF S6A mutant. We found that two groups exhibit a phenotype similar to the unphosphorylatable ADF mutant, whereas the remaining groups did not. This suggests that a subset of CDPKs could be responsible for phosphorylating ADF. "
(a) University of Massachusetts Amherst, Dept. of Biology

P19023 Plant tubulin phosphorylation on tyrosine residues
Blume, Yaroslav B.-presenter cellbio@cellbio.freenet.viaduk.net(a) Yemets, Alla I. (a) Sulimenko, Vadym (b) Sulimenko, Tetyana (b) Chan, Jordi (c) Lloyd, Clive W. (c) Dráber, Pavel (b)
"Phosphorylation by protein tyrosine kinases plays an important role in the regulation of many cellular processes in eukaryotes including plant development, signal transduction of phytohormones, and plant cell cycle control. In this study we used an immunochemical approach to demonstrate that both α- and β-tubulins, from two different plant species *Nicotiana tabacum* and *Daucus carota*, isolated both by immunoprecipitation and from polymeric microtubules, as well as from different cytoskeleton extracts are phosphorylated on tyrosine residues. Using two different microtubule fractions, cold-sensitive and cold-stable, we established that dynamic cold-sensitive microtubules are more intensively phosphorylated on tyrosine residues than cold-stable ones. Our results suggest that tyrosine phosphorylation, a newly found posttranslational modification of plant tubulin, may play a determinant role in the dynamics of polymerization and depolymerization of microtubules, including their sensitivity to low temperature in plant cells."
(a) Institute of Food Biotechnology and Genomics, Kiev (b) Institute of Molecular Genetics, Prague (c) Department of Cell and Developmental Biology, John Innes Centre, Norwich

P19024 Mechanisms of cortical microtubule organization
Dixit, Ram-presenter ramdixit@wustl.edu(a) Can Eren, Ezgi (b) Gautam, Natarajan (b)
"The cortical microtubule cytoskeleton of plants is a highly dynamic and interconnected system whose organization regulates cell shape. This system consists of a large population of microtubule elements that are constantly turning over and that interact with each other to form ordered patterns with morphogenetic properties. These patterns, which are robust and responsive to environmental and developmental cues, arise due to self-organizing properties. Most of the current research in the field has focused on genetic and biochemical characterization of molecules that regulate this process, but little has been done to develop a quantitative framework for how the various molecular activities impact pattern formation. We are combining live-cell imaging and computer simulations to explore the factors responsible for robustness in this dynamic array. We are exploiting the wealth of mutants that affect cortical microtubule organization to test and validate our computer models. In addition, we have embarked on developing a cell-free reconstitution system using purified components to systematically build complexity and provide a testing arena for predictions derived from our models."
(a) Washington University (b) Texas A&M

SESSION P20 – DNA REPLICATION, RECOMBINATION & REPAIR

P20001 "Progeny of stressed plants exhibit dramatic changes in genome stability, methylation pattern, stress tolerance and metabolites profile"
Kovalchuk, Igor-presenter igor.kovalchuk@uleth.ca(a) Boyko, Alex (a) Kathiria, Palak (a) Yao, Youli (a) Blevins, Todd (b,b)
"The fact that plants are able to quickly adapt to stress may suggest the involvement of epigenetic mechanisms of inheritance. We hypothesized that epigenetic alterations are a general mechanism of plant adaptation to stress, and are the initial mechanism of permanent genomic changes leading to genome evolution. We used transgenic *Nicotiana tabacum* and *Arabidopsis thaliana* plants carrying luciferase-based substrate for the analysis of homologous recombination frequency (HRF) and methylation patterns. We exposed plants to abiotic (temperature, water, salt, UV) and biotic (viral and bacterial pathogens) stresses and analyzed changes in the progeny. We found the progeny of stressed plants to show changes in genome stability, reflected by higher level of HRF, as well as changes in global genome hypermethylation and loci-specific hypomethylation, as shown using MeDIP, cytosin extention and COBRA assays. Changes in HRF persisted when plants were propagated with stress and were less dramatic when plants were propagated without stress. Changes at the genome and epigenome were paralled by multiple changes in plant physiology, including higher tolerance to stress, changes in metabolic profile, in the level of phenolic compounds as well as the expression of DNA repair and stress tolerance genes. Since these experiments suggested possible role of epigenetic machinery in transgenerational changes, we performed the same experiments in *dcl2*, *dcl3* and *dcl4* mutants, and indeed found the mutants to be partially impaired in establishment of transgenerational changes, including the increase in HRF and stress tolerance. This work suggests that epigenetic mechanisms indeed may play essential role in plant adaptation."
(a) University of Lethbridge (b) Biology Department, Washington University

P20002 The NAC domain transcription factor Suppressor of Gamma Response 1 (Sog1) governs programmed response to DNA damage
Britt, Anne B.-presenter abbritt@ucdavis.edu(a) Yoshiyama, Kaoru (a) Furukawa, Tomoyuki (a) Conklin, Phillip (a) Curtis, Marc (b) Hays, John (b)
"All living things possess mechanisms to detect the presence of DNA damage and transduce that signal to induce a variety of responses. These responses include the activation of repair, the arrest of the cell cycle, and the induction of programmed cell death. In Arabidopsis the response to chromosome-breaking agents includes the robust upregulation of hundreds of genes, including many genes that are clearly involved in DNA repair. This is a specific response to double strand breaks: the spectrum of genes induced by gamma radiation does not include the genes known to be induced by a variety of other abiotic stressors, and the transcriptional response is entirely dependent on the PI3K-like protein kinase ATM, a protein known to be activated by double strand breaks. Such a response is unprecedented- mammals and yeast do not exhibit this robust and specific induction of repair-related genes in response to double strand breaks. As a result of a search for mutants defective in gamma-induced cell cycle arrest, a line carrying a 'gamma resistant' mutation, termed sog1, was identified. This mutation was mapped and cloned, and SOG1 was revealed to be a member of the large family of NAC domain transcription factors. Further analysis of sog1 revealed that this gene, like ATM, is required for the transcriptional response to gamma radiation. Here we will present evidence that SOG1 is also required for the programmed, tissue-specific cell death response to DNA damage observed in Arabidopsis. Thus SOG1, although unrelated to the mammalian transcription factor and tumor suppressor

TP53, evolved independently in multicellular plants to play the same essential role in governing DNA damage response. "
(a) University Of California (b) Oregon State University

P20003 Rapid repair of DNA double strand breaks in plants
Kozak, Jaroslav (a) Angelis, Karel J.-presenter angelis@ueb.cas.cz(a)
"By studying repair kinetics of DNA double strand breaks (DSB) in Arabidopsis NHEJ mutants *atlig4* and *atku80* we have identified a novel, rapid repair pathway. Half-life ($t_{1/2}$) of DSB survival in nuclear DNA of mutants is 5.7 and 5.6 min, respectively in comparison to 8 min in Arabidopsis wt. Rapid DSB repair pathway depends on structural maintenance of chromosomes protein 6 (MIM, $t_{1/2}$ = 57 min) and our results suggest involvement of LIG1 ($t_{1/2}$ = 13.5 min). Similarly as in Arabidopsis rapid DSB repair also exists in apical cells of moss *Physcomitrella patens*. Majority of induced DSB are rapidly removed not only in wt ($t_{1/2}$ = 5 min), but surprisingly also in *pprad51* mutated in both alleles of moss *RAD51* ($t_{1/2}$ = 10 min). These findings suggest that rapid repair of DSB is a common feature of plants. Acknowledgements: MESY Czech Republic projects 1M0505 and LC06004, EU project COMICS LSHB-CT-2006-037575. "
(a) Institute of Experimental Botany AS CR

P20004 NAP1 family proteins are involved in nucleotide excision repair in Arabidopsis thaliana
Dong, Aiwu-presenter aiwudong@fudan.edu.cn(a) Zhu, Yan (a) Liu , Ziqiang (a,b) Shen, Wen-Hui (b)
"Nucleosome Assembly Protein 1 (NAP1) is conserved in yeast, animals and plants, which facilitates the in vitro assembly of nucleosomes as a histone H2A-H2B chaperone. The Arabidopsis thaliana genome encodes four NAP1 and two NAP1-RELATED-PROTEIN (NRP) proteins, and our previous work demonstrated that AtNRP1 and AtNRP2 are required for maintaining postembryonic root growth. Now we find that AtNAP1;4 is weakly expressed in root segments and pollens, while the other three NAP1 genes are universally expressed. The Arabidopsis NAP1 family proteins are localized mainly in the cytoplasm and weakly in the nucleus, can bind histones H2A/H2B specifically and form homomeric and heteromeric protein complexes. But to our surprise, the simultaneous knockout of the three abundantly expressed NAP1 genes, AtNAP1;1, AtNAP1;2 and AtNAP1;3, did not affect the normal growth of the plants under our laboratory standard growth conditions. While the loss of NAP1 in Drosophila melanogaster leads to embryonic lethality or poorly viable adults, and the knockout of the mouse neuron-specific NAP1-homolog-2 gene is embryo lethal at the mid-gestation stage. In the triple mutants nap1;1-1 nap1;2-1nap1;3-1 and nap1;1-1 nap1;2-1nap1;3-2, the expressions of more than 400 genes were down-regulated, including some genes involved in DNA repair. The triple mutants exhibited a UV-C hepersensitivity as well as reduced efficiency of in vitro repair of UV-damaged DNA. The ChIP analysis showed that AtNAP1 proteins directly target to the promoter regions of several nucleotide excision repair (NER) pathway genes. We propose that NAP1 family proteins in Arabidopsis act as H2A/H2B chaperones and are involved in modulating DNA repair."
(a) Department of Biochemistry, Fudan University (b) Institut de Biologie Moleculaire des Plantes, Laboratoire Propre du CNRS

P20005 "Arabidopsis cohesion establishment factor, CTF7, is essential for embryo, but not endosperm development"
Makaroff, Christopher A.-presenter makaroca@muohio.edu(a) Jiang, Ling (a) Yuan, Li (a) Xia, Ming (a)
"Ctf7 is an essential gene in yeast that is required for the formation of sister chromatid cohesion. While recent studies on Ctf7 have provided some insights into how sister chromatid cohesion is established in yeast, how specifically CTF7 facilitates the formation of cohesion is unknown. Likewise, essentially nothing is known about how sister chromatid cohesion is established in plants. In this report we describe the isolation and characterization of Arabidopsis CTF7. Arabidopsis CTF7 is similar to Saccharomyces cerevisiae Ctf7 in that it lacks an N-terminal extension, exhibits acetyltransferase activity and can complement a yeast ctf7 temperature-sensitive mutation. CTF7 transcripts are found throughout the plant with the highest levels present in buds. Seeds containing a T-DNA insertion in CTF7 exhibit mitotic defects in the fertilized zygote. Interestingly, the endosperm developed relatively normally in ctf7 seeds, suggesting that CTF7 is not essential in mitosis for endosperm nuclei. Minor defects were observed in female gametophytes of CTF7+/- plants, while pollen development appeared relatively normal. Furthermore, plants that over-express CTF7 exhibit female gametophyte lethality. Therefore, while proper levels of CTF7 are important for female gametophyte and embryo development, CTF7 is not essential for the establishment of mitotic cohesion during microgametogenesis or during endosperm development."
(a) Miami University

P20006 Suppression of single strand-specific telomere binding protein NtGTBPs results in developmental abnormalities in tobacco
Lee, Yong Woo-presenter blackwand@hanmail.net(a) Kim, Woo Taek (a)
"Proper telomere maintenance is essential for vitality during cell division. Many telomere binding proteins are identified and characterized, but telomere function is still uncertain in higher plants. Here, we searched telomere single stranded binding proteins (TSSBP) in tobacco using sequence homology with human hnRNPa1 and tobacco NtGTBP1, both of which were known to be TSSBP, and identified highly homologous NtGTBP2 and NtGTBP3. Gel retardation assays indicated that all three NtGTBPs specifically bind to plant single stranded telomere sequence, TTTAGGG. CHIP assay also showed that NtGTBP1 is associated with telomeres in tobacco. To examine the in vivo functions, we established *NtGTBP1-RNAi* lines, which showed markedly decrease in its mRNA level. Pulsed-field gel electrophoresis showed that these RNAi plants exhibited significantly longer telomeres compared to wild type, indicating that telomere length inversely correlates with the amount of functional NtGTBP1. *NtGTBP1-RNAi* plants have smaller leaves, shorter stem, and earlier flowering phenotypes with frequent formation of abnormal anaphase bridges in pollen mother cells compared to the wild type plant. In these *NtGTBP1-RNAi* lines, increased t-circles, which abundantly exist in alternative lengthening of telomeres (ALT)-positive cancer cells, were detected by 2-dimensional gel electrophoresis. Since NtGTBP1 blocked strand invasion of single stranded telomere repeat to the double stranded repeat in vitro, telomere lengthening in *NtGTBP1-RNAi* plants may be due to the single stranded invasion mechanism-based ALT pathway. These results suggest that HnRNP homologs have critical roles in proper telomere maintenance in tobacco."
(a) Department of Biology, College of Life Science and Biotechnology, Yonsei University, Seoul 120-749, Korea.

SESSION P21 – DORMANCY

P21001 Differential expression of carbohydrate metabolism genes during bud dormancy changes in leafy spurge (*Euphorbia esula*)
Chao, Wun S.-presenter wun.chao@ars.usda.gov(a) Serpe, Marcelo (b)
"Underground adventitious buds of leafy spurge undergo three well-defined phases of dormancy, para-, endo-, and ecodormancy, throughout the year. In this study, relationships between carbohydrate metabolism and bud dormancy were examined and real-time PCR was used to determine if shifts in carbohydrate contents correlate with the expression levels of some carbohydrate metabolism genes. Our results indicated that many carbohydrate metabolism genes were differentially-regulated after paradormancy release and in response to seasonal signals. Among these genes, a specific β-amylase transcript increased 100-fold after growth induction and increased 16,000-fold from July to December. This β-amylase was represented by two genes, *Ee-BAM1* and *Ee-BAM2*. The deduced amino acid sequences of these two genes are very similar at the N-terminal end but

are disparate at the C-terminal. Both contain a nearly identical, predicted 48-amino acid plastid transit peptide. Only *Ee-BAM1*, not *Ee-BAM2*, can be amplified by PCR using gene-specific primers, indicating that *Ee-BAM2* is organ specific and/or not abundant. Immunoblot analyses identified a 29-kD and a 35-kD protein; the 29-kD protein could be the mature Ee-BAM1 where the transit peptide was cleaved. Unlike transcript expressions, both 35-kD and 29-kD proteins were constitutively expressed in growth-induced and seasonal samples. Immunolocalization indicated that Ee-BAM1 is in the cytosol of cells constituting leaf primordium and procambium at the tip of the bud. Ee-BAM1 also surrounds the amyloplasts in mature cells toward the base of the bud. These observations implicate that Ee-BAM1 may have dual functions; it serves as nutrient reserve in the cytosol and acts as degrading enzyme at the surface of amyloplasts. "
(a) USDA-Agricultural Research Service (b) Boise State University

P21002 Potato tubers treated with sprout inhibitors exhibit transcript profiles that have similarities to the dormant state.
Campbell, Michael A-presenter mac17@psu.edu(a) Suttle, Jeffrey C (b)
"The compounds chlorpropham (CIPC) and 1,4-dimethylnaphthalene (DMN) are used to suppress sprout growth and prolong the storage of potato tubers. However, the mechanism of action for these chemicals is poorly defined. Microarray analysis and quantitative real-time PCR were utilized to compare the transcript profiles of dormant, nondormant, and potato tubers treated with CIPC, and DMN. Natural progression from the dormant to the nondormant state demonstrated a decrease in ABA inducible transcripts particularly in the BURP class of proteins. Exposure to CIPC and DMN resulted in the expression of similar class of ABA inducible transcripts suggesting that sprout inhibitors may function via a prolongation of dormancy induced growth suppression. CIPC treatment resulted in decreased expression of transcripts associated with plastid development and metabolism. DMN resulted in an increase in expression of transcripts encoding for proteins involved with osmotic regulation, specifically osmotin and germin-like proteins. Levels for transcripts encoding for genes involved with cell division such as PCNA, KIP1, and KIP2, were measured using quantitative PCR. PCNA expression was linked to rapidly dividing meristems, while KIP1 and KIP2 expression was associated with dormant meristems or meristems treated with CIPC or DMN."
(a) School of Science, Penn State Erie (b) USDA ARS, Northern Crop Science Laboratory

SESSION P22 – ECOPHYSIOLOGY

P22001 Why does photosynthetic nitrogen use efficiency decrease with increasing leaf mass per unit area?
Evans, John R-presenter john.evans@anu.edu.au(a)
"The photosynthetic capacity of leaves is strongly related to their nitrogen content. Variation between species in this relationship is associated with variation in LMA: leaves with greater LMA tend to have lower photosynthetic nitrogen use efficiency (PNUE, photosynthetic capacity per unit nitrogen). Greater LMA is also associated with longer leaf lifespan. Persistence may come at a cost to photosynthetic rate by diverting nitrogen away from photosynthetic proteins and/or by restricting CO2 diffusion to Rubisco. Factors which have been put forward to explain variation in PNUE are: 1. lower Ci (stomatal conductance) 2. lower Cc (mesophyll conductance) 3. less Rubisco per unit leaf N 4. poorer Rubisco kinetic parameters 5. photosynthesis not measured under saturating irradiance. Data from multiple studies are compared to assess the relative importance of each of these factors."
(a) RSBS, Australian National University

P22002 Kudzu (*Pueraria lobata*) grown at suboptimal temperatures exhibits incomplete photosynthetic acclimation
Coiner, Heather A-presenter h.coiner@utoronto.ca(a) Sage, Rowan F (a)
"Global warming is resulting in poleward shifts in species distributions, and invasive species may be among the first to respond. Kudzu (Pueraria lobata) is an invasive vine in North America that reduces biodiversity by rapidly overtopping and killing vegetation by shading. Kudzu is moving northwards from the southeastern US, but the physiological mechanisms underlying this movement are poorly understood. One possible mechanism is that kudzu cannot fully acclimate photosynthesis to growth at suboptimal temperatures and that this depressed photosynthetic rate negatively impacts growth. A warming climate would remove this constraint. To test this hypothesis, we grew kudzu plants from a Mississippi and a New York population in cool (22/14^{o}C day/night) and warm (30/22^{o}C) treatments in growth cabinets. We measured photosynthetic leaf gas exchange to test for evidence of acclimation to cool growth temperatures. Photosynthetic rates at the daytime growth temperatures were depressed for cool-grown plants at the thermal optimum, but were similar at 10^{o}C. Sensitivity to a 90% reduction in O_2 concentration down to 10^{o}C ruled out triose-phosphate limitation at low temperatures. The temperature response of photosynthesis flattened out in cool-grown plants, which would be consistent with the hypothesis that Rubisco is limiting at cool growth temperatures. These results suggest that kudzu is unable to acclimate to suboptimal growth temperatures. This could act as a drag on photosynthetic capacity during cool parts of the growing season, thereby limiting its invasive potential until climates warm."
(a) Department of Ecology and Evolutionary Biology, University of Toronto

P22003 Seagrasses under stress: linkages with epiphytic biofilms and eutrophication ?
Cammarata, Kirk V-presenter kirk.cammarata@tamucc.edu(a) Sweatman, Jennifer (a) Chilton, Valerie K (a) Helander, Erik (a) Dovalina, Stephanie (a) Ufkes, Francis (a) Graham, Venis (a)
" Seagrasses are in decline globally. Epiphytes (bacteria, algae) are implicated in seagrass declines due to shading effects. However, biofilms present complex physical/chemical environments with spatiotemporal variation and biological interactions. Imaging and genomic methods are used to study linkages between nutrients, epiphytes and seagrass stress. A fluorescence-based imaging method quantifies photosynthetic epiphytes. High resolution images reveal accumulation patterns along the age gradient of the leaf. The method utility is extended with artificial substrates to image green algal/cyanobacterial epiphytes and capture recruitment temporally. Comparison of Halodule epiphytes from 2 Texas coastal bays showed 25-fold differences in epiphyte loading, and seagrasses at different depths at the same site revealed 5-fold more epiphytes and greater abundance of green algae on shallow seagrasses. The method facilitates micro-landscape analyses suitable for routine monitoring and data archiving. Linkage between the epiphyte landscape and environmental conditions is explored by DNA-based species characterization because diversity/ richness will be affected by biogeochemical conditions and climate change. Bacterial epiphyte populations on seagrasses from 2 Texas bays were compared by 16S rDNA profiling. Representative epiphyte species assemblages profiled by denaturing gradient gel electrophoresis (DGGE) suggest bacterial preferences for both seagrass host and location. Analysis of a bacterial clone library suggests an interface between aerobic and anaerobic conditions. An indirect pathway of eutrophication effects on seagrasses, mediated by epiphytic biofilms, is proposed. "
(a) TX A&M University-Corpus Christi

P22004 The serpentine syndrome: is calcium the only factor limiting growth?
Palm, Emily R-presenter eniniane@u.washington.edu(a) Van Volkenburgh, Elizabeth (a)
"Natural environments vary in their biotic and abiotic composition. Plants respond with phenotypic differences in morphology and physiology. In serpentine soils, with characteristically low calcium (Ca) to magnesium (Mg) ratio and level of productivity, is calcium the limiting factor for plant growth? Mimulus guttatus is the ideal study system to address this question, with locally adapted populations occurring both on and off serpentine soils. Cuttings are being grown hydroponically in aerated solutions mimicking normal (Ca:Mg of 4.0) and serpentine soils (Ca:Mg of 0.04). Simultaneous measures of gas exchange and fluorescence are performed, allowing for the isolation of potential impacts to supply (gas exchange) or demand (fluorescence) functions of photosynthesis. Effects of Ca and Mg on biochemical factors are assessed by measures of chlorophyll concentrations and Rubisco activity. Shoot and root biomass, growth rate and reproductive output data are collected over the course of exposure to defined growth solutions. Leaf expansion rates are measured as a proxy of the effects of Ca and Mg on potential photosynthetic surface area. Preliminary data suggests that growth, overall biomass and photosynthetic function remains consistent in adapted plants across a range of Ca:Mg values, while non-adapted plants exhibit reduced growth and photosystem efficiency in response to decreasing values of Ca:Mg. We intend to find that low Ca leads to a reduction in chlorophyll concentration, photosynthetic capacity and overall growth. This response to soil Ca:Mg ratios may suggest a possible mechanism for the inability of non-adapted plants to survive on serpentine soil and a potential explanation for the limited distribution of serpentine adapted plants on normal soil. "
(a) Department of Biology, University of Washington

P22005 Evidence of functional trade-off to drought in tree species of Chilean temperate rainforest.
Jimenez-Castillo, Mylthon-presenter mylthonjimenez@uach.cl(a) Rivera, Renato (a)
http://www.ecolevol.cl
"As consequence of global climate change, the rainfall have decreased significantly in south-central Chile in the last decades, and predictive models anticipate an increment of this tendency in the future. The goal of this study was to evaluate the hydraulic functioning of seven tree species under drought and wet conditions; in order to anticipate future hydraulic limitations in the forest as consequence of drought increasing. We measured transpiration (T), specific hydraulic conductivity (K_S), leaf hydraulic conductivity (K_L), and build vulnerability curves. Our results showed significant differences in transpiration rate and differential response to water availability among species. Several species showed more drought tolerance in the waterless area compared with wet area, but less hydraulic conductivity, evidencing a functional trade-off for water limitations. Because hydraulic efficiency is associated with competitive advantages, this result anticipate a possible differential response of species to predicted increment of water deficit and could be an important factor determinig competence and forest structure under future climatic scenarios."
(a) Universidad Austral de Chile

P22006 "Ecophysiological responses of *Abies sachalinensis* seedlings to two contrasting environments in a sub-boreal forest of Hokkaido, Japan."
Bontempo e Silva, Edgard A.-presenter silva@pop.lowtem.hokudai.ac.jp(a) Hara, Toshihiko (a) Sumida, Akihiro (a) Ono, Kiyomi (a) Kodama, Yuji (a) Nakai, Taro (a) Uemura, Shigeru (b)
http://www.lowtem.hokudai.ac.jp/plantecol/home-e.html
"In cold climate areas, canopy coverage can protect seedlings from environmental stresses, such as radiant frost and cold-induced photoinhibition. The comprehension of how plants respond to such pressures in the event of a canopy opening, or when colonizing an open area, is important to explain the present species distribution and to predict shifts in forest dynamics in response to climate change. The influences of environmental conditions on the photosynthesis of *Abies sachalinensis* (Sakhalin Fir) seedlings at two contrasting sites, under a deciduous canopy and in a wide canopy gap, were studied by making photosynthetic light curves, measuring chlorophyll fluorescence, growth, soil temperature, soil water content, carbon/nitrogen ratio, and gathering meteorological data. The measurements were made on September 2007, and on the period from May to October 2008, at a sub-boreal forest in the Hokkaido Island, Japan. Seedlings were photoinhibited (*sensu* Demmig, 1987) in both sites in May and June, but at the open canopy site the photoinhibited state lasted until late July. *Abies* growing at the closed canopy site showed stronger photoinhibition than those at the open canopy site, but recovered quickly after the canopy's new leaves flushed, reflecting its acclimation to the shaded condition. Although fluorescence values were positively correlated to photosynthetic rates, seedlings showed similar photosynthetic rates at both sites on spring and summer. This work's results corroborate with *Abies'* reported status as a shade-tolerant and late successional tree. This species' ecophysiological responses and acclimation capacity at seedling stage can help to explain its life history strategy and role in Hokkaido's sub-boreal forest dynamics. "
(a) The Institute of Low Temperature Science, Hokkaido University. (b) Field Science Center for Northern Biosphere, Hokkaido University.

P22007 The adaptive radiation of leaf venation in Hawaiian *Chamaesyce* (including disjunct veins)
Sporck, Margaret J.-presenter maggiesporck@gmail.com(a) Sack, Lawren (b)
http://www.botany.hawaii.edu/gradstudentpages/Maggie_Sporck.htm
"The diversity of leaf venation architecture within and across lineages is gaining increasing interest as a source of functional adaptation to contrasting environments. Hawaiian *Chamaesyce* (Euphorbiaceae) are a group of C_4 eudicots that radiated from one colonizing species into nearly 30 taxa. This group includes a variety of life forms, from creeping woody sub-shrubs to trees over six meters tall, with taxa adapted to diverse habitats, from rain forest to dry forest to coastal vegetation. The leaves of the taxa in this group vary strongly, 80-fold in leaf size and eight-fold in leaf mass per area. One study (Herbst, Science, 1971) pointed out a unique qualitative trait in this group-'disjunct minor veins,' unattached to the vein network and surrounded by mesophyll cells. However, no study has quantified venation architecture or its relationship to environment for this radiation. For 26 native *Chamaesyce* taxa, we cleared leaves chemically and quantified numerous traits relating to venation architecture, including densities of all vein orders (i.e., length/area), and of disjunct veins. We tested for correlation of taxon venation traits with climate and habitat and with other aspects of leaf structure and composition. We hypothesized that venation would vary with climate and habitat, with greater vein density supplying leaves of taxa at higher temperatures and greater exposure, and that disjunct vein formation would be associated with moist rainforest habitats, as these C_4 species might not suffer from the loss of vein length. We found that Hawaii's isolated location and strong climatic gradients have led to strong diversification in venation characteristics, and we explore possible hypotheses for the evolution and physiological significance of this diversity."
(a) University of Hawaii, Botany Department, 3190 Maile Way, Honolulu, Hawaii, 96822 (b) University of California, Los Angeles, Ecology and Evolutionary Biology, 621 Charles E. Young Drive, Los Angeles, CA, 90095

P22008 Structural versus functional leaf trait coordination in the adaptive radiation of Hawaiian violets
Sack, Lawren-presenter lawrensack@ucla.edu(a) Havran, J. Christopher (b) Nakahashi, Christopher D (c) McKown, Athena D (a) Ballard, Harvey E (d)
http://www.eeb.ucla.edu/Faculty/Sack/

"Plants function in given environments based on coordination and optimization of numerous leaf traits. We studied leaf trait integration for eight rare taxa of Hawaiian *Viola*, an adaptive radiation that includes herbs, shrubs and treelets, ranging from bog to forest to cliff habitats. We quantified leaf size and shape; composition; venation architecture; mesophyll, xylem and epidermal anatomy; hydraulic conductance; photosynthetic CO_2 response parameters, and drought tolerance traits. We found strong leaf diversification, and tested hypotheses for structural and functional leaf trait coordination. Structural or anatomical trait linkages held strongly across all eight taxa (e.g., correlation of xylem vessel, guard cell and epidermal cell sizes). *Functional* trait linkages typically held only among the five taxa of wet areas (3-4 m mean annual rainfall), with the three species of warmer, drier, and/or extremely wet sites breaking from these trends. For the five wet area taxa, we found strong linkage among carbon and water flux-related traits, including leaf hydraulic conductance, stomatal pore area, venation density and photosynthetic rate per area. We also found a coordination among traits relating to nutrient and carbon economics, and among traits related to drought tolerance. Our findings demonstrate the rapid evolution of trait coordination for taxa radiated within a narrow range of given environmental variables, and the decoupling of traits when species radiated outside of that range. Leaf trait relationships can also inform conservation, indicating the range of shared and specialized habitats important for preservation of the whole lineage."
(a) University of California Los Angeles, Department of Ecology and Evolutionary Biology (b) Campbell University, Department of Biology (c) University of Hawaii at Manoa, Department of Botany (d) Ohio University, Department of Environmental & Plant Biology

P22009 The determinants and significance of stomatal responses to vapor pressure deficit in native Hawaiian wet and dry forest species
Nakahashi, Christopher D-presenter cdn@hawaii.edu(a) Sack, Lawren (b)
http://www.botany.hawaii.edu/gradstudentpages/Chris_Nakahashi.htm
"Vapor pressure deficit (VPD) responses may be a fundamental determinant of species distribution and tolerance to drought. By hypothesis, stomatal responses of more drought tolerant species should have more responsive stomata to dry air. Using a gas exchange system we measured stomatal conductance at two different VPDs for 21 native Hawaiian wet and dry forest species grown in a common garden, and calculated indices of VPD response. We tested whether species with high maximum rates of gas exchange showed stronger VPD responses. We also aimed to test whether species with higher stomatal density and/or with smaller stomata showed stronger responses to vapor pressure deficit. We found a strong variation of VPD responses across species from wet and dry forests, and that dry forest species were significantly more responsive to VPD than wet forest species. We explore hypotheses for the determinants of stomatal vapor pressure deficit responses, their relationship to leaf anatomy, and their role in integrated leaf function and ecology. These data may also be useful for conservation and restoration projects given the need for ecophysiological information for plants of threatened dry and wet forests in Hawaii. "
(a) University of Hawaii at Manoa, Department of Botany (b) University of California Los Angeles, Department of Ecology and Evolutionary Biology

P22010 "Stoichiometry and physiological traits of mosses across an elevation and temperature gradient on Mauna Loa, Hawaii: testing predictions from global theory"
Waite, Mashuri-presenter mashuri@hawaii.edu(a) Sack, Lawren (b)
"Recently, general hypotheses have been framed relating nutrient stoichiometry to physiology for many organisms across resource gradients. However, these theories have not been tested on mosses despite their wide ecological ranges and importance in ecological fluxes. To test these theories, six moss species were sampled along an elevational gradient on Mauna Loa, Hawaii. N and P concentrations were expected to decline with increasing elevation and decreasing mean annual temperature (MAT), as shown previously for *Metrosideros polymorpha*, the dominant tree species across the gradient. Alternatively, mosses were hypothesized to follow global trends for vascular plants across latitudes and elevational gradients, where N is independent of MAT, but P increases, and N:P decreases, with decreasing MAT. Further, based on theory and results for vascular plants, in mosses less negative δ13C was expected with higher elevation, higher irradiance, and lower MAT. We found for the mosses that N was independent of elevation and MAT, consistent with the global trend for vascular plants. Further, consistent with these trends, P was positively correlated with elevation, and negatively correlated with MAT, while N:P showed the opposite trends. These results confirmed the global trends for vascular plants, and contrasted with the distinctive trends found for *M. polymorpha* at these sites. As predicted, for δ13C, the mosses showed less negative values at higher elevation and higher irradiance, consistent with local trends for *M. polymorpha* and global trends for vascular plants. These results indicate the potential for convergent physiological responses driving stoichiometric trends among mosses and vascular plants, operating across local as well as global resource gradients."
(a) Botany Department, University of Hawaii (b) Dept. of Ecology and Evolutionary Biology, University of California Los Angeles

P22011 "Reduced synthesis and accelerated degradation of ribulose-1,5-bisphosphate carboxylase/oxygenase is key to photosynthetic acclimation to elevate CO_2 in rice (*Oryza sativa* L. cv.Notohikari)"
Seneweera, Saman-presenter samans@unimelb.edu.au(a,a) Norton, Rob M (b,b) Suzuki, Yuji (b,b) Makino, Amane (b,b)
http://www.unimelb.edu.au/
"Initial stimulation of photosynthesis (*A*) at elevated $p(CO_2)$ often declines after prolonged exposure to elevated CO_2. This process known as *A* acclimation, is closely associated with a decline in leaf nitrogen (N) and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco, E.C.4.1.1.39) content. The suppression of Rubisco synthesis at elevated CO_2 is a major factor for *A* acclimation and is suggested to be mediated through soluble sugars. We tested the hypothesis that *A* acclimation to elevated CO_2 is regulated by factors other than the accumulation of carbohydrates using flag leaf blade of rice *Oryza sativa* L. cv.Notohikari). Plants were hydroponically grown in artificially illuminated growth chambers at either 39 or 100Pa of $p(CO_2)$. Gas exchange measurements, the amount of Rubisco synthesized and degraded, the levels of rbcL and rbcS mRNAs, sucrose content, nitrogen (N) influx and efflux were measured. Growth at 100 Pa of $p(CO_2)$ suppressed light saturated *A*, Rubisco and leaf N content in all development stages of the flag leaf blade. The *A* rate declined at elevated CO_2 during leaf expansion up until leaf to senescence and the decline correlated well with leaf Rubisco content. In addition, Rubisco synthesis decreased, while Rubisco degradation was accelerated at elevated CO_2. No relationship was found between *A* and sugar content. However, the amount of Rubisco synthesized was well correlated with the N influx in to the leaf blade. Hence, these results suggest that a decrease in N influx in to the leaf blade is a key to reduction in Rubisco synthesis and *A* acclimation to elevated CO_2. Accelerated degradation of Rubisco also contributed to the *A* acclimation to elevated CO_2. "
(a) Melbourne University, Private Box 260, Horsham, VIC 3401, Australia (b) Tohoku University, 1-1 Tsutsumidori-Amamiyamachi, Aoba-ku, Sendai, 981-8555 Japan

SESSION P23 – EDUCATION & OUTREACH

P23001 With Plants Darwin Was Wrong and Darwin Was Right
Al-Jamali, Abbas F.-presenter jamali@just.edu.jo(a)
"**Darwin was wrong; Darwin was right. Both hold true with plants**. He was **wrong** in saying that grape(*Vitis vinifera*) tendrils are used **EXCLUSIVELY** for climbing. Grape tendrils can be a potentially formidable, **versatile wrestling tool** in the grapes struggle for light as evidenced by the pictures which will be presented. The red bract around the growing point of a rubber plant (*Ficus elastica*) is being bound shut and pulled aside resulting in more essential light reaching the grape vine! i.e. tendrils are **not** exclusively for climbing. Darwin was **right** when he predicted the further spread of cardoon (*Cynara cardunculus*), a plant the tender midribs of whose leaves is the pride of the cuisine of its Mediterranean origin, farther than Argentina where Darwin saw it covering vast areas. Cardoon is now considered a noxious weed in California! He was very conscious of the devastating results on native populations, human, animal and plant, of more competitive invading species such as the successful phytocolonialist, cardoon. Pictures of Three of the weapons which make cardoon a formidable phyto-foe: self mulching, storage crown,and hypersensitve reaction will be presented. "
(a) Jordan University of Science & Technology

P23002 Undergraduate-level Inquiry: Benefits and challenges of engaging in classroom-based research
Dolan, Erin L-presenter edolan@vt.edu(a) Alkaher, Iris (a) Hlousek-Radojcic, Alenka (b)
http://www.prep.biotech.vt.edu
"Partnership for Research and Education in Plants for Undergraduates (PREP-U) engages students in research in the contexts of their courses. They address the unanswered question of how disabling genes in *Arabidopsis thaliana* influences the plants' interactions with two herbivores, two-spotted spider mites (*Tetranychus urticae*) and root-knot nematodes (*Meloidogyne incognita*). Students design and conduct their own investigations to determine differences in herbivory of wild-type versus mutant plants and then report their findings to scientists interested in the genes being studied. Here we focus on students' perceptions of the benefits and challenges of participating in classroom-based research and its relevance for teaching and learning. PREP-U was implemented in one plant physiology and four introductory biology courses in fall 2008. Twenty-five sets of pre/post interviews were conducted with participating students. Content analysis was done to identify emergent categories and major themes. Students reported a wide range of benefits and challenges and perceived that some aspects were both benefits and challenges. For example, students indicated the importance of independent work, but also asked for more guidance and support. Students valued the opportunity to understand how science is done, but noted their struggles to develop rationales and explain results. Our results point to the complexity of inquiry teaching in undergraduate classrooms. Instructors must achieve a balance between preparing students to conduct investigations and allowing them freedom to make progress independently. We believe that the extent of instructors' involvement, especially how they gauge students' needs to inform their instructional decisions, influences students' perceptions of research."
(a) Virginia Tech (b) Richard Bland College

P23003 Bring Your Own Cassava: Understanding the diversity of cassava in Puerto Rico by providing research-based laboratory experiences to minority undergraduate students
Siritunga, Dimuth S.-presenter siritunga@uprm.edu(a) Montero, Milly (a) Seda, Jean (a) Rodriguez, Lorraine (a) Franklin, Carrero-Martinez (a)
"Lab modules, broadly entitled Bring Your Own Cassava (BYOC), are being implemented in both undergraduate Genetics course and Cell Physiology course at the University of Puerto Rico. Our project has an expected impact of approx. 700 students per year. Cassava, a crop of immense importance to humans living in the poorest places in Africa, South America, Central America and Asia, is widely consumed by Puerto Ricans though not as a staple food. Despite this wide consumption, the genetic diversity of cassava in the Caribbean is poorly understood. In order to assess the genetic diversity of cassava in Puerto Rico (PR), the BYOC modules request the undergraduate students to bring their own cassava leaves from their respective townships. Students enrolled in the Genetics lab course apply modern molecular biology techniques to evaluate the genetic make up of their own samples. The students extract DNA from their unknown samples followed by PCR amplification for microsatellite markers and agarose gel electrophoresis. The poly-acrelamide gel electrophoresis and marker assessment is performed by senior personnel. The pooled data from all sections is discussed with the students educating them on genetic diversity assessment related to conservation. The BYOC module for the Cell Physiology lab course similarly permits the students to evaluate the accessions of cassava from the PR germplasm using a combination of cellular and biochemical techniques. Students characterize the cellular structure of root and leaf cells of different accessions as well as unknown samples previously characterized in the BYOC genetics. In-depth assessment of content learning and perception is being conducted both pre- and post-course. The development of these lab modules is being supported by the CCLI-NSF."
(a) Department of Biology, University of Puerto Rico Mayaguez, Mayaguez, PR

P23004 Advances in Biosciences Education for Community Colleges: The journey from summer workshop to year-round independent research project
Neupane, Kabi R-presenter kabi@hawaii.edu(a) Lum, Jamie M (a,b) Messa-Oh, Christine (a,b) Perez, Pierriden A (b) Christopher, David A (b)
"The Advances in Biosciences Education (ABE) workshop was formed to maximize broader impacts under a NSF-sponsored research grant entitled 'Functional Genomics of the Protein Disulfide isomerase Family in Arabidopsis Plants.' Five faculty and 24 students from four community colleges lacking research programs received hands-on training with experimental materials and techniques employed in the ongoing research grant. The three-week long workshop held each summer for four years provided basic training in recombinant DNA, molecular biology, genomics, bioinformatics and state-of-the art fluorescence and electron microscopy by using *Arabidopsis* as the experimental material. By pairing students with their teachers, the workshop format fostered collaborative problem solving, helping students prepare for graduate-level research or the job market, while giving teachers an opportunity to hone their professional skills and develop new educational resources. As an outcome of the workshop, two undergraduate students and a community college faculty mentor pursued an independent project during the academic year on an economically important tropical ornamental plant (*Anthurium adreanum*) in Hawaii. They utilized their knowledge gained from the workshop to characterize and identify >600 genes from senescing tissues of Anthurium. Several senescence-related genes were present in transcriptome, which are currently being assembled for publication and for use for undergraduate education and research."
(a) Leeward Community College (b) University of Hawaii

P23005 Development and assessment of didactic packages including DVDs on plant biology experiments for rural schools in Mexico
Reynaga-Pena, Cristina G.-presenter creynaga@ira.cinvestav.mx(a) Valderrama-Chairez, Maria L. (b) Tiessen, Axel (a)

http://www.sientelaciencia.com
"An important number of public elementary schools in Mexico are schools in rural areas, where didactic resources are very limited. This work intends to facilitate the first scientific experience of children in those schools to diverse topics such as plant and cell biology among other. Currently, there is a great need for the development of didactic materials for teaching science to vulnerable populations in Mexico such as children in rural areas. In the context of our present education and outreach activities we have implemented a 3-day workshop for children of rural areas, where we include a section on Plant Biology. Most experiments were chosen by the precondition of needing only easily available materials. We have included experiments on plant cells, photosynthesis, metabolism, tropisms, DNA extraction, plant pigments and pH determination. From our experience, the presentation of experiments by a competent scientist usually gives the student a different perspective, encouraging them to continue doing experiments in the long term. So, to multiply and expand the experience, we are videotaping and editing a series of DVDs that will be distributed among all possible schools to help the rural school teacher. Currently, we are about to deliver our first DVD in 1500 schools of the State of Guanajuato, Mexico. The impact on the children's attitude to science as well as the utility for the teacher as a didactic resource will be evaluated."
(a) Centro de Investigacion y de Estudios Avanzados del IPN Unidad Irapuato (b) CUCBA, Universidad de Guadalajara

P23006 Partnership for research and education in plants for undergraduates
Hlousek-Radojcic, Alenka-presenter alenkahr@rbc.edu(a) Lewis, Ed (b) Dolan, Erin L (c)
"The Partnership for Research and Education in Plants for Undergraduates (PREP-U) engages students in classroom-based research to address the unanswered question of how disabling genes in Arabidopsis thaliana influences the plants interactions with two herbivores, two-spotted spider mites (Tetranychus urticae) and root-knot nematodes (Meloidogyne incognita). Students design and conduct investigations to determine the effects of herbivory on wild-type versus mutant plants and then report their findings to scientists interested in the genes being studied. Thus, PREP-U aims to establish research collaborations that bring together education and research communities while contributing to the body of knowledge about gene function in Arabidopsis, especially related to plant-herbivore interactions. PREP-U instructional resources include introductions to the organisms and experimental/analytic tools, research literature that provides context, rationale for and implications of the students experiments, student guidelines for preparing and revising research proposals, progress and final reports, and grading rubrics. PREP-U has been pilot tested in general biology laboratory courses, plant biology / physiology lecture courses, and research-based behavioral ecology lab courses. Materials are being revised based on student work and instructor reflections as well as pre/post-testing that examine changes in students knowledge. We seek additional collaborators interested in becoming part of a larger study to determine how research experiences in classroom settings prepare undergraduates to reap the benefits of research internships. "
(a) Richard Bland College of the College of William and Mary (b) UC Davis (c) Virginia Tech

P23007 Carbohydrate oxidase identification and characterization as an undergraduate research project
Maki, Jennifer A-presenter jmaki@css.edu(a) Lindgren, Khrystyne (a)
"Engaging undergraduates in research broadens their potential career opportunities in the sciences. The College of St. Scholastica is a small, private liberal arts college with the main focus on teaching and somewhat limited financial and physical research infrastructure. Our undergraduates are very pre-professionally and medically minded, to the extent that they are often unaware of what graduate school entails. My laboratory provides a unique experience for the brightest and most curious students, some of whom discover that graduate school is a better fit for them than medical or pharmacy school. In the lab they learn experimental design including appropriate controls, endure the frustration of failed experiments, and relish the exhilaration of success. The project we are working on incorporates a number of biochemistry and molecular biology techniques and is relatively inexpensive. Carbohydrate oxidases, enzymes involved in plant defense via production of hydrogen peroxide, were first reported in lettuce, sunflower and tobacco. We have identified carbohydrate oxidase activity in multiple plant families, including geranium, jade, and rabbits foot fern utilizing a native in-gel colorimetric assay. Further, we have developed a purification scheme that involves homogenization, precipitation, and size exclusion chromatography followed by identification of activity by a horseradish peroxidase-linked assay. Optimization of this process will be discussed, along with future plans to sequence and clone the carbohydrate oxidase genes of interest into yeast. This will allow for heterologous expression and characterization of the enzymes via kinetics, fluorometry and DNA/protein sequence analysis. "
(a) The College of St. Scholastica

P23008 Teaching plant biology through research: The Freshman Research Initiative at The University of Texas at Austin
Clark, Gregory Bland-presenter gbclark@uts.cc.utexas.edu(a) Roux, Stanley J (a) Shear, Ruth I (a) Simmons, Sarah L (a)
"At large research institutions, undergraduate students typically first encounter faculty research as upper-division students. We have begun a large-scale reinvention of our undergraduate research paradigm. Our new model, the Freshman Research Initiative (FRI), involves large numbers of freshmen, and is integrated with the curriculum. Each year, freshmen are recruited into an eighteen-month set of courses that promote critical thinking, interaction with faculty, hands-on experimentation, data interpretation, student presentation, publication, and peer mentoring. One of the 20 different research areas offered to students in the FRI program is the Plant Biology Discovery Lab. The objectives of this lab are for freshman students: to carry out novel experiments and discover new findings on a question of significant current interest in plant biology; to learn methods of experimental design, data gathering, data interpretation, and data presentation; to develop skills of devising alternative hypotheses to interpret experimental results; to learn, through experimentation and data interpretation, basic principles of stimulus-response coupling in plants. Our class is addressing the question of what signaling steps mediate the effects of extracellular ATP and ADP (eATP and eADP) on the polarized growth of single-celled root hairs. The experiments constitute a novel test of a relatively new hypothesis which predicts that eATP and eADP can influence plant growth and development by functioning like hormones, as they do in animal cells. We will present details about the impact of the FRI program on the academic performance of participating students and results from the research performed by students in our class addressing the role of eATP/eADP in Arabidopsis root hair growth."
(a) University Of Texas

P23009 Identification of Expressed Sequences in Fruit Transcriptome of Breadfruit (*Artocarpus altilis*)
Dang, Nong C-presenter nong@hawaii.edu(a) Chen, Nancy J (b) Paull, Robert E (b) Neupane, Kabi R (a)
"More than 100 expressed sequence tags (ESTs) were isolated from ripening breadfruit, *Artocarpus altilis*. The randomly selected cDNA clones were partially sequenced and their putative gene function identified by comparison with the GeneBank database. The BLAST search showed that 86% genes in the breadfruit cDNA library had matching sequences in the GeneBank whereas 14% clones had no homology. Putative protein function could be attributed to 38% of the clones that included glycoside hydrolases, peptidases, S-Adenosyl Memthionine (SAM) synthase, different transcription factors and translation elongation factors. Additional clones are currently being isolated and sequenced for potential molecular markers as well as genes involved in fruit ripening."
(a) Leeward Community College (b) University of Hawaii at Manoa

P23010 A one semester carbonic anhydrase-based biotechnology project in the advanced undergraduate plant science classroom.
Wagner, Ryan L.-presenter Ryan.Wagner@millersville.edu(a)
"Stimulating interest in the plant sciences at a small liberal arts PUI (primarily undergraduate institution) can present a formidable challenge requiring the development of course curricula that transcend the standard expectations. To meet this demand for innovative and exciting content in advanced plant science courses, a one semester (15 week) biotechnology project has been developed in which the student generates and analyzes transgenic plants expressing a foreign gene. A chimeric periplastic carbonic anhydrase (*AtlCah1*) gene was developed by fusing an *Arabidopsis thaliana* leader sequence to the *Chlamydomonas reinhardtii Cah1* gene. Using standard molecular techniques, the GOI (gene of interest) is analyzed, isolated, and inserted into the binary vector *pCB302-3* (Xiang et. al., 1999). *Agrobacterium tumefaciens* is then transformed with the *pCB302-AtlCah1* construct and used in conjunction with the Floral Dip method (Clough and Bent, 1998) to transfect *Arabidopsis*. Seeds are collected and germinated and then transgenic plants are selected based on herbicide resistance conferred by the presence of the bar gene. Western blot analysis is performed to confirm the presence of the protein product. Upon completion of the biotechnology project, the student will have taken the gene of interest (GOI) from conceptual sequence analysis to recombinant protein expression and analysis in transgenic *Arabidopsis*. Using the data gathered through the semester, the students complete the project by writing a primary literature style paper. To date, this biotechnology project has been successfully integrated into our Plant Biochemistry course during the last three offerings (Spring 2007, 2008, 2009) and continues to be enhanced with new input from the students."
(a) Millersville University of Pennsylvania

P23011 Politics and Agricultural Biotechnology in Hawaii
Miyasaka, Susan C-presenter miyasaka@hawaii.edu(a) Shintaku, Michael (b) Patino, Mario (c)
"To ensure sustainability of agriculture in Hawaii, research is needed to improve resistance to pests and diseases using biotechnology. However, recent legislation in Hawaii has been passed to restrict research on genetic engineering of certain crops. In November 2008, the Hawaii County Council passed Bill 361 that makes it unlawful for any person to test, propagate, cultivate, raise, plant, grow, introduce or release genetically engineered (transgenic) or recombinant DNA taro (kalo) or recombinant DNA coffee. The purpose of the bill was to protect the taro (kalo) and coffee industry from genetic engineering and preserve agriculturally-based practices and cultural traditions associated with taro (kalo) and coffee within the County of Hawaii. The language of the bill is very broad and could be interpreted to prohibit molecular biological techniques for purposes other than genetic engineering. Many oral and written testimonies presented before the Hawaii County Council were emotional and without scientific basis. Clearly, the current controversy provides a teachable moment when there is both interest and need to educate the public about genetic engineering. To address this problem through education, a place-based based course on Agricultural Biotechnology will be offered for middle school and high school science and agriculture teachers during June 2009, using the context of genetic engineering of papayas (*Carica papaya*) for resistance to Papaya Ringspot virus. This course is being taught by instructors from the University of Hawaii Manoa, University of Hawaii Hilo, and Kamehameha Schools. Information gained from the legislative experience and from teaching this class will be presented."
(a) University of Hawaii - Manoa (b) University of Hawaii - Hilo (c) Kamehameha Schools

P23012 Implementation of a 'Darwin Project' in an introductory biology course
Peng, Hui-Mei-presenter mei2468@isu.edu.tw(a)
"Using textbooks as a mean of teaching is a common practice in many college introductory courses. Students know and remember facts are doing well in the paper and pen exams. This simple method of teaching is easy. However, it fails to nurture students' passion to science and miss the opportunity to develop students' ability of critical thinking. To fulfill the purposes of science education, we have introduced a 'Darwin Project' in the General Biology course. On the one hand, this project is to celebrate the 200th anniversary of Charles Darwin's birth. On the other hand, the inquiry-based project could engage students in active learning. After observing and recording the interactions between plants and environment, students have learned to collect relevant information and read scientific papers. Because of special geographic conditions, Taiwan has tropical, subtropical, temperate, coniferous, and even tundra flora. The frequent earthquakes and typhoons also aid to shape the island's ecosystems. In the classroom, we have in-depth discussion on how Darwin's natural selection theory might have played on island's biodiversity and in the speciation of endemic species under the unique conditions in Taiwan."
(a) I-Shou University

P23013 Grounding in botany
Kirouac, Martha-presenter mkirouac@huntington.org(a) Kerkman, Mike (a)
http://huntington.org
"Imagine your wish list for a high school science lesson: standards-aligned; inexpensive; engaging; inquiry-based. At the Huntington Botanical Gardens, we show teachers how to make these wishes come true by teaching with plants. As part of the Huntington's mission to promote botanical science, we have developed a professional development program for classroom biology teachers. Every year since 2004, 20 high school teachers are invited to participate in the *Grounding in Botany* (GIB) program. The goals of the year-long program, which is funded through the National Science Foundation and the Arthur Vining Davis Foundations in partnership with the California Institute of Technology and University of California, are to:

- enhance teachers' knowledge of plant science,
- refine methods of teaching using inquiry-based lab experiences,
- foster the use of plants as model systems in the classroom,
- support implementation of the California State Science Content Standards,
- make intercurricular connections, particularly among science, math, and technology, and
- provide a connection between high school science classrooms and current scientific research.

GIB meets these goals through a combination of lectures, laboratories, group activities, debates, demonstrations, problem solving, hands-on experiments, and the development of inquiry-based instructional materials, on topics ranging from scientific logic, cell biology, and genetics to ecology and evolution. Participants begin the program with an intensive four-week summer institute and leave armed with 32 hands-on labs and the confidence to teach them. Not only do participants gain more content knowledge in botany, but evaluation has revealed that they also gain the skills to translate that knowledge to their students in meaningful ways. "
(a) The Huntington Library, Art Collections and Botanical Gardens

P23014 "Integrating biology and chemistry, basic and applied plant biology in undergraduate research"
Blauth, James-presenter james_blauth@redlands.edu(a) Schrum, David (b)
"The Merck-AAAS Undergraduate Science Research Program supports interdisciplinary (biology & chemistry) research at primarily undergraduate institutions. A team of biologists and chemists at the University of Redlands was awarded a grant in 2003. The plan for our (Blauth and Schrum) project was to explore restoration of disturbed desert habitats using local woody legumes (Fabaceae) partnered with local rhizobia and mycorrhizas (mutualistic soil microbes). Project phases were: identify disturbed site to revegetate and reference site for comparison; compare woody plant communities, soil physicochemical properties, rhizobia and mycorrhizas across the sites; determine which factors may be limiting for revegetation; test plant-soil-microbe combinations to see if nutrient availability in disturbed site is adequate; collect local seeds and soil, grow microbe-colonized seedlings, and transplant into disturbed site; monitor transplants and woody recruits, soil microbes, soil properties, and animal community to assess this approach to ecological restoration. Students participated through research courses and an in-house summer research program. Student work included field ecology, microbe characterization and ID, chemical analysis of soil samples and plant tissues, and growing rhizobia and seedlings. Students experienced a mix of plant- and microbiology and analytical chemistry techniques as well as basic and applied aspects of the project. Outcomes included many student participants, posters at undergraduate and disciplinary conferences, students going on to graduate programs, and transplanting and initiating monitoring in our disturbed site. Considerations for funding, initiating, and conducting interdisciplinary research projects with undergraduates will be discussed."
(a) University of Redlands, Biology (b) University of Redlands, Chemistry

P23015 An adaptable undergraduate molecular biology lab module that integrates use of genomic resources with bench experiments to pursue original research questions
Vernon, Daniel M.-presenter vernondm@whitman.edu(a) Shafer, Michelle (a) Forsthoefel, Nancy R (a)
"We have designed an undergraduate molecular biology lab module that integrates use of computer-based genomic resources with bench experiments and facilitates integration of teaching and research. Our goal was to develop a ~5 week project focused on eukaryotic gene structure and expression that: 1) involves original experiments; 2) provides training in core molecular techniques; 3) promotes an integrative biology perspective; 4) exposes undergraduates to plants and plant genomics; and 5) promotes writing skills. Importantly, the project framework had to be flexible enough to allow original experiments each year, but consistent enough to minimize lab preparation time and effort. Provided with a fragment of *Arabidopsis* gene sequence, students identified the gene using BLAST searches, and used TAIR resources to define gene and mRNA structures. They then searched for information on gene function, formulated a hypothesis about differential expression in developmental, environmental, or physiological contexts, and designed PCR primers to test their hypothesis. In subsequent weeks, students tested primers on genomic DNA, isolated RNA, and performed RT-PCR. Alternatively, students used other plant genomic resources to identify orthologs of their *Arabidopsis* gene in another species, and used that species for their PCR experiments. Results were formally written up in manuscript style. Evaluations indicated that despite technical difficulties, students appreciated doing real experiments in a teaching laboratory. By incorporating genes related to their own research interests, faculty can use this module to integrate their research with undergraduate instruction. Project information will be made available at http://people.whitman.edu/~vernondm/ *Supported by NSF grant 0616166 to DMV*"
(a) Whitman College

P23016 SYMBIOSIS: Teaching Biology and Mathematics as an Integrated Course for Undergraduate Students
Kumar, Dhirendra-presenter kumard@etsu.edu(a) Joplin, Karl H. (a) Govett, Aimee L. (c) Miller, Hugh A. (a) Moore, Darrel J. (a) Yampolsky, Lev (a) Jones, Thomas C. (a) Knisley, Jeff R. (b) Seier, Edith (b) Helfgott, Michel (b) Gardner, Robert B. (b)
"Traditionally biology and mathematics courses are stand alone courses. There are sometimes minor applications of mathematics in some of the upper division biology courses. At the Department of Biological Sciences, East Tennessee State University, biology for majors is taught in 3 semester introductory courses with associated labs which provide students with hands on experience. Besides, these students also have a mathematics requirement of Probability & Statistics, Calculus I and Calculus II courses. Most of the students who take these courses are biology majors, pre-med, pre-pharmacy and nursing students. Usually the biology students tend to shy away from learning mathematics and mathematics students tend to avoid learning biology. This leaves the biology students lacking in mathematics skills which has become increasingly apparent with the recent developments in molecular biology and biochemistry. Large numbers of datasets generated from recent developments in genomics, proteomics, interactomes, microarray analysis etc are now available for analysis by the students. Most students find it difficult to handle these biological datasets. Most mathematics courses are not directed to solve biological problems. To overcome this deficiency in the students we at the Department of Biological Sciences and the Department of Mathematics at ETSU have developed a novel course and curriculum (SYMBIOSIS) to teach an integrated course of biology and mathematics to the students at the freshman level. SYMBIOSIS has received funding from Howard Hughes for the development and teaching of the course. This course is currently being taught to two cohort of students. "
(a) Department of Biological Sciences, East Tennessee State University (b) Department of Mathematics, East Tennessee State University (c) ETSU Center for Mathematics and Science Education

P23017 Peer-tutors as integrators across introductory science courses
Shinkle, James R. (a) Brodl, Mark-presenter MARK.BRODL@trinity.edu(a)
"As a part of a broader curriculum integration program, science peer-tutors (PTs) assist in lecture sections of introductory science and mathematics courses supporting the 'biomedical' curriculum. Initial funding was through a grant from the Howard Hughes Medical Institute (HHMI), with the university taking over support this year. PTs have worked with students in first year biology, first and second year chemistry, calculus-I, introductory (not always taken first year) neuroscience, physics and psychology courses and an upper level mathematical modeling course for both Biology and Mathematics students. The PTs have all completed the course they tutor for and usually a number of other science courses in multiple departments. They attend lectures for their assigned course, hold office hours, and provide supplementary problems, review sessions and other enrichments. PTs also use course Moodle sites to provide supporting information. Grading done by PTs is limited to small assignments and types of student work that help PTs understand where their class is having difficulties. In addition to facilitating learning in their assigned course, PTs use their experience with other science courses to help their students appreciate the importance of current material in relation to the overall experience of the science curriculum. Because the PTs have had courses across this curriculum they also help faculty to take better advantage of synergies between courses. The notes taken by the PTs are archived and serve as a resource for tracking curricular developments and hence are an element of program assessment. This presentation will review our experience over the last four years, including assessment data from student and peer-tutor questionnaires and interviews. Sponsored by HHMI."
(a) Trinity University

P23018 Differential gene regulation in japonica and indica rice and their F1 hybrids- a course in applied plant molecular biology
Terzaghi, William B. (a) Shah, Jay-presenter jay.shah@wilkes.edu(a) Grow, Casey (a) Kocher, Neil (a) Papayannakos, Christopher

(a) Deng, Xing Wang (b)
"Hybrid vigor is a well-know phenomenon that is essential for agriculture, yet is poorly understood. The recent publication of the complete genomic sequences of the indica and japonica subspecies of rice enables the study of hybrid vigor at the molecular level. We therefore used an experimental investigation of hybrid vigor as the basis for a course. We used bioinformatics tools to identify 5 genes which may be involved in hybrid vigor whose mRNA sequences differ by small indels between indica and japonica. All five genes, phytoene synthase (Os06g51290), malic enzyme (Os01g52500), alternative oxidase (Os02g21300), phosphoglucomutase (Os03g50480) and S-adenosyl methionine synthetase (Os01g22010) are expressed in both indica and japonica according to MPSS and microarray data. We designed primer sets flanking these indels allowing us to identify which parental alleles are expressed in F1 hybrids. We used these primers to clone the genomic sequences for these regions into pBlueScript, and verified the inserts by sequencing. RT-PCR analysis of RNA from 10 day old indica and japonica seedlings, and their reciprocal F1 hybrids shows differential expression of Os01g22010, Os01g52500 and Os02g21300 according to the maternal parent. This analysis will provide further insight into the molecular basis of hybrid vigor."
(a) Wilkes University (b) Yale University

P23019 Spectral quality and template suitability of onion DNA prepared by the spindling technique
Schmid, Katherine M-presenter kschmid@butler.edu(a) Hoops, Geoffrey C (b)
"DNA collected by spindling following alcoholic precipitation, although a graphic demonstration of the genetic material, has limitations as an experimental material. The high pectin that contributes to the visible 'DNA' spindle obtained from tissues such as onion and soft fruits also can inhibit enzyme activity and contribute to erratic spectral quality. Addition of calcium salts to the extraction buffer promotes production of a firm debris pellet in a low speed table-top centrifuge, leaving a supernatant giving an obvious A_{260} peak. Off-scale short wavelength absorbance remaining after elimination of light scattering due to insoluble contaminants has been traced to EDTA, and can be adjusted by titration with calcium. However, effective PCR of promoter and coding sequence segments of anthocyanidin synthase genes is possible after high speed microcentrifugation to remove residual debris from DNA resuspended in 10 mM Tris 1 mM EDTA pH 8.0 following alcoholic spindling from a classical saline citrate EDTA buffer."
(a) Butler University, Dept. of Biological Sciences (b) Butler University, Dept. of Chemistry

P23020 Undergraduate Research as a Tool to Channel Hispanic Students to Science Career
Louzada, Eliezer S-presenter elouzada@ag.tamu.edu(a) Hilda , Del Rio Sonia (a) Persans, Michael M (b)
"The objective of this project was to provide hands-on research experiences for undergraduate students from the highly Hispanic community of the Lower Rio Grande Valley in Texas. The Hispanic community is known by the lower education attainment, compared to the white non-Hispanics, which induces a low household income in communities highly populated by this minority group. The participation of Hispanics in science careers is also very low nationally. Considering that the Hispanics are projected to comprise 25% of the USA population by 2030, investment needs to be made to attract new scientists from this ethnical group. To address this issue at the local level, Texas A&M University-Kingsville Citrus Center (TAMUK-CC) initiated an undergraduate research internship in agricultural biotechnology with the objective of attracting undergraduate students from the University of Texas at Brownsville and the University of Texas Pan-American to graduate studies at TAMUK-CC. We provided over 60 research internship and so far more than 50% were channeled to graduate school including 11 at the doctoral level, additionally, many students are currently science teachers at local high schools. The project started in 2001 and has been funded by the Hispanic Serving Institutions Education Grants Program-USDA. Additional information will be provided. "
(a) Texas A&M University Kingsville (b) The University of Texas Pan-American

P23021 ChloroFilms: Plant Biology Videos Powered by YouTube
Cosgrove, Daniel-presenter dcosgrove@psu.edu(a)
http://chlorofilms.org/
"**ChloroFilms.org** is an educational and outreach resource promoting greater appreciation and understanding of plant life, specifically through the production of informative, creative and entertaining videos posted on **YouTube**. In the spring of 2009 Chlorofilms organized its first video contest with cash prizes; over 60 video 'shorts' were entered in the contest. Our judges agreed - some were amazing and some were beyond painful. Check out the web site to view the winning videos - they're great for classroom use or as a warm-up before lecture or seminar. Or come by the exhibit to enjoy some popcorn and showings of the winning vids. We will prepare a pamphlet with tips about using 'YouTube', 'Animoto.com' or 'Animate.com' videos in class, as well as pointers and resources for making a winning video for the next contest, planned for December 2009. "
(a) Penn State University

P23022 Thinking in numbers: Infusing quantitative reasoning into biology education
Shiu, Shin-Han-presenter shius@msu.edu(a) Ebert-May, Diane (a)
"The rapid influx of biological data such as genome sequences, large-scale functional studies of every cell component, and ecological data are transforming how we think and conduct biological research. Sieving through this sea of information requires not only knowledge in biology but also abilities to think quantitatively. The major challenges, however, are that current biological curricula offers little or no emphasis on quantitative understanding of biological concepts. Furthermore, students often choose to pursue biology to avoid quantitative sciences. To explore how quantitative thinking can be integrated into biology curricula, we developed two instructional modules using backward design with a learning objective that students will be able to comprehend and apply basic quantitative concepts to different topics in introductory biology. The modules were used in two courses with distinct class sizes and prerequisites (15 second-year and 450 first-year students). In the small class setting, after two 1.5 hours class sessions, students improved significantly in their ability to develop hypotheses, examine data, and draw quantitative conclusions about their findings. In the large enrollment class, however, students had difficulties in graphing, formulating hypothesis, using numbers to justifying their conclusions, and applying the quantitative concepts learned in one setting to new situations. We found that integrating quantitative thinking into a biology curriculum is challenging and requires time, practice, and feedback to students. For instructors not familiar with quantitative topics, the integration is achieved through collaborative efforts between quantitative biologists and the instructor to institute small, gradual changes one concept/meeting/class at a time."
(a) Michigan State University

SESSION P24 – EMBRYOGENESIS

P24001 Micropropagation studies in *Astragalus holmgreniorum*
McGowan, Brett (a) Fry , Aaron (a) Babaoka, Julianne (a) Searle, Ally (a) Van Buren, Renee (a) Kopp, Olga Ruiz-presenter

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http://www.uvu.edu/profpages/olgakopp
"*Astragalus holmgreniorum* (Holmgren Milk-vetch), an endemic species of Washington County, Utah and Mohave County, Arizona has been listed as a federally endangered species since 2001 due to its rarity and declining population. Threats to the species stem from habitat destruction arising from commercial and residential development, overgrazing by livestock, off-highway vehicle use, and mining operations. In an attempt to develop a micropropagation technique that could be used in the recovery efforts for the species, we report successful induction of shoots and embryos from leaf and petiole explants. Incubation of explants in Murashige and Skoog medium amended with 2,-4 D and BA resulted in organogenesis and embryogenesis. Morphogenetic callus resulted in the production of plantlets that will be induced to root. Current work focuses on the effects of varying concentrations of NAA, IBA, and IAA on root formation. Ultimately, we hope that this research may be used to facilitate the recovery of the species by providing a stock of plants which could be used to establish new populations."
(a) Utah Valley University

P24002 Key signaling steps driving embryo formation from somatic cells in the model legume *Medicago truncatula*.
Rose, Ray J.-presenter Ray.Rose@newcastle.edu.au(a) Mantiri, Feky R. (a) Kurdyukov, Sergey (a) Chen, Shih-Kuang (a) Wang, Xin-Ding (a) Nolan, Kim E. (a) Sheahan, Michael B (a)
http://www.cilr.uq.edu.au
"The model legume *Medicago truncatula* (genotype 2HA) can produce somatic embryos (SEs) when leaf explants are cultured on a basal nutrient medium containing the plant hormones auxin and cytokinin. We have obtained evidence for a series of key signaling steps in the induction and development of somatic embryos. The first signals perceived by the explant on excision are reactive oxygen species (ROS), which are essential for the first cell divisions. Secondly, the stress hormone ethylene is synthesised. The auxin and cytokinin in the culture medium, together with the newly synthesized ethylene, have specific roles in SE induction. SEs derive from two types of stem cells; stem cells derived from dedifferentiated mesophyll cells and vein procambial cells. These stem cells become committed embryo stem cells, in part due to the action of the WUSCHEL transcription factor, whose expression is cytokinin-dependent. Transcription of the *SERK1* (*SOMATIC EMBRYO RECEPTOR KINASE1*) gene follows and marks cells that will differentiate into SEs. Ethylene, auxin and cytokinin are necessary for the induction of the transcription factor MtSERF1 (SOMATIC EMBRYO RELATED FACTOR1) which signals embryo development after about two weeks in culture. The first SEs become visible to the eye after about four weeks in culture, just after the establishment of CLAVATA3 (CLV3) expression and the WUSCHEL-CLV3 feedback loop."
(a) The University of Newcastle, School of Env. and Life Sciences

P24003 Genome-wide analysis of reveals gene expression and metabolic network dynamics during embryo development in Arabidopsis and Brassica napus
Datla, Raju-presenter raju.datla@nrc-cnrc.gc.ca(a) Xiang, Daoquan (a) Wang, Edwin (b) Venglat, Prakash (a) Tibiche, Chabane (b) Yang, Hui (a) Keller, Wilf (a) Selvaraj, Gopalan (a)
"Embryogenesis is an important developmental phase in the life cycle of seed plants. During this phase, the zygote undergoes a well-ordered series of cell divisions, followed by region-specific cell differentiation leading to an embryonic body plan to establish shoot and root apical meristems, central axis, cotyledons, and the hypocotyl. Despite advances in the identification embryo patterning mutants in recent years, the global genetic program involved in the regulation of early events after fertilization are largely unknown. To address gene expression patterns during the key stages of embryogenesis, embryos isolated from single cell zygote to mature stages have been used in model system Arabidopsis and crop plant Brassica napus. The ESTs, microarray, and proteomic based approaches have been applied to generate genome-wide expression datasets for key stages of embryo development. Analysis of these results identified developmental and stage specific programs that are connected to gene expression and metabolic regulation during embryogenesis. These studies also showed coordinated, regional and chromosome-specific expression patterns in the different stages of embryo development. By combining the omic data with KEGG metabolic database, we constructed stage-specific metabolic sub-networks mapped with differentially regulated genes in Arabidopsis and B. napus. Our findings indicate that during plant embryogenesis, changes in metabolic activities are associated with changes in network structures and topologies, and the interactions of core-pathway, pathway-pathway and gene/expression-networks. Together these studies provide new regulatory insights into embryogenesis in model plant Arabidopsis and crop species B. napus."
(a) Plant Biotechnology Institute (b) Biotechnology Research Institute

P24004 Development Dynamics of Wheat (*Triticum aestivum* L.) Microspores in Culture
Zheng, Ming Y.-presenter ming.zheng@gordon.edu(a) Hurlbut, Tiffany (a) Camp, Russell (a) Reczek, Stanley (a)
http://www.gordon.edu
"Doubled haploid production via microspore culture is a technique known to accelerate conventional breeding by shortening the breeding cycle and increasing the selection efficiency. Past research indicates that some microspores spontaneously abort before completing their development towards mature embryoids. Such abortions drastically decrease the yield of mature embryoids, thus making the microspore culture less efficient. Present research attempts to identify and quantify at which developmental stage(s) microspores are most susceptible to embryogenic abortion. Embryogenic microspores were isolated from pretreated wheat (*Triticum aestivum* L.)tillers and cultured in induction medium. The development of embryogenic microspores was monitored over a 35 day period. At days 7, 10, 14, 21, 28, and 35 the developing microspores were counted and categorized based on their developmental phase. Our results showed that 44% of the embryogenic microspores halted their development at various stages. Of the 44% total abortion rate, 20% halted at multicellular stage, 17% arrested their development as pre-embryoids, and 7% were terminated at the immature embryoid stage. Identifying factors that arrest microspore development and subsequent reduction in embryogenic abortions will significantly improve the yield of doubled haploid production."
(a) Gordon College

P24005 The effects of light on development of true shoots in excised immature cotton embryos (Texas Marker-1) cultured in vitro
Arnold, Marianne K (a) Gould, Jean H-presenter gould@TAMU.edu(a)
"Cotton is an important crop worldwide. In addition to providing a better understanding of regulation in early cotton embryo development, the culture of excised immature cotton embryos in vitro (embryo rescue) is an important tool in cotton breeding, used to recover viable progeny from wide crosses. Historically, excised immature cotton embryos are cultured in the dark, and the function of light in immature cotton embryo development has not been studied. Although the cotton was the dicot model used to understand the role of ABA in embryo development in the 1970s, recent research on dicot embryo development has focused on Arabidopsis and legumes. There are important developmental differences between the embryos of these species and cotton with respect to the presence of chloroplasts and their role in embryo development, early embryo cell divisions, length of embryogenesis, as well as, development of cotyledons and nutrient uptake. We initiated a study to determine expression patterns in immature cotton embryos (Texas Marker-1) dpa 15 onward, expecting a compromised ability to survive and to develop into normal plants based on

reports in the literature. We found that the most important factor influencing embryo survival and development was the presence of light. Excised immature cotton embryos (1.5 to 4 mm in length, or 16 to 17 dpa) are capable of continued development and production of true shoots at a significantly greater frequency when cultured in light, rather than in darkness. As expected, the color of light produced different effects. Red light inhibited growth and shoot development. Far red light, and blue light did not inhibit shoot development, while blue light promoted greening. "
(a) Texas AM University

P24006 Developmental characterization of *Arabidopsis thaliana* mutants affected in DDB1 proteins
Bernhardt, Anne-presenter annexbernhardt@gmail.com(a,b) Mooney, Sutton (b) Hellmann, Hanjo (b)
"DNA Damaged Binding 1 (DDB1) is a highly conserved protein of around 125 kDa. It serves as a substrate adaptor subunit to a CUL4-based E3 ubiquitin ligase within the ubiquitin proteasome pathway. Originally DDB1 was identified in context with DNA repair processes; however, based on a set of three beta-propellers, the protein is able to mediate various protein-protein interactions, suggesting that it participates in many developmental and physiological processes in the plant. *Arabidopsis* encodes for two closely related DDB1 proteins, named DDB1a and DDB1b. While loss-of DDB1a is not leading to obvious developmental defects, loss-of DDB1b results in embryo lethal phenotypes. Here we described a novel *ddb1b* mutant that is partially functional, and show that both DDB1a and DDB1b are critical for embryogenesis, photomorphogenesis, and root development."
(a) Freie University Berlin, Angewandte Genetik, Germany (b) Washington State University Pullman, USA

P24007 An integrated transcriptome and proteome analysis of microspore embryogenesis in *Brassica napus*
Whittle, Carrie A (a) Malik, Meghna R (a,b) Wan, Lianglu (a) Ross, Andrew (a,c) Krochko, Joan E-presenter Joan.Krochko@nrc-cnrc.gc.ca(a)
"Microspore-derived embryos (MDE) are important model systems for the study of plant embryo development and totipotency, and are highly effective tools for plant breeding. At present, much is unknown about the molecular mechanisms that underlie the shift between unicellular microspores (which would normally develop into pollen) and fully functional multi-cellular embryos. In order to reveal molecular factors associated with microspore embryogenesis in *Brassica napus*, cDNA libraries were constructed and sequenced, and large-scale proteome analysis conducted by one-dimensional SDS-PAGE and liquid chromatography-tandem mass spectrometry (LC-MS/MS), using extracts obtained from microspore-derived embryos (induced microspores) and *in vitro* pollen controls (non-induced microspores) at various stages of development. A total of 42 362 ESTs were obtained, clustered and resolved into 12 761 unigenes; 5507 of these unigenes are expressed specifically in microspore-derived embryos (not in pollen controls) and 4461 unigenes are specific to the pollen control cultures. From our proteomics data collection, a total of 14 923 non-redundant proteins were identified for the microspore-derived embryos while 12 154 were identified for the pollen control tissues. Comparative analyses of the transcriptome and proteome of induced microspore cultures and pollen controls revealed key gene sets, transcription factors and molecular processes associated with microspore embryogenesis, as well as putative embryogenesis markers. Altogether, the transcriptome and proteome datasets described here serve as a foundation for further studies to reveal genes and gene pathways associated with embryogenesis."
(a) NRC-Plant Biotechnology Institute, Saskatoon, Saskatchewan, Canada, S7N 0W9 (b) Agrisoma Biosciences Inc., 110 Gymnasium Place, Saskatoon, Saskatchewan, Canada, S7N 0W9 (c) Institute of Ocean Sciences, 9860 West Saanich Road, Sidney, British Columbia, Canada, V8L 4B2

P24008 RCD1 And SRO1 Play Key Roles In Embryogenesis And Vascular Development
Teotia, Sachin-presenter teotia.2@osu.edu(a) Lamb, Rebecca S (a)
"RADICAL-INDUCED CELL DEATH1 (RCD1) and SIMILAR TO RCD ONE1 (SRO1) are the only two proteins encoded in the *Arabidopsis* genome containing both a putative poly (ADP- ribose) polymerase (PARP) catalytic domain and a WWE protein-protein interaction domain. Proteins with a similar domain structure have been found in other eukaryotes, including humans. PARPs mediate attachment of ADP-ribose units from donor NAD+ molecules to target proteins and have been implicated in a number of processes including DNA repair, apoptosis, transcription, and chromatin remodeling. Both *RCD1* and *SRO1* are expressed in all plant organs. *rcd1-3* plants have pleiotropic developmental defects, most notably reduced stature. *sro1-1* plants display some subtle developmental defects in the roots but otherwise develop normally. The lack of major developmental phenotypes in *sro1-1* mutants could be due to redundancy with *RCD1*. However, while *rcd1-3* plants are early bolting, *sro1-1* plants flower late. This suggests that the genes do not always function equivalently. 70% of *rcd1-3; sro1-1* seeds do not germinate; those that do produce dwarf plants with abnormal flowers and short siliques. The seeds that do not germinate contain defective embryos with either very shortened or absent hypocotyls. In addition, their precambial and ground meristem cells divide abnormally, producing misshapen embryos. Consistent with the defects in vascular and ground cells evident in embryos, the stems of *rcd1-3; sro1-1* adult plants have a wider cortex layer, and reduced pith as well as vascular patterning defects. The leaves of *rcd1-3; sro1-1* has fewer vascular strands and organization of the mesophyll is disrupted. Characterization of the embryonic and postembryonic defects in vascular and ground tissues is being presented."
(a) Department of Plant Cellular and Molecular Biology; Molecular, Cellular and Developmental Biology Program, The Ohio State University

P24009 Expression dynamics of *PIN1* and *WOX* genes during Norway spruce (*Picea abies*) somatic embryogenesis
Palovaara, Joakim-presenter joakim.palovaara@hik.se(a) Hallberg, Henrik (a) Stasolla, Claudio (b) Luit, Bert (b) Hakman, Inger (a)
"Auxin and its polar transport, mainly established by the PIN family of efflux membrane transporters, as well as the WOX family of transcription factors are all essential for plant embryo patterning and development. It has been proposed that there is a connection between the spatial separation of *WOX2* and *WOX8* and polar auxin transport (PAT) in the formation of the apical-basal axis in *Arabidopsis* embryos and that both are involved in *PIN1* regulation. In spite of the central role of *PIN* and *WOX* genes in embryo pattern formation, their expression patterns have not been extensively investigated in plants outside the angiosperm taxa. Recent analyses by us suggest that both PAT and *WOX*-related genes play fundamental roles also in Norway spruce embryo patterning. Many aspects of growth and development are different between gymnosperms and angiosperms, including certain features of their embryogeny, making analysis of fundamental processes that appear conserved between the two groups evolutionary important. In this study, we describe the expression pattern of both *PIN1* and *WOX* genes during spruce somatic embryo development and in early seedlings growth, and show that in some respect, such as during the formation of the apical root meristem, their expression do not follow the same pattern as observed in *Arabidopsis*."
(a) School of Pure and Applied Natural Sciences, University of Kalmar (b) Department of Plant Science, University of Manitoba

SESSION P25 – EMERGING MODEL SYSTEMS

P25001 *Zostera marina* populations from the U.S. Eastern Seaboard demonstrate reduced levels of heterozygosity over related populations
Campanella, James J-presenter james.campanella@montclair.edu(a) Bologna, Paul (a) Rosenzweig, Eric (a) Smith, Stephanie M

(a) Smalley, John V. (b)
"*Zostera marina* (eelgrass) can be found in the North Atlantic on the coast of Europe and on the east and west coasts of North America. Over the last 30 years, this once robust species has been reduced to sparse patchy populations due to disease and anthropogenic effects. In order to better understand the consequences of this devastation on the population genetics of the species, we have analyzed the population structure of western Atlantic *Z. marina*, employing microsatellite DNA polymorphisms. Although high Fixation Index values suggest moderate genetic differentiation among most of the *Z. marina* populations, within population diversity was low. This lack of diversity was supported by a general dearth of observable heterozygotes in these populations; mean Hobs values (0.14-0.46) were lower than the mean Hexp values (0.57 -0.81) . Additionally, the mean FIS values in these populations were positive, again indicating a surfeit of homozygotes. Allelic Richness suggests Chesapeake Bay has the greatest internal genetic diversity of the populations studied. Genetic diversity appears lower in these American populations than in comparable populations, suggesting reproductive fitness problems in the future. There is evidence of demographic bottlenecking and particularly low genetic diversity in Long Island. Northern Maine had the highest effective population size, suggesting a possible use in future restoration projects. The large number of apparent homozygotes in these populations may also be generated by the presence of microsatellite null alleles for which there is evidence."
(a) Montclair State University (b) Bergen Community College

P25003 *Brachypodium distachyon*: a new model for the grasses
Vogel, John-presenter john.vogel@ars.usda.gov(a) Mayer, Klaus (b) Rokhsar, Daniel (c) Schmutz, Jeremy (d) Mockler, Todd (e) Huo, Naxin (a) Gu, Yong (a) Garvin, David (f) Bevan, Michael (g)
http://brachypodium.pw.usda.gov/
"*Brachypodium distachyon* (Brachypodium) is rapidly emerging as a model system to study questions unique to the grasses. This emergence is coincident with an increased need for basic research in grass biology to develop perennial grasses as a source of renewable fuel. The list of genomic resources available to Brachypodium researchers is increasing exponentially. We recently completed the sequencing and analysis of the entire Brachypodium genome using a whole genome shotgun sequencing strategy based on Sanger sequencing. The vast majority (99.6%) of the 272 Mb of genomic sequence was assembled into 10 scaffolds ranging from 8 to 38 Mb. These scaffolds were then verified and arranged into five chromosome scale assemblies using a high-density SNP-based linkage map. Automated annotation of this compact genome revealed ~25,500 genes strongly supported by transcriptome sequencing. With the Brachypodium sequence in hand we can examine the relationship between genomes from the three major groups of grasses; the Panicoids (Sorghum), the Bambusoids (rice) and the Pooids (Brachypodium). When combined with other tools developed for Brachypodium (insertional mutants, microarrays, diverse inbred lines, physical map, BAC libraries and BAC end sequences, EST sequences, and mutagenesis protocols), the complete genome sequence will allow plant biologists to efficiently utilize this new model system."
(a) USDA-ARS Western Regional Research Center (b) Munich Information Center for Protein Sequences (c) DOE Joint Genome Institute (d) Hudson Alpha Institute of Biotechnology (e) Oregon State University (f) USDA-ARS Plant Science Research Unit (g) John Innes Centre

P25004 Development and application of functional genomic tools for *Brachypodium distachyon*
Bragg, Jennifer N.-presenter jennifer.bragg@ars.usda.gov(a) Wu, Jiajie (b) Steinwand, Michael (a) Gu, Yong (a) Lazo, Gerard (a) Anderson, Olin (a) Vogel, John (a)
"*Brachypodium distachyon* (Brachypodium) is an excellent model for studying the basic biological processes underlying the traits that determine the utility of grasses as energy, grain and forage crops. Several important genomic resources have been developed for Brachypodium, including the recently completed whole genome sequence, ESTs, a high-density genetic linkage map and germplasm resources. In addition, we have developed key functional genomic tools including an extremely efficient Agrobacterium-mediated transformation protocol (average efficiency 44%) and an optimized chemical mutagenesis (EMS) protocol. We have created over 3,000 T-DNA mutants as part of a project to create >7,500 sequence-indexed T-DNA mutants. These mutants will be freely available to researchers studying grasses and grains. We are currently screening the EMS and T-DNA populations with near-infrared spectroscopy (NIR) to identify mutants with altered cell wall composition. To date, we have identified over 60 mutants and are in the process of determining what is altered in the cell wall. We have also initiated a screen to identify mutants with increased stem density, an important trait for biomass crops, and have identified six mutants with stems up to 40% denser than wild type. An overview of the T-DNA tagging and mutant screening projects will be presented."
(a) USDA, Agricultural Research Service, Western Regional Research Center (b) University of California, Davis

P25005 Diploid strawberry (*Fragaria vesca*) a reference species for the Rosaceae family
Slovin, Janet P.-presenter janet.slovin@ars.usda.gov(a) Shulaev, Vladimir (b) Folta, Kevin M. (c) Davis, Thomas M. (d) Sargent, Daniel J. (e) Folkerts, Otto (b)
"The fresh and processed products of the Rosaceae plant family (almonds, apples, apricots, blackberries, peaches, pears, plums, cherries, strawberries, raspberries, roses) in the U.S. are valued at over $7 billion. Expansion of the genomics, genetics, and germplasm knowledge base of flower, fruit, and nut development, ripening, senescence, and microbial contamination is essential for maximizing and maintaining quality of these crops. Of the major Rosaceous crop plants, only strawberry has the efficient transformation systems that permit rapid elucidation of gene function. The octoploid (2n=8x=56) genome of the cultivated strawberry, *Fragaria* x *ananassa* is among the most complex of any crop species. However, the ~200 Mb size of the basic (x=7) strawberry genome ranks among the smallest of any cultivated crop species. The diploid woodland strawberry, *F. vesca*, has been developed as a system for rapid discovery in strawberry genetics and genomics, and as a reference plant for the Rosaceae family. Advantages of *F. vesca* include its self-fertility, fecundity, small plant size, short generation time (~3.5 months), amenability to genetic transformation, diverse germplasm base, and very small genome. *F. vesca* has been sequenced to ~20x coverage with 454 technology. In addition to a sequenced genome, a diploid genetic map is rapidly being populated with markers, documented inbred lines are available, and a highly efficient transformation system facilitates insertion mutagenesis and direct assessment of gene function with overexpression or RNAi. A well-characterized *F. vesca* system enables us to begin developing useful assays to evaluate genes for their function in plant stress responses, fruit quality, disease resistance and a host of other horticulturally relevant traits. "
(a) USDA ARS Beltsville (b) Virginia Bioinformatics Institute, Virginia Tech (c) Horticultural Sciences Dept., University of Florida (d) Dept. Plant Biology, University of New Hampshire (e) Plant Breeding and Genetics, East Malling Research

P25006 *Brachypodium distachyon* Transcriptomics
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http://mocklerlab.cgrb.oregonstate.edu/
"*Brachypodium distachyon* is a model for temperate grasses and bioenergy crops. To facilitate genomic studies in Brachypodium we used Illumina (Solexa) sequencing to sample a collection of cDNA libraries representing a diverse array of tissues, treatments and developmental stages. The resulting EST data were aligned to the Brachypodium genome and used to assemble transcriptional units, including alternative splice variants. The

high depth of sequencing and broad unbiased coverage available from the Illumina platform increases the chance of identifying low abundance transcripts. Our analysis provides a comprehensive view of the Brachypodium transcriptome and facilitates annotation efforts. We used our empirical annotation of the transcriptome to aid design of a versatile oligonucleotide microarray platform that includes exon scanning and genome tiling features. We are using these arrays to generate a Brachypodium expression atlas comparing tissues over development, diurnal and circadian time-courses, and stress conditions. This atlas will provide a hypothesis-generating foundation for elucidating the transcriptional networks underlying traits of major importance economically important crops including wheat, barley and potential bioenergy grass crops. "
(a) Department of Botany and Plant Pathology and Center for Genome Research and Biocomputing, Oregon State University, Corvallis, Oregon, 97331 (b) Waksman Institute of Microbiology, Rutgers, Piscataway, NJ, 08854

P25007 Unraveling the complexity of rootstock-scion interactions in Rosaceae
Koepke, Tyson A-presenter tkoepke@wsu.edu(a) Krishnan, Vandhana (b) Duncan, Christina (c) Whiting, Matthew (d) Kalyanaraman, Ananth (b) Dhingra, Amit (a)
http://www.genomics.wsu.edu
"Many important crops are grown as composite plants where rootstocks are known to mediate scion traits such as stature, disease resistance and yield. The rootstock genotype exerts control over floral bud numbers in Prunus avium, sweet cherry, in addition to several other traits. These experiments focus on floral bud number because it is a precisely quantifiable phenotypic trait with high variation among the rootstock/scion combinations sampled. Quantitative transcriptome profiling of developing floral bud tissues through high throughput sequencing has begun to reveal transcripts with putative roles in this phenomenon. Of the initial assemblies on 454 reads, less than 5 percent had similarity to sequences in GenBank. Of this 5 percent, approximately 25 percent showed significant differences in expression levels among the samples. These expression differences were categorized as rootstock dependent, time dependent and both rootstock and time dependent and examined further. Results from these experiments will be presented. "
(a) Molecular Plant Sciences, Department of Horticulture, Washington State University (b) Department of Computer Sciences, Washington State University (c) Department of Civil Engineering, Washington State University (d) IAREC, Department of Horticulture, Washington State University

SESSION P26 – EMERGING TECHNOLOGIES

P26001 Precise genome modification in the crop species *Zea mays* using zinc-finger nucleases
Shukla, Vipula K-presenter vkshukla@dow.com(a) Doyon, Yannick (b) Choi, Vivian (b) Rock, Jeremy (b) Ying-Ying , Wu (b) Katibah, George (b) Gao, Zhifang (a) McCaskill, David G (a) Simpson, Matthew A (a) Blakeslee, Beth (a) Greenwalt, Scott A (a) Butler, Holly J (a) Miller, Jeffrey C (b) Hinkley, Sarah J (b) Lei, Zhang (b) Rebar, Edward J (b) Gregory, Philip D (b) Urnov, Fyodor D (b) DeKelver, Russell C (b) Moehle, Erica A (b) Worden, Sarah E (a) Mitchell, Jon C (a) Arnold, Nicole L (a) Gopalan, Sunita (b) Xiandong, Meng (b)
"Agricultural biotechnology is limited by the inefficiency and unpredictability of conventional random mutagenesis and transgenesis. Here we report a broadly applicable, versatile alternative: the use of designed zinc-finger nucleases (ZFNs) that induce double-stranded breaks (DSB) at their target locus. We have used ZFNs to modify endogenous loci in plants of the crop species *Zea mays*, and show that ZFNs designed to cleave a maize gene disrupt their target via DSB repair by error-prone non-homologous end-joining. We further demonstrate that simultaneous expression of ZFNs and delivery of a simple heterologous donor molecule leads to high frequency targeted addition of an herbicide-tolerance gene at the intended locus. Modified maize plants transmit these genetic changes to the next generation. Insertional disruption of one target locus, *ZmIPK1*, results in both herbicide tolerance and alterations of the inositol phosphate profile in developing seeds. ZFNs can be utilized in any plant species amenable to DNA delivery; therefore our results establish a new paradigm in plant genetic manipulation for basic science and agricultural applications."
(a) Dow AgroSciences, LLC (b) Sangamo BioSciences

P26002 A novel strategy to express multiple genes from a poly cistronic construct driven by a single promoter in transgenic plants
Maiti, Indu B.-presenter imaiti@uky.edu(a) Pattanaik, Sitakanta (a) Raha , Sumita (a) Bhattacharyya, Somnath (a) Das, Narayan (a)
"For modification of plants to have multiple desired traits, and for engineering entire metabolic and regulatory pathway or redirecting complex biosynthetic pathways involving multiple genes, it will be necessary to introduce several genes into transgenic plants. We have developed novel expression cassettes for constitutive expression of multiple genes from the polycistronic (di- and tri-cistronic) construct in transgenic plants. The polycistronic expression cassettes, designed for regulated expression of multiple genes, are composed of Peanut chlorotic streak virus (PClSV) full-length transcript promoter-leader and the p7R (antisense orientation of PClSV ORF VII) sequence as intervening sequence, and either GFP, GUS or CAT as reporter genes. The PClSV full-length transcript promoter-leader sequence and the p7R act as an intron in this process, and are involved in precise splicing of the polycistronic units. This has been tested both in transient protoplasts expression experiments and in stably transformed transgenic plants. Molecular analysis of spliced transcripts from chimeric di- and tri-cistronic constructs showed processed mRNAs with defined 5'- and 3'- ends. The p7R also acts as a polyA signal and processes transcripts in this context. The efficient translation of polycistronic mRNAs has potential value in plant metabolic engineering."
(a) University of Kentucky

P26003 Technologies for studying ocean biodiversity
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http://www.mbari.org
"Conservation of terrestrial biodiversity has advanced as a global issue over the last few decades; now concern for ocean biodiversity, beyond traditional interests in exploitable natural resources, is gaining momentum. Over 2,000 Census of Marine Life researchers in 80 countries have added 5,300 new species to the list of known marine organisms over the past five years. Yet we have hardly scratched the surface of the greatest part of the biggest ecosystem on Earth, the ocean which may hold multitudes of undescribed species, from large animals to marine microbes. Overfishing, pollution, climate change and ocean acidification are altering ocean ecosystems in ways we do not understand and yet the future ocean cannot be predicted given the current lack of quantitative data. The challenge is to find cost-effective tools and techniques that address the temporal scale and scope of the problem as well as the spatial scale of those ecosystems. Cameras on remotely operated vehicles now routinely document the ocean, but autonomous vehicles with artificial intelligence and imaging and sampling capabilities promise to lower the cost of ocean exploration. The Environmental Sample Processor from MBARI, a portable undersea genetics lab, can help chart the distributions of known species including harmful algal blooms by detecting and quantifying those organisms in real time and transmitting data back to land. Software programs streamline data processing and analysis (e.g., the Video Annotation and Reference System). These examples and future development efforts show potential for

enhancing biodiversity studies of the ocean. "
(a) MBARI - Monterey Bay Aquarium Research Institute

P26004 New viral vectors for cereals and legumes
Kearney, Christopher-presenter Chris_Kearney@baylor.edu(a) Liu, Zun (a)
http://www.baylor.edu/Biology/index.php?id=15000
"Viral vectors for cereals and legumes have been created from *Foxtail mosaic potexvirus* (FoMV) and *Sunn hemp mosaic tobamovirus* (SHMV), respectively. To enhance environmental safety, viral genes were eliminated, resulting in the base vectors FECT (FoMV elimination coat protein and triple gene block) and SHEC (SHMV elimination coat protein). In fact, these incapacitated vectors express only trace amounts of marker unless co-expressed with a silencing suppressor, in which case they express GFP at 40% TSP (FECT) or 15% TSP (SHEC) in agroinoculated *Nicotiana benthamiana*. An estradiol-inducible FECT/p19 version strongly displays this on/off characteristic via chemical induction. Xylanase and a full-sized, immunologically functional IgG have also been expressed in FECT. SHEC expresses GUS easily via agroinoculation in several legumes, at levels 6x greater than a 35S/GUS construct. FECT expression in grasses will be investigated with either protoplasts or transgenic plants. "
(a) Baylor University, Dept. of Biology

P26005 Agrobacterium tumefaciens-mediated transformation of maize (Zea mays) inbred lines MO17 and H99
Lee, Hyeyoung-presenter leehye@missouri.edu(a)
"Maize (Zea mays) is not only the most widely grown crop in the U.S. but also used for studying genetics and molecular biology as a model plant. Particularly, maize inbred lines have been used extensively as primary genetic resources for genetic improvement and experimental material for maize genome and functional genomics studies. Genetic transformation of maize inbred lines employing Agrobacterium tumefaciens-mediated transformation plays unique role in maize genetic improvements and genomic studies. However, transformation of maize inbred lines particularly those suitable for maize functional genomics have been very challenging. Here, we explored regeneration and transformation in four maize inbred lines, B73, H99, and MO17 using the basal area of shoot tips as starting explants. We further modified regeneration and transformation processes. Briefly, maize seeds were germinated on MS basal salts for 5-7 days in light. The explants were excised from germinating seeds and placed on regeneration media which employed 0.5 mg/l of 2,4-D and 2.2 mg/l of picloram. The cocultivation media were amended with 3.3mM of L-cysteine and 1mM of dithiothreitol. We compared the impact of 10% vs. 50% MS salts of inoculation and co-cultivation media on transformation in MO17 and H99. Our results showed that the basal area of shoot tips was very suitable for efficient regeneration and transformation in these two maize inbred lines through somatic embryogenesis. Molecular analysis of putative transgenic events derived from these two inbred lines is underway and results will be presented."
(a) University of Missouri-Columbia

P26006 DNA Barcoding of *Cassia spp* using *trn*H-*psb*A intergenic region of chloroplast genome
Damodaran, Suresh-presenter suresh.d.gen@gmail.com(a) Arunraj, Rex (a) Natarajan, Purushothaman (a) D, Narasimhan (b) Madasamy, Parani (a)
"DNA barcoding involves species identification using conserved DNA sequence that contains enough phylogenetic information to discriminate the species. Its robust nature makes it as a potential tool in the identification of new and cryptic species. DNA barcoding prevails to be valuable addition to the morphological taxonomy, as it facilitates biodiversity studies and forensic analyses of several plant species. Rare plant species possessing valuable medicinal properties can be safeguarded from extinction through this identification tool. Due to slow rate of mutation, chloroplast intergenic regions are considered as suitable loci for DNA barcoding of plants. To be useful in DNA barcoding, the locus should be divergent enough to discriminate all the species of a genus and at the same time conserved enough within a species in order to identify all the individuals of a species with single barcode. For the current study, 20 species of *Cassia* were collected from different parts of Tamil Nadu, India. Genomic DNA was isolated from all the samples and *trn*H-*psb*A intergenic region was PCR amplified. Size of the amplified fragment varied between 279 bp to 481 bp. DNA sequencing and multiple alignment using ClustalW showed species specific sequences which can be used as DNA barcodes. In order to ascertain that the DNA barcode is not variable within the species, 9 samples from *Cassia tora* and 3 samples from *Cassia occidentalis* were analysed for *trn*H-*psb*A intergenic region. It showed 99-100% identity with the DNA barcode of respective species. Barcoding of other species of *Cassia* and analysis of intra-species variation is under progress. "
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P26007 Transient protein expression in three *Pisum sativum* (green pea) varieties
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http://www.fraunhofer-cmb.org/
"The expression of therapeutic proteins in plants, both transiently and via permanently transformed lines, has been demonstrated by a number of groups. Transient plant expression systems, due to high expression levels and speed of production, have gained wide acceptance. Expression vectors based on a Tobacco Mosaic Virus (TMV) are the most commonly utilized and the primary plant used is *Nicotiana benthamiana*. *N. benthamiana* has two limitations for its use, one is its slow growth, and second is its low biomass. To address these limitations we screened a number of legumes for transient protein expression. Using the Alfalfa Mosaic Virus (AMV) and the Cucumber Mosaic Virus (CMV) vectors delivered via *Agrobacterium*, we were able to identify three *Pisum sativum* (green pea) varieties that demonstrated protein expression: speckled, yellow, and bill jump peas. Speckled pea was selected for expression optimization due to greater biomass and minimal tissue damage after infiltration. Initial GFP expression levels were low, so to address this we took a number of approaches such as changing growth conditions, utilizing the inhibitor of silencing p19, with the most dramatic results obtained when the CMV-based vector system was employed. Through these strategies GFP expression levels increased from 10 +/- 2.41 mg/kgFW to 420 +/- 26.24 mg/kgFW. We were also able to express three therapeutic proteins indicating promise for this system in the production of biopharmaceuticals. The first protein expressed was the protective antigen domain four fused to lichenase (LicKM-PAD4), which is an antigen targeted for an anthrax vaccine as well as human growth hormone (hGH) and hemagglutinin from Influenza A/Wyoming/3/03 (H3N2) (HAWY1) an antigen for generating protective immunity. "
(a) Fraunhofer Center for Molecular Biotechnology

P26008 Genome assembly validation using optical restriction map
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http://www.biotech.ufl.edu/

"High throughput next-generation DNA sequencers such as 454 Life Sciences GS FLX Titanium and ABI SOLiD have certainly revolutionized our approach to DNA sequencing. However, these sequencing techniques due to its *in-vitro* nature and short read length also bring new problems in genome assembly and finishing. Genome finisher has a tough time in arranging contigs or validating assembly in absence of physical map. Here we are emphasizing the importance of optical map to validate bacterial genome sequencing. An optical map is a single complete genome restriction map derived from a number of partial restriction fragment maps information. Basically, whole-genome optical maps are ordered restriction maps generated by spreading whole chromosomes onto treated glass surfaces containing many channels, followed by its digestion with restriction enzymes. About 50 to 100 contiguous restriction fragment of size measuring up to one-third of the whole chromosome are selected. These overlapping partial chromosome contigs are combined by alignment software using contiguous fragment sizes. The contiguous fragments of one optical map now aligned and compared to the in silico chromosome map of a sequenced reference strain. The optical map allowed us to identify several assembly errors, which is not possible without any mapping data. Despite the advantages of so-called long sequence (~250 bp) pyrosequencing reads and clone end-pairing data, 454 assembly contains error because of the presence of numerous highly repetitive sequences. We, thus conclude that, in order to ensure the accuracy of finished genome sequences optical mapping is an important tool to validate *de-novo* assemblies generated by next-generation sequencers."
(a) Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL-32611

P26009 Launch Vector system: A novel platform for subunit vaccine development and production
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"There has been a great deal of development in recombinant protein expression technologies in the past 30 years with a variety of in vivo and in vitro systems emerging and each having strengths and weakness in their ability to express a range of foreign proteins. The demand for recombinant biopharmaceutical proteins and industrial enzymes is expected to rise dramatically in the near future. However, the current capacity and cost of production for most recombinant proteins limits their availability. Thus, it is important to evaluate different production systems and choose one that ensures a functional, yet cost-effective, product. Most genes can be expressed in numerous systems, so it is essential to determine which system offers the most advantages for production. No one system will likely be ideal for all proteins. Practical considerations for each individual recombinant protein produced will determine the choice of production system. We have developed accelerated production platform that is applicable for expressing a broad range of monomeric and multimeric proteins, including therapeutic enzymes, monoclonal antibodies and vaccine antigens. This is accomplished by using launch vectors that enable the use of non-genetically modified plants for target production. Combination of launch vectors and non-genetically modified plants creates a highly competitive production platform that brings a new concept to biomanufacturing."
(a) Fraunhofer Center for Molecular Biotechnology

P26010 Prickly Pear and Nopalitos (*Opuntia* spp.) Dessert such a Marmalade
Valderrama-Chairez, Maria L.-presenter leonor.valderrama@cucba.udg.mx(a) Montppellier y Montes de Oca, Briseida (b) Mendoza-Beas, Eduardo A. (b) Mendoza-Beas, Irma A. (b) Carreon-Amaya, Javier (b) Juarez, Mayolo S. (c)
"Cactus pear (*Opuntia* spp.) provides a wide range of products for human and animals, also contributes to biodiversity by providing feed, protection and habitat for wildlife in these areas and were used as medicine since prehispanic times. Now a day, exist scientific evidence that the nopalitos (Cactus cladodes) and prickly pear intake promotes health (antioxidant properties, antihyperlipidemic and effect, healty lipophilic fiber, effect in cancer chemoprevention), this makes them attractive candidates for the preparation of products with nutraceutical characteristics. In developing fruit marmalade several factors combine to ensure quality preparation and after a period of shelf life. This, we developed a prickly pear and nopalitos dessert such a marmalade using various combinations of prickly pear pulp and nopalitos, without adding sucrose neither pectin nor citric acid. It is interesting to evaluate our new product development, since we only use ingredients like cactus pulp and nopalitos, unlike the common jams and marmalade, as established by the Mexican Official Standard of food and fruit products (NOM-F - 131-1982). The formulated marmalades with different quantities of fruit pulp and cladodes were evaluated by a sensory panel; the characteristics evaluated include: color, flavor, aroma, sweetness, texture an fruit pieces. The marmalade chosen had the greater acceptance by the sensory panel by the statistical analysis of the results o the sensory evaluation. Future it will test on biological models to assess its effects on health."
(a) Dpto. Salud Publica CUCBA, Universidad de Guadalajara (b) Division de Estudios de Postgrado e Investigacion, Instituto Tecnologico de Tlajomulco, Jal. (c) Dpto. de Ingenieria Quimica y Bioquimica,Instituto Tecnologico de Celaya

P26011 A simple and sensitive high-throughput GFP screening in plants
Hily, Jean-Michel (a,b) Liu, Zongrang-presenter Zongrang.liu@ars.usda.gov(a)
"Green fluorescent protein (GFP) has been used widely as a powerful bioluminescent reporter, but its visualization by existing methods in tissues or whole plants and its utilization for high-throughput screening remains challenging in many species. Here, we report a fluorescence image analyzer-based method for GFP detection and its utility for high-throughput screening of transformed plants. Of 3 detection methods tested, the Typhoon fluorescence scanner was able to detect GFP fluorescence in all Arabidopsis thaliana tissues and apple leaves, while regular fluorescence microscopy detected it only in Arabidopsis flowers and siliques but barely in the leaves of either Arabidopsis or apple. The hand-held UV illumination method failed in all tissues of both species. Additionally, the Typhoon imager was able to detect GFP fluorescence in both green and non-green tissues of Arabidopsis seedlings as well as in imbibed seeds, qualifying it as a high-throughput screening tool, which was further demonstrated by screening the seedlings of primary transformed T0 seeds. Of the 30,000 germinating Arabidopsis seedlings screened, at least 69 GFP-positive lines were identified, accounting for an approximately 0.23% transformation efficiency. About 14,000 seedlings grown in 16 Petri plates could be screened within an hour, making the screening process significantly more efficient and robust than any other existing high-throughput screening method for transgenic plants. "
(a) Appalachian Fruit Research Station, USDA-ARS (b) New York State Agricultural Experiemntal Station, Cornell University

P26012 An upright robotic positioning device for high throughput machine vision studies of plant phenotypes
Subramanian, Ram-presenter ram@cae.wisc.edu(a) Spalding, Edgar P (b) Ferrier, Nicola J (a)
"Techniques for defining phenotypes quantitatively and in detail are needed in plant biology because phenotypes are a major source of information about gene function. With roughly 10^4 genes and corresponding mutants per model organism, automation is needed to address the function of a significant portion of the genome. We describe here a system for high throughput monitoring, image acquisition and analysis of seedlings growing on vertical Petri dishes. The system consists of two major units. The first is a fixture with a cassette capable of housing a grid of 36 Petri dishes vertically (6 rows by 6 columns), each dish containing multiple seedlings. The fixture is uniformly illuminated with white LED lights for growth and infrared LEDs provide backlight for imaging. The second is a dual camera robot (3 axis gantry) system. The robot has a workspace (extent of motion) large enough to actively position (servo) the camera along the vertical x,y plane to all positions of interest in the sample grid. Camera one with a low magnification lens is used for calibration and camera two with a high magnification lens is used for servoing and image gathering. Custom developed

software performs the servoing task, ensures that the root and root tip (region of present interest) are in focus, records the location on the grid and saves the image file onto the data server for further analysis. Once the grid locations of interest are determined the image acquisition is repeated at regular time intervals for each seedling. We are currently working to achieve a continuous workflow by utilizing multiple pre-planted fixture cassettes, and to expand analyses to include other regions of the seedling. The goal is to automate the analysis of changes in seedling root and shoot size and shape over time."
(a) Dept of Mechanical Engineering, University of Wisconsin (b) Dept of Botany, University of Wisconsin

P26013 New purification and mass spectrometric approach for cytokinin analysis
Novak, Ondrej-presenter ondrej.novak@upol.cz(a) Lenobel, Rene (a) Dolezal, Karel (a) Strnad, Miroslav (a)
"The identification and quantification of plant hormones in plant tissues are necessary for physiological studies of their metabolism and mode of action. The major problem associated with plant hormone analysis is that the amount of phytohormones present endogenously in plant tissues is very low, usually in the range of fmol to pmol/g fresh weight. Development of simple purification of real samples by batch immunoextraction (Hauserova *et al.*, 2005) and application of new analytical approaches based on UPLC separation (Novak *et al.*, 2008) makes possible a new direction in plant hormone research. A fast chromatography technique, the ultra performance liquid chromatography (Acquity^{TM} UPLC,Waters) was coupled to triple quadrupole mass spectrometer (Xevo^{TM} TQ MS,Waters) equipped with an electrospray interface (ESI) and the unique performance of collision cell (ScanWave^{TM}). Small amount (*1 mg*) samples of 10-day-old *A. thaliana* plants were purified by stop-to-go-microextraction follow by an immunoaffinity step and process was completed by fast chromatographic analysis of naturally occurring cytokinins (bases, ribosides, O- and N-glucosides, and nucleotides) in 5 minutes. In multiple reaction monitoring mode, the detection limit for most of cytokinins was close to 50 amol and achieved linear range was at least five orders of magnitude. The method provides substantial improvements in terms of robustness, sensitivity, selectivity, convenience, through-put and cost-effectiveness over previous methods published. In conclusion, we believe that UPLC-ESI(+)MS/MS technology can be used for fast and sensitive quantitative analysis showing reproducibility in the plant hormones profiling (cytokinins, auxins, abscisic acid, gibberellins, brassinosteroids etc.) in different plant extracts."
(a) Palacky University, Laboratory of Growth Regulators

P26014 "Physiology Assessment of Small Plants using Red, Green, Blue LED Light Source and new Whole Plant Arabidopsis Chamber "
Morgan, Patrick B.-presenter pat.morgan@licor.com(a) Hupp, Jason R. (a) McDermitt, Dayle K. (a)
http://www.licor.com/env/
"To capitalize on the wealth of genetic and molecular data available for Arabidopsis, a new chamber for the LI-COR LI-6400XT Portable Photosynthesis System allows rapid assessment of photosynthetic and respiration rates of small rosette plants, short grasses and seedlings. Traditional, clamp-style gas exchange chambers hamper photosynthetic studies because of difficulties enclosing leaves of small, low-stature or rosette plants. The new Whole Plant Arabidopsis (WPA) chamber alleviates this issue by enclosing the entire plant within the chamber. Utilizing a novel flow path and blocking techniques to suppress belowground gas exchange, the WPA isolates aboveground CO_2 fluxes unlike traditional whole plant chambers, in which net gas exchange is the sum of growth media, container fluxes, plant assimilation and respiration. Injections of 80 µ;g CO_2 (250 µ;l pure CO_2) into the growth media just below the surface were suppressed by blocking and positive pressure (not detectable at 0.3 ppm resolution). Designed for high throughput to assist with genetic screening, the WPA chamber measures Arabidopsis plants with total leaf area as small as 1.5 cm^2. Photosynthetic efficiency and responses to narrow-band red, green and blue light was assessed in wild-type Arabidopsis (Col-0) using independently-controlled LEDs within the light source. Arabidopsis leaf absorption was significantly lower in the green wavelengths, but whole plant light responses were similar for white, red and green light. Leaf level measurement confirmed that this was a function of similar quantum efficiencies of CO_2 fixation (ΦCO_2) in the different wave bands. Physiological studies of different gas exchange pathways can now be undertaken for collections of knockout and knock-down mutants available in Arabidopsis."
(a) LI-COR Biosciences - Environmental

P26015 The powerful technology for inducing deletion mutations in *Arabidopsis thaliana*
Kazama, Yusuke-presenter ykaze@riken.jp(a) Liu, Yang (a,b) Hirano, Tomonari (a) Ohbu, Sumie (a) Hayashi, Yoriko (a) Matsuyama, Tomoki (c) Abe, Tomoko (a)
"Ion beams consist of ions accelerated to about 50% of the light speed in the RIKEN ring cyclotron, and have a high linear energy transfer (LET). This high LET irradiation is an excellent technology for inducing mutations to improve horticultural and agricultural crops. Previously, we optimized the conditions of ion beam irradiation used to induce mutations at high frequency in *A. thaliana*, and demonstrated that a LET of 30 keV/µ;m was the most effective for inducing albino mutants. Under this condition, the incidence of albino mutants was three times greater than that after irradiation with a LET of 22.5 keV/µ;m. In this study, we screened morphological mutants from the M_2 progenies after 22.5-keV/µ;m irradiation and 30-keV/µ;m irradiation to investigate the effect of LET on DNA deletion sizes. We focused on elongated hypocotyl (*hy*) and glabrous (*gl*) mutants for detection of DNA mutations, because the genes responsible for these phenotypes have been well characterized. With 22.5-keV/µ;m irradiation, three deletions (1, 2, and 3 bp) were identified from three hy mutants, and one deletion (47 bp) and one base change (G→A) were found from two *gl* mutants. With 30-keV/µ;m irradiation, three deletions (2, 5, and 815 bp) were identified from three *hy* mutant, and two independent 1-bp deletions were detected in two *gl* mutants. In addition, a chromosomal translocation was detected from one *gl* mutant. In this mutant, chromosome 3 and 5 were connected to each other on *TTG1*, a gene responsible for the gl phenotype. These findings demonstrated that ion beams predominantly cause DNA deletions, and that 30-keV/µ;m irradiation tends to induce greater DNA damage than 22.5-keV/µ;m irradiation. Many other morphological mutants were also collected. These mutant lines will be presented."
(a) RIKEN, Nishina Cent. for Accel. Based Sci. (b) Inst. Modern Phys., Chin. Acad. Sci. (c) RIKEN Plant Breeding & Cell Engn. Res. Unit

SESSION P27 – ENVIRONMENTAL PHYSIOLOGY

P27001 Flowering induction pathway in rice
An, Gynheung-presenter genean@postech.ac.kr(a) Ryu, Choong-Hwan (a) Choi, Heebak (a) Lee, Shinyoung (a) Kim, Song Lim (a) Lee, Yang-Seok (a) Jin, Ping (a) Yang, Jung-Il (a) Choi, Sang Chul (a) Jeong, Hee Joong (a) Cho , Lae-Hyeon (a)
"Rice is an excellent model system in elucidating the flowering signal pathways of the plants that prefer short-day (SD) for flowering. We have recently identified *OsMADS51* that is a SD-specific inducer by inducing expression of *Ehd1* and *Hd3a*. We also identified *OsID1* that also positively regulate *Ehd1* under both SD and long day (LD). In addition, two MADS box genes *OsMADS50* and *OsMADS56* that function antagonistically to control LD-specific flowering have been studied. There are more than a dozen of genes that belong to the CO family. One of the genes is *Hd1* that was reported to be an activator in SD and inhibitor in LD. We are investigating roles of the gene using a new *hd1* mutant. We also study other CO-

like genes. We observed that *CO-like gene 4* (*COL4*) is a constitutive repressor acting upstream of *Ehd1*. Interaction among these regulatory factors is under investigation. We are also investigating several genes that appear to involve in chromatin remodeling. One of the genes is an ortholog of *AtTRX1* and the *OsTRX1* gene conferred late lowering when expression of the gene was disrupted by mutations. Another chromatin factor has the jumanji domain and the *Osjmj* gene appears to involve in flowering since mutation of the gene resulted in late flowering in both SD and LD. We will present a model on the complex network of flowering pathways in the facultative SD-flowering plant. "
(a) Pohang University of Science and Technology, Pohang, Korea

P27002 "Peroxidase Activity in relation to lipid and sugar compositions in ungerminated, cotyledon and embryo Tissues of *Vigna unguiculata* L. Walp seed grown under stress temperatures"
Islam, Shahidul-presenter islams@uapb.edu(a) Carvajal,, Carmen R R. (b) Garner, James O. (a)
"Twenty five cultivars were screened for germination at low (10C), moderate (30C), and at stress (40C) temperatures. Three cultivars were chosen, variety 'Texas Cream 40' was able to germinate at stress and low temperatures; 'Black Crowder' demonstrated acceptable germination at stress temperatures but was negatively affected at low temperature; 'Mississippi Purple' had low percent germination at all temperatures studied. Sugar compositions and peroxide activities were determined in whole ungerminated seed, cotyledon and embryo tissues of these cowpea cultivars. The main sugars founds in cowpea seed were sucrose, raffinose, and stachyose. Sugar contents were affected by cultivar, type of tissue, and temperature. Sucrose content was higher in embryo tissue of cultivars with low percent germination, and reduced in the cultivar with higher percent germination. This suggested that sucrose was used during germination. The amount of sucrose decreased greatly at 30C and increased again at 40C. Raffinose and stachyose contents were higher in ungerminated seed. In germinated seed, raffinose and stachyose contents were found only in cotyledon tissues at 10C. The most abundant fatty acids in cowpea seed were palmitic acid (16:0), palmitoleic acid (16:1), stearic acid, oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), and arachidic acid (20:0). The results show that the long-chain fatty acid appears to be important in the cowpea seed germination process. Peroxidase activities were affected by cultivars, type of tissue and temperature. The highest peroxidase activity was found at low temperature (10C) in embryo tissue of the cultivar with the highest germination. High peroxidase activity was related to ability of seed to germinate at low temperature."
(a) Dept. of Agriculture, Plant Science, University of Arkansas Pine Bluff, Mail Slot 4913, Arkansas, 71601 (b) Mississippi State University; Mississippi

P27003 "Light duration and temperature determine sprouting in buds of the aquatic plant, *Potamogeton crispus*"
Gleason, Florence Kowalczyk (a,b) Heuschele, Deborah J-presenter heus0023@umn.edu(a,b) Haque, Sakinah (a,b) Manteuffel, Adam (a,b) Newman, Raymond N (a,c)
"*Potamogeton crispus* is an invasive aquatic macrophyte in North America. Originating in Eurasia,this plant forms vegetative buds (turions) during late spring that usually sprout in autumn but can persist in a viable dormant state for at least 4 years. Preliminary data on new turions collected in Minnesota in June indicate that summer light conditions (16:8, L:D) at 24 C promote sprouting at a rate of 25%, while under fall conditions (10:14) sprouting rate increases to 40%. In the dark, the average sprouting rate was 4%. Very few turions sprouted at 4^{o} under dim light. New turions were found to retain chlorophyll even after 6 weeks in the dark. Turions were photosynthetically active under both spring and fall light regimes. Sugar levels were generally low but turions contained high concentrations of starch. In total darkness, turions catabolize starch indicating that these buds are not dormant. We conclude that new turions are metabolically active during the summer months and continue to synthesize and store carbohydrates. Fully dormant turions were not obtained over the 6 months of observation suggesting that full dormancy is only initiated in turions that do not sprout in their first year. "
(a) University Of Minnesota (b) Department of Plant Biology (c) Department of Fisheries, Wildlife and Conservation Biology

P27004 Growth environment may change the sensitivity of adaxial and abaxial stomata to light?
Wang, Yin-presenter wangy@biol.s.u-tokyo.ac.jp(a) Noguchi, Ko (a) Terashima, Ichiro (a)
http://www.biol.s.u-tokyo.ac.jp/users/seitaipl/index_e.html
"In most amphistomatous leaves, stomata on the adaxial (upper) and abaxial (lower) surfaces are developed in different light environments in terms of both intensity and wavelength composition. According to our recent study, adaxial stomata were always less sensitive to both white and monochromatic light than abaxial stomata (Wang *et al.*, 2008, PCE, 31:1307). In this study, to investigate effects of growth light environment on light response of stomata, we artificially inverted leaves of sunflower (*Helianthus annuus* L.) at their expanding stage, and measured gas exchange properties after their expansion with a laboratory-constructed system. We also treated both normal and inverted leaves with 3(3,4-dichlorophenyl)-l,l-dimethylurea (DCMU) to inhibit photosynthesis, and compared stomatal behaviors with those in the uninhibited leaves. Compared with normal leaves, abaxial stomata of inverted leaves became more sensitive to white and blue light, but their sensitivities to red and green light did not show marked changes. On the other hand, adaxial stomata of inverted leaves became less sensitive to light. After DCMU treatment, adaxial stomata became almost insensitive to light. Also the abaxial stomata almost lost sensitivity to red light, indicating the strong relationship between photosynthesis and the red light response. On the other hand, blue light and green light responses in abaxial stomata were inhibited but partly, implying involvement of the light response pathway(s) independent of photosynthesis. Interestingly, the decrease in the sensitivity was smaller for green light than for blue light."
(a) Department of Biological Sciences, Graduate school of Science, The University of Tokyo

P27005 *Arabidopsis thaliana* (L.) Heynh. having altered expression of mitochondrial pyruvate dehydrogenase kinase show enhanced oil biosynthesis under elevated CO_2.
Weraduwage, Sarathi M-presenter sweraduw@uoguelph.ca(a) Rauf, Shezad A. (a) Micallef, Malgre C. (a) Taylor, David C. (b) Grodzinski, Bernard (a) Micallef, Barry J. (a)
"Productivity of plants grown under long-term elevated CO_2 is often less than predicted; this reduced response is believed to be caused by end-product inhibition of photosynthesis through source-sink imbalance. We hypothesized that *Arabidopsis* transgenic lines with elevated activity of mitochondrial pyruvate dehydrogenase (mtPDH) through antisense repression of mtPDH kinase (mtPDHK) will show enhanced growth rates and productivity under high CO_2 compared to controls due to greater sink strength. To test this hypothesis control and transgenic lines expressing antisense mtPDHK constructs were grown at ambient (380ppm) or high (700ppm) CO_2. Data revealed a significant increase in sink size (increased seed weight and number) in transgenics at high CO_2. Analyses of fatty acid and seed oil showed a significant increase in fatty acid and oil biosynthesis under high CO_2 in transgenics. Significant increases in harvest indices at high CO_2 for constitutive lines 10^4 and 3^1 demonstrated their improved capacity to utilize photosynthate more efficiently under high CO_2. Interestingly, RT-PCR and Northern analyses showed lines 10^4 and 3^1 to have moderately suppressed mtPDHK expression, suggesting a dosage effect. We believe that an increase in dark respiration due to suppressed mtPDHK may increase metabolic carbon flux to seed oil biosynthesis and possibly to other biosynthetic processes necessary for plant growth and development, thereby enhancing sink activity and productivity under high CO_2. Also, the trend of increasing proportions of Very Long Chain Fatty Acid (VLCFA) and decreasing Long Chain Fatty Acid with increasing fatty acid content in transgenics suggests that most carbon going through mtPDH for

fatty acid synthesis is going to VLCFA synthesis in the endoplasmic reticulum. "
(a) Department of Plant Agriculture, University of Guelph (b) Plant Biotechnology Institute, National Research Council of Canada

P27006 *Arabidopsis* response to the environmental carcinogen benzo[a]pyrene
Irikura, Beth-presenter irikura@hawaii.edu(a) Paull, Robert E. (a)
" *Arabidopsis* was used to investigate the mechanisms by which phytoremediation of the ubiquitous environmental carcinogen benzo[a]pyrene (BaP) may occur. Phenotypic observations indicated these plants tolerated BaP. HPLC and comet assays suggested that root uptake and possible translocation occurred under sterile conditions. Microarray profiling revealed differences in gene transcription 24 h and 4 wks after exposure of 4 wk-old plant shoots grown in sterile media containing 50 ppm BaP. The experiment was repeated using non-sterile soil, and selected genes were examined by qRT-PCR. Based on concordant results from the two experiments, it appears that plant BaP response may involve homologs of genes used for BaP metabolism in other organisms. Transcripts of a cytochrome P450, an ABC transporter, and a UDP-glycosyltransferase increased in both experiments after 24 h exposure. A different ABC transporter with high homology to MRP1 (overexpressed in drug-resistant cancer cells) decreased after 4 wks. A laccase, peroxidase, and an alpha-dioxygenase with homology to human COX-2 (induced by BaP metabolites), were up-regulated at 4 wks. Long-term BaP exposure also increased expression of the homolog for Napsin A (a lung cancer marker). Known plant stress response genes were affected, and comparison with other stress profiles indicated similarity to 24 h cold response, with corresponding effects on circadian genes. We also measured increased strictosidine synthase expression, suggesting that plants could potentially produce anticancer compounds in response to a carcinogen, as these enzymes are in the pathway that produces vincristine and vinblastine in *Catharanthus roseus*."
(a) Department of Tropical Plant & Soil Sciences, University Of Hawaii

P27007 Gene expression and antioxidative defense enzymes to lead stress in subcellular leaves compartments of *Lepidium sativum*
Bafeel, Sameera O.S (b) Ibrahim, Mohamed M.-presenter mibrahim@ksu.edu.sa(a)
"A rapid changes in gene expression in plants in response to stress are important for environmental adaptation. A mRNA differential display technique was used in this study to analyze alterations in gene expression in *Lepidium sativum* in response to different concentrations of lead. The relationship of differential expression patterns and to the identification of cDNAs induced during lead toxicity were determined. Polymorphism has been detected under lead toxicity and the differentially expressed genes in stressed seedlings was strongly and rapidly induced under higher lead concentrations(400 and 600 ppm), whereas induction was delayed and transcripts accumulated to a low level under lower concentrations(100 and 200 ppm). A great variations in isoforms of different antioxidant enzymes as superoxide dismutase (SOD; EC1.15.1.1), catalase (CAT;EC1.11.1.6.) and ascorbate peroxidase (APx; EC1.11.1.11) were detected in response to lead treatments. Heavy metal toxicity increased protein degradation at higher concentrations, but the increase was significantly lower at lower concentrations. The results presented indicate that protein degradation are interlinked upon metal treatments and are concentration dependent. Within such response patterns, the gene expression is a valuable stress marker in ecophysiological studies. "
(a) King Saud University, Riyadh, Saudi Arabia (b) King Abdulaziz University, Jeddah, Saudi Arabia

P27008 Identification of Phosphate (Pi)-responsive genes in cucumber by suppression subtractive hybridization
Priya, Padmanabhan v (a) Kashchandra , Raghothama G (b) sahi, shivendra v-presenter shiv.sahi@wku.edu(a)
"Phosphate (Pi) is an essential macronutrient that plays a central role in many physiological and biochemical processes in plants. Application of phosphorus-rich manures to the soil often exceeding plant P- requirements has become routine in many parts of the US. The excess P run-off from agricultural lands leads to eutrophication and other serious water quality issues. Mining of excess soil P with the help of plants capable of extracting P from soil has been proposed as an attractive strategy for remediation of P-contaminated soils. Several plants were screened in P-enriched soils to identify P-hyperaccumulator plants that can be used in remediating P-contaminated soils. Studies revealed that cucumber (*Cucumis sativus*) showed high P accumulation in their shoot as well high biomass. A suppression subtractive hybridization (SSH) analysis was used to evaluate the phosphate (Pi) responsive gene expression in this plant. RNA was isolated from shoot and roots of plants grown under P-sufficient and P-deficient conditions and used for library synthesis. All the positive clones with inserts from the library were differentially screened by dot blot hybridization. Differentially expressed clones were then selected for sequencing. A number of Pi-responsive cDNAs were identified which include ubiquitin conjugating protein, phenylalanine ammonia lyase, metallothionein-like protein, PRLI-interacting factor A mRNA beta 1, 3-glucanase, translation elongation factor 1a, carbohydrate oxidase."
(a) Department of Biology, western kentucky university (b) Department of Horticulture and Landscape Architecture, Purdue University

P27009 "The major flowering time gene, *Flowering Locus C*, regulates seed germination in *Arabidopsis thaliana*."
Chiang, George C.K.-presenter ckchiang@fas.harvard.edu(a) Barua, Deepak (a) Kramer, Elena M. (a) Amasino, Richard M. (b) Donohue, Kathleen (c)
"*FLOWERING LOCUS C* (*FLC*) is a major regulator of flowering responses to seasonal environmental factors. Here we document that *FLC* also regulates another major life-history transition--seed germination--and that natural variation at the *FLC* locus and in *FLC* expression is associated with natural variation in temperature-dependent germination. *FLC*-mediated germination acts through additional genes in the flowering pathway--*FT*, *SOC1*, and *AP1*--before involving the ABA catabolic pathway (via *CYP707A2*) and GA biosynthetic pathway (via *GA20ox1*) in seeds. Furthermore, *FLC* regulation of germination is largely maternally controlled, with *FLC* peaking and *FT*, *SOC1*, and *AP1* levels declining at late stages of seed maturation. High *FLC* expression during seed maturation is associated with altered expression of hormonal genes (*CYP707A2* and *GA20ox1*) in germinating seeds, indicating that gene expression before the physiological independence of seeds can influence gene expression well after any physical connection between maternal plants and seeds exists. The major role of *FLC* in temperature-dependent germination documented here reveals a much broader adaptive significance of natural variation in *FLC*. Pleiotropy between these major life stages therefore likely influences patterns of natural selection on this important gene, making *FLC* a promising case for examining how pleiotropy influences adaptive evolution."
(a) Department of Organismic and Evolutionary Biology, Harvard University (b) Department of Biochemistry, University of Wisconsin-Madison (c) Department of Biology, Duke University

P27010 NaCl-induced expression of glutathione reductase in roots of rice (*Oryza sativa* L.) seedlings is mediated through hydrogen peroxide but not abscisic acid
Hong, Chwan-Yang-presenter cyhong@ntu.edu.tw(a) Chao, Yun-Yang (b) Yang, Min-Yu (a) Kao, Ching Huei (b)
"Reactive oxygen species play an important role in NaCl stress. Plants tolerant to NaCl stress may evolve certain strategies to remove these ROS, thus reducing their toxic effects. Therefore, the expression patterns of the gene family encoding glutathione reductase (GR, EC 1.6.4.2) were analyzed in roots of etiolated rice seedlings in response to NaCl stress. Semi-quantitative RT-PCR was applied to quantify the mRNA levels for one cytosolic (OsGR2) and two chloroplastic (OsGR1 and OsGR3) isoforms of glutathione reductase identified in the rice genome. The expression of

OsGR2 and OsGR3 but not OsGR1 was increased in rice roots treated with 150 mM NaCl. Treatment with 150 mM NaCl also induced the expression of an ABA inducible OsRab16A gene, and the expression increased with increasing concentrations of ABA, which suggests that ABA may be involved in this response in rice roots. In fact, exogenous application of ABA enhanced the expression of OsGR2 and OsGR3 in rice roots. On inhibiting ABA accumulation with sodium tungstate (Tu), an inhibitor of ABA biosynthesis, the expression of OsGR2 and OsGR3 was still induced by NaCl; therefore, NaCl-triggered expression of OsGR2 and OsGR3 in rice roots is not mediated by accumulation of ABA. However, NaCl treatment could induce H_2O_2 production in rice roots, and H_2O_2 treatment resulted in enhanced OsGR2 and OsGR3 induction. On inhibiting the NaCl-induced accumulation of H_2O_2 with diphenylene iodonium, the expression of OsGR2 and OsGR3 was also suppressed. Moreover, the increase in H_2O_2 level was prior to the induction of OsGR2 and OsGR3 in NaCl-treated rice roots. Thus, H_2O_2, but not ABA, is involved in regulation of OsGR2 and OsGR3 expression in NaCl-treated rice roots."
(a) Department of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan (b) Department of Agronomy, National Taiwan University, Taipei, Taiwan

P27011 Increased Antioxidant Defense and Glyoxalase Systems by Proline and Glycinebetaine Confer Salt Tolerance in Cultured Tobacco Cells
Hoque, Md. Anamul-presenter anamul71@yahoo.com(a) Banu, Mst. Nasrin Akhter (a) Okuma, Eiji (a) Watanabe-Sugimoto, Megumi (a) Amako, Katsumi (b) Matsuoka, Ken (c) Shimoishi, Yasuaki (a) Nakamura, Yoshimasa (a) Murata, Yoshiyuki (a)
"Salt stress impairs reactive oxygen species (ROS) and methylglyoxal (MG) detoxification systems, and causes oxidative damage to plants. Up-regulation of the antioxidant defense and glyoxalase systems offered by proline or glycinebetaine alleviates the NaCl-induced oxidative damage in plants. In this study, we investigated the effects of exogenously applied proline and glycinebetaine on cell growth and viability, ROS accumulation, and activity of enzymes and expression of genes involved in the antioxidant defense and glyoxalase systems in cultured tobacco BY-2 cells exposed to NaCl stress. Salt stress caused a significant inhibition of the growth of BY-2 cells, whereas both proline and glycinebetaine significantly mitigated this inhibition, and that proline was more effective than betaine. Salt stress induced reactive oxygen species (ROS) accumulation, and increased lipid peroxidation, protein carbonylation, nuclear deformation and cell death but decreased the activity of enzymes involved in the antioxidant defense and MG detoxification systems. Exogenous application of proline or glycinebetaine contributed to the reduction of ROS accumulation, lipid peroxidation and protein carbonylation, and suppression of nuclear deformation and cell death under salt stress. Furthermore, both compounds alleviated the reduction of activities of enzymes involved in the antioxidant defense and MG detoxification systems. The mRNA levels of some ROS-scavenging antioxidant defense genes were also induced by proline or glycinebetaine under salt stress. These results suggest that both proline and glycinebetaine confer tolerance to NaCl stress in tobacco BY-2 cells by protecting cellular components and increasing antioxidant defense and glyoxalase mechanisms."
(a) Okayama University (b) Kobe Gakuin University (c) Kyushu University

P27012 "Heat-shock dependent oligomeric status alters the function of a plant-specific thioredoxin-like protein, AtTDX"
Chae, Ho Byoung-presenter truedaisy@hanmail.net(a) Moon, Jeong Chan (a) Lee, Sun Yong (a) Kim, Je Hein (a) Son, Bo Hwa (a) Lee, Hye Bin (a) Lee, Kyun Oh (a) Lee, Sang Yeol (a)
"We found that Arabidopsis AtTDX, a heat-stable and plant-specific thioredoxin (Trx)-like protein, exhibits multiple functions, acting as a disulfide reductase, foldase chaperone, and holdase chaperone. The activity of AtTDX, which contains 3 tetratricopeptide repeat (TPR) domains and a Trx motif, depends on its oligomeric status. The disulfide reductase and foldase chaperone functions predominate when AtTDX occurs in the low molecular weight (LMW) form, whereas the holdase chaperone function predominates in the high molecular weight (HMW) complexes. Because deletion of the TPR domains results in a significant enhancement of AtTDX disulfide reductase activity and complete loss of the holdase chaperone function, our data suggest that the TPR domains of AtTDX block the active site of Trx and play a critical role in promoting the holdase chaperone function. The oligomerization status of AtTDX is reversibly regulated by heat shock, which causes a transition from LMW to HMW complexes with concomitant functional switching from a disulfide reductase and foldase chaperone to a holdase chaperone. Overexpression of AtTDX in Arabidopsis conferred enhanced heat shock resistance to plants, primarily via its holdase chaperone activity."
(a) Division of Applied Life Science (BK21 Program) and EB-NCRC, Gyeongsang National University, JinJu, GN660-701, Korea

P27013 Antimicrobial Activity of a Heat-Stable Protein from *Arabidopsis thaliana*
Jung, Ji Hyun-presenter henia1@nate.com(a) Lee, Jung Ro (a) Kim, Sun Young (a) Lee, Deok Ho (a) Jeon, Ju Mi (a) Cha, Jae Ho (a) Harmoko, Rickno (a) Lee , Kyun Oh (a) Lee, Sang Yeol (a)
"A heat-stable protein with antimicrobial activity was isolated from Arabidopsis thaliana plants by buffer-soluble extraction and two chromatographic procedures. The results of MALDI-TOF analysis revealed that the isolated protein shares high sequence identity with aspen SP1. To determine the exact antimicrobial properties of this protein, a cDNA encoding the protein was isolated from an A. thaliana leaf cDNA library and named AtHS1. AtHS1 mRNA was induced by exposure to external stresses, such as salicylic acid and jasmonic acid. We also analyzed the antimicrobial activity of recombinant AtHS1 expressed in Escherichia coli. This protein inhibited pathogenic fungal strains, except for Phytophthora infestans and Phytophthora nicotianae, and it exhibited antibacterial activity against E. coli and Staphylococcus aureus. These results suggest that AtHS1 shows good potential for use as a natural material in the study of antimicrobial agents. [Supported by EB-NCRC & BK21 program]"
(a) Environmental Biotechnology National Core Research Center, PMBBRC & Division of Applied Life Science (BK21)

P27014 Characterization of cyclophilin with antifungal activity from Chinese Cabbage
Lee, Young Mee-presenter totoro9985@lycos.co.kr(a) Park, Jin Ho (a) Sung, Nu Ri (a) Jung, In Jung (a) Yoo, Jea Yong (a) Kang , Chang Ho (a) Lee , Kyun Oh (a) Lee , Sang Yeol (a)
"An antifungal protein that inhibits the growth of filamentous fungal pathogens was isolated from Chinese cabbage (Brassica campestris L. ssp. pekinensis) by affinity chromatography on Affi-gel blue gel and ion exchange chromatography on CM-Sepharose. The N-terminal amino acid sequence of the protein was highly homologous to that of plant cyclophilins and consequently the protein was denoted as CyP. To understand the antifungal activity of CyP, we isolated a cDNA encoding its gene from a Chinese cabbage leaf cDNA library. The Chinese cabbage genome bears more than one CyP gene copy and CyP mRNA is highly expressed in all tissues except the seeds. Recombinant CyP catalyzed the cis trans interconversion of the Ala Pro bond of the substrate, which indicates this protein has peptidyl prolyl cis trans isomerase activity. It also inhibited the growth of several fungal pathogens."
(a) Environmental Biotechnology National Core Research Center, PMBBRC & Division of Applied Life Science (BK21)

P27015 Heat-shock and redox-dependent functional switching of an h-type
Shin, Mi Rim-presenter tsj4rain@hanmail.net(a) Jung, Young Jun (a) Fanata, Wahyu Indra (a) Park, Dong-Jin (a) Ko, Ki Seong (a) Kim,

Woe Yeon (a) Lee, Kyun Oh (a) Lee, Sang Yeol (a)
"A large number of thioredoxins (Trxs), small redox proteins, have been identified from all living organisms. However, many of the physiological roles played by these proteins remain to be elucidated. We isolated a high molecular weight (HMW) form of h-type Trx from the heat-treated cytosolic extracts of Arabidopsis suspension cells and designated it as AtTrx-h3. Using bacterially expressed recombinant AtTrx-h3, we find that it forms various protein structures ranging from low and oligomeric protein species to HMW complexes. And the AtTrx-h3 performs dual functions, acting as a disulfide reductase and as a molecular chaperone, which are closely associated with its molecular structures. The disulfide reductase function is observed predominantly in the LMW forms, whereas the chaperone function predominates in the HMW complexes. The multimeric structures of AtTrx-h3 are regulated not only by heat-shock but also by redox status. Two active Cys residues in AtTrx-h3 are required for disulfide reductase activity, but not for chaperone function. AtTrx-h3 confers enhanced heat-shock tolerance in Arabidopsis, primarily through its chaperone function."
(a) Environmental Biotechnology National Core Research Center, PMBBRC & Division of Applied Life Science (BK21)

P27016 Arabidopsis copper-zinc superoxide dismutase (SOD) can be activated by copper chaperone for SOD1-independent pathway via glutathione
Jinn, Tsung-Luo (a) Huang, Chien-Hsun -presenter f93b42012@ntu.edu.tw (a) Kuo, Wen-Yu (a)
"Superoxide dismutase (SOD) catalyzes the disproportion of the superoxide anion into H_2O_2 and O_2 molecules. The major pathway for CuZnSOD activation is known to depend on copper chaperone for SOD1 (CCS), with a minor pathway independent of CCS observed in human. Here, we demonstrated that the CCS-independent CuZnSOD activation also exists in planta. Partial CuZnSOD activities were presented in *Arabidopsis thaliana Atccs*Δ, and the residual activities was about 6 percent to 30 percent comparing to WT. To further identify which CuZnSOD has CCS-independent activity, yeast expression system was performed. From observations of SOD activity assay and viability on dropout lysine medium, AtCSD1 and AtCSD3, but not AtCSD2, had CCS-independent activities. These activities were influenced when glutathione concentration was changed by treating *Atccs*Δ flowers with 1-chloro-2, 4-dinitrobenzene (CDNB, chelator of glutathione), reduced glutathione or γ-glutamylcysteine (precursor of glutathione). The physiological importance of CCS-independent pathway was evaluated by root length of WT, *Atccs*Δ and *Atcsd1*Δ. When grew on 1/2 MS medium, *Atcsd1*Δ root was shorter than WT, and *Atccs*Δ was the same as WT. However, when plants were treated with 0.2 nM paraquat, *Atccs*Δ was more sensitive to glutathione depletion than WT and *Atcsd1*Δ, indicating higher requirement for the CCS-independent activity under severe oxidative stress. Moreover, in AtCSD1, residues 141 and 143 were important in CCS-independent activation, with the same effect in human and yeast SOD1. Prolines in these positions will prohibit the CCS-independent activation of CuZnSOD."
(a) Institute of Plant Biology and Department of Life Science, National Taiwan University

P27017 Functional and expression analyses of Arabidopsis aminopeptidase during plant development
Waditee, Rungaroon-presenter waditee@riken.jp(a) Nakano, Takeshi (b,c) Takabe, Teruhiro (d) Tsujimoto, Masafumi (a)
"Aminopeptidases constitute a diverse set of peptidases which cleave amino acids from the N terminus of oligopeptides. They are widely distributed in all living organisms and linked to a variety of cellular processes. Here, we report the characterization of an aminopeptidase *Arabidopsis thaliana mutant, pep1,*which exhibits a reduction in vegetative growth and shows symptom of early leaf senescence. The accelerated senescence in the mutant is accompanied by accelerated decrease of photosynthetic yield and chlorophyll contents. Under stress conditions, germination frequency of the *pep1*mutant was significantly reduced compared with the wild-type seeds. Transcript levels of senescence-related genes (SENESCENCE-ASSOCIATED GENE 12, DARK INDUCIBLE 1) and autophagy genes (ATGs) were enhanced in the *pep1* mutant. Metabolite analysis of the *pep1* mutant revealed that several amino acids and gamma-aminobutyrate, a non-protein amino acid, were significantly changed. We suggest the alterations in these metabolites contribute to the stress tolerance of and cause early ageing in the *pep1* mutant."
(a) Laboratory of Cellular Biochemistry, RIKEN Advanced Science Institute (b) Plant Chemical Biology Research Unit, RIKEN Advanced Science Institute (c) JST-PRESTO, Kawaguchi (d) Research Institute of Meijo University, Nagoya

P27018 "Analysis of Ca $^{2+}$-binding proteins, CCaP1 and CCaP2, in Arabidopsis thaliana"
Ouchi, Yuya-presenter ouchi.yuya@g.mbox.nagoya-u.ac.jp(a) Nagatani, Akira (b) Maeshima, Masayoshi (a)
"We found novel Ca^{2+}-binding proteins in *A. thaliana*: namely, CCaP1 (cytoplasmic Ca^{2+}-binding protein), CCaP2 and CCaP3, which are composed of 152, 138 and 95 amino acid residues (Plant Cell Physiol., 2007). These three proteins have no common motif found in other proteins or enzymes. Three CCaP proteins are localized in the cytoplasm when expressed as GFP-fusion proteins. The promoter-GUS analysis revealed that *CCaP1* was predominantly expressed in petioles and *CCaP2* and *CCaP3* in roots. In this study we examined effect of the growth conditions on the expression of CCaP genes. When plants were grown in the dark for >20 h, the mRNA levels of CCaP1 and CCaP2 were increased approximately 10-fold of that of the daytime level. These high levels were decreased to the daytime level by light illumination for <5 h. We also examined the transcription levels in *phy* and *cry* mutants individually and found that the expression of CCaPs was partially affected by these light-related genes. The same responses of CCaP genes to the dark were observed in germinating seedlings. Then we tested the effect of photosynthesis and senescence on their gene expression. A photosynthesis inhibitor (DCMU) enhanced the expression of CCaP1 and a senescence inhibitor (cytokinins) suppressed the expression of CCaP2 in the dark, suggesting that expression of *CCaP1* and *CCaP2* is under regulation of photosynthesis and senescence, respectively. To examine the shoot-root relationship in the enhancement of CCaP2 expression in the dark, we cut shoots off and quantified the mRNA. There was no induction of *CCaP2*. Now we are trying to determine the wavelength of light required for suppression of transcription and the gene-expression profiles in *ccap1*, *ccap2*, and their over-expression mutant lines."
(a) Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan (b) Graduate School of Science, Kyoto University, Kyoto, Japan

P27019 "Leaf gas exchange, antioxidant and yield responses of field-grown alfalfa (*Medicago sativa* L.) subjected to water deficit"
Maruthavanan, Janakiraman-presenter johnnym@nmsu.edu(a) Klypina, Nina (a) Ray, Ian (a) Sterling, Tracy (a)
"Water deficit is a major environmental factor limiting crop production in arid/semi-arid regions. The effect of water deficit on water relations, gas exchange, antioxidants, shoot and root biomass yield was characterized by withholding irrigation on six alfalfa (*Medicago sativa* L.) accessions varying in water use efficiency and yield potential, grown near Las Cruces, NM in 2006 and 2007. Well watered (baseline) measurements were taken on non-stressed plants within 5-10 days after irrigation (DAI) and water deficit (Stress-1 & 2) on visibly wilting plants between 15-20 DAI in successive growth cycles. In both years, baseline gas exchange was similar across alfalfa accessions, but varied under water deficit, dropping by 18 to 53 %. Levels of antioxidants increased under water deficit conditions. Alfalfa accessions expressed conserved drought response, where decreased net photosynthesis was associated with limited water availability, increasing leaf temperature and vapor pressure deficit, decreasing stomatal conductance and transpiration; non-stomatal factors like limitations in phosphorylation, RuBP regeneration and Rubisco activity may also have significant role. Increased antioxidants accumulation might be associated with photoprotection of photosystem II under excess light. Water deficit reduced shoot biomass between 65 to 80 % regardless of alfalfa accessions. Wilson had highest shoot and root biomass, while Falcata and MFC 192 had lowest shoot and root biomass, indicating that drought tolerance may be conferred to plants with extensive deep root system. Further, root

carbohydrate and amino acid analysis might reveal their osmoprotective role under drought."
(a) New Mexico State University

P27020 "Physiological and molecular changes in *Oryza meridionalis* Ng., a heat-tolerant species of wild rice"
Atwell, Brian J-presenter batwell@rna.bio.mq.edu.au(a) Scafaro, Andrew P (a) Haynes, Paul A (a)
"*Oryza meridionalis* Ng. is a wild relative of *Oryza sativa* L. found throughout northern Australia where temperatures regularly exceed 35°C in the monsoonal growing season. The tolerance of *O. meridionalis* to high temperatures was tested by exposing seedlings to 45°C for 24 h and comparing growth and photosynthetic rates with rates in *O. sativa spp. japonica* cv. Amaroo. Elongation rates of the third leaf of *O. meridionalis* declined by 47% over 24 h at 45°C compared with a 91% decrease for *O. sativa*. Net photosynthesis was significantly higher in *O. sativa* at 27°C whereas the two species had the same assimilation rates at 45°C. The leaf proteome and expression levels of individual heat responsive genes provided insight into the heat response of *O. meridionalis*. After 24 h of heat exposure many enzymes involved in the Calvin Cycle were more abundant, while mRNA of their genes generally decreased. On the other hand, a key enzyme in photosynthetic electron transport had both reduced abundance and gene expression, suggesting that the light reaction was highly susceptible to heat stress. Rubisco activase was strongly up-regulated after 24 h of heat, with the large isoform particularly affected. The protective proteins Cpn60, Hsp90 and Hsp70 all increased in both protein abundance and gene expression."
(a) Macquarie University

SESSION P28 – EPIGENETICS

P28001 Broad impact of gene associated transposable elements in epigenetic gene regulation
Li, Feng-presenter lifeng@berkeley.edu(a) Baker, Babara (a,b)
"Transposable Elements (TE) are genetic parasites and proliferate by duplicating themselves and inserting into host genome. In higher Eukaryotes such as human, maize and rice, over half of their genetic materials consist of TEs and their derivatives. Mobilization of TE is deleterious to the host because TE insertion could disrupt an essential gene function for survival. Recently RNA silencing pathway has been extensively investigated as a mechanism controlling TE activity in higher Eukaryotes. During RNA silencing, TE specific small RNAs, such as siRNAs or piRNAs, guide silencing machinery to shut down the expression of TE gene at either transcriptional or post-transcriptional level, consequently blocking TE transposition. However the importance of TE small RNA mediated host gene regulation is much under appreciated. Miniature Inverted-Repeated Transposable Elements (MITE) and Short Interspersed Nuclear Elements (SINE) are non-autonomous TE, most of which lost autonomous partner and are no longer transposable. We found MITE and SINE are amplified to high copy number during the evolution and associated with thousands of host genes in potato and tobacco plants, for which we name them gene associated transposable element (GATE). Using High-through-put sequencing, genetic and molecular approaches, we showed that GATE small RNAs regulate their nearby genes through RNA silencing pathway, most of which are stress and development responsive. Significantly GATE insertions in many of these genes are conserved cross species. Our data indicate that TE insertion is not always bad to their host but also an important driving force to create new gene regulatory mechanisms during evolution."
(a) University Of California Berkeley (b) Plant Gene Expression Center, ARS, USDA

P28002 Defining the Combinatorial Interplay of Chromatin Modifiers in the Epigenetic Network
Lam, Eric-presenter ericL89@hotmail.com(a) Luo, Chongyuan (a) Watanabe, Naohide (a) Durgin, Brittany (a)
http://aesop.rutgers.edu/~lamlab/
"Epigenetic regulators (epi-regulators) do not choose their targets randomly in the genome. How epi-regulators are appropriately directed to their proper targets in the genome across development stages remains unclear. Genomic location might be one of the determinants that affect the recruitment of epi-regulators. To test this possibility, we took advantage of Chromatin Charting (CC) Lines, each of which harbors an identical transgene cassette integrated at different locations in the Arabidopsis genome. Some lines display epigenetic silencing of maker genes (Luciferase and/or NPTII). We searched for epi-regulators that are involved in transgene silencing in 4 selected CC lines that contain insertions within a 100 kbp region of Chr2 by suppressing 8 candidate regulators (LHP1, MOM1, CMT3, DRD1, DRM2, SUVH2, CLF and HD1) systematically. We discovered that the integration position of transgenes could influence the 'choice' of different subsets of epi-regulators that are responsible for the observed epigenetic silencing. Also, the relative importance of a particular epi-regulator can vary among tissues (shoot and root), with regard to silencing transgene targets. Lastly, using molecular phenotypes (altered expression of transgenes and endogenous loci) induced by suppression of epi-regulators, we suggested a novel and high-throughput method in predicting interaction between epi-regulators via hierarchical clustering of regulators based on similarities between molecular phenotypes. "
(a) Rutgers, State University of New Jersey

P28003 Genome-wide gene expression analysis revealed multiple function of *HDA6* in *Arabidopsis*
Yu, Chun-Wei-presenter d95b42004@ntu.edu.tw(a) Wu, Keqiang (a)
"Histone acetylation and deacetylation play an important role in epigenetic controls of gene expression. HDA6 is a RPD3-type histone deacetylase in *Arabidopsis*,which is known to control transgene and rRNA gene expression, DNA methylation, jasmonate response, senescence and flowering in *Arabidopsis*. In this study, we analyzed genome-wide gene expression of *HDA6* RNA-interfering plants using Affymetrix GeneChiP. Compared with wild-type plants, it was found that 438 genes were up-regulated and 46 genes were down-regulated in *HDA6* RNA-interference plants. Genes involved in flowering, gene silencing and stress response as well as some transposable elements showed increased expression and histone hyperacetylation in *HDA6* mutant plants, suggesting that HDA6 controls these genes expression by deacetylating their chromatins. A specific subset of flowering related gene including the major flowering repressor, *FLOWERING LOCUS C (FLC),* was up-regulated in *HDA6* mutant plants indicating that HDA6 play an important role in flowering. The flowering time of *axe1-5/flc-3* double mutants was earlier than that of *axe1-5* plants, indicating that the late-flowering phenotype of *axe1-5* was FLC dependent. In addition, we have identified several genes as the potential targets of HDA6."
(a) National Taiwan University,Institute of Plant Biology

P28004 *Arabidopsis* actin-related protein ARP5 in multicellular development and DNA repair
Kandasamy, Muthugapatti K-presenter kandu@uga.edu(a) Elizabeth, McKinney C (a) Richard, Meagher B (a)
"ACTIN-RELATED PROTEIN5 (ARP5) is a conserved subunit of the INO80 chromatin-remodeling complex in yeast and mammals. We have characterized the expression and subcellular distribution of *Arabidopsis thaliana* ARP5 and explored its role in the epigenetic control of multicellular development and DNA repair. ARP5-specific monoclonal antibodies localized ARP5 protein to the nucleoplasm of interphase cells in *Arabidopsis* and *Nicotiana tabacum*. ARP5 promoter-reporter fusions and the ARP5 protein are ubiquitously expressed. A null mutant and a severe knockdown allele

produced moderately dwarfed plants with all organs smaller than wild type. The small and slightly deformed organs such as leaves and hypocotyls were composed of small sized cells. The ratio of leaf stomata to epidermal cells was high in the mutant, which also exhibited a delayed stomatal development compared to wild type. Mutant plants were hypersensitive to DNA damaging reagents including hydroxyurea, methylmethane sulfonate and bleocin demonstrating a role for ARP5 in DNA repair. A wild type transgene fully complemented all developmental and DNA repair mutant phenotypes. Despite the common participation of both ARP4 and ARP5 in the INO80 complex, ARP4- and ARP5-deficient plants displayed only a small subset of common phenotypes and each displayed novel phenotypes suggesting that in *Arabidopsis* they have shared and unique functions."
(a) University of Georgia

P28005 Repression of *FT* chromatin by functionally redundant histone H3 lysine 4 demethylases in *Arabidopsis*
Jeong, Ju-Hee-presenter jjh30096@snu.ac.kr(a,b) Song, Hae-Ryong (a,b) Ko, Jong-Hyun (a,b) Jeong, Young-Min (a,b) Kwon, Young-Eun (a) Seol, Jae-Hong (a) Amasino, Richard M. (b,d) Noh, Bosl (b,c) Noh, Yoo-Sun (a,b)
"Methylation is an important posttranslational modification of histone proteins that affects chromatin-based processes including transcription, DNA repair, X-chromosome inactivation, and epigenetic inheritance. Until recently, histone methylation has been considered an irreversible modification, but the identification of histone demethylases has revealed that this modification can be dynamically regulated. So far, the majority of histone demethylases characterized have come from mammals and yeasts. The largest group of these histone demethylases shares the Jumonji (Jmj) C domain as their catalytic core. However, little has been known on the role of Jmj proteins in *Arabidopsis*. There are 21 genes encoding Jmj proteins in the *Arabidopsis* genome. Our previous studies have shown that two of these Jmj proteins, EARLY FLOWERING6 (ELF6) and RELATIVE OF ELF6 (REF6), have distinctive roles in the regulation of floral transition through affecting the photoperiod pathway and *FLOWERING LOCUS C*, respectively. Here we report that another *Arabidopsis* Jmj protein is involved in photoperiodic flowering by affecting the transcription of *FT*. We found that the early flowering phenotype of the mutant lacking this protein is accelerated by an *elf6* mutation. Furthermore, this Jmj protein as well as ELF6 associated directly with *FLOWERING LOCUS T* (*FT*) chromatin. Trimethylation levels of histone H3 lysine 4 (H3K4) at *FT* locus were increased in these *Arabidopsis jmj* single and double mutants, indicating that these two *Arabidopsis* Jmj proteins have redundant roles as H3K4 demethylases at *FT* locus. Consistent with this, the Jmj protein showed an H3K4-specific demethylase activity *in vitro*. "
(a) School of Biological Sciences, Seoul National University, Seoul 151-747, Korea. (b) Global Research Laboratory for Floral Regulatory Signaling, Seoul National University, Seoul 151-747, Korea. (c) Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju 660-701, Korea. (d) Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706, USA.

P28006 "Role of *unstable factor for orange1*, a novel epigenetic modifier in histone modifications at *pericarp color1* of maize"
Wang, PoHao-presenter puw116@psu.edu(a) Surinder , Chopra (a)
"Epigenetic gene regulation is associated with DNA methylation, chromatin modification, and small RNA based silencing. In this study, we address epigenetic mechanism involved in gene regulation of *pericarp color1* (*p1*) gene of maize. *p1* gene encodes a Myb transcription factor and regulates the accumulation of phlobaphenes (flavonoid pigment) in floral tissues. *P1-wr*, one of *p1*'s natural alleles, has a multicopy gene structure that is transcriptionally regulated by a mechanism correlated with extensive DNA methylation and results in tissue-specific pigmentation. *P1-wr* plants thus have white pericarp and red cob glume phenotype. Additionally, *Unstable factor for orange1 (Ufo1)*,a spontaneously dominant mutation, is a trans-acting modifier, and upregulates the expression of *P1-wr* leading to enhanced accumulation of phlobaphenes in floral tissues. Evidence also shows that *Ufo1* is involved in epigenetic regulation of *P1-wr* and its epiallele *P1-wr**, as well as epialleles of *P1-rr*, and *P1-pr*^{TP}. DNA blot and bisulfite sequencing data from *P1-wr* and *P1-wr Ufo1* plants show that increased phlobaphene accumulation correlates with increased transcription and decrease in DNA methylation of *P1-wr*. Evidence has shown that DNA methylation and histone modification usually interplay each other and regulate the gene expression collectively. In this study, we investigate the role of *Ufo1*-mediated epigenetic mechanisms on the expression of *P1-wr* allele and other *p1* epialleles (*P1-wr**, *P1-rr* and *P1-pr*^{TP}) in histone modification. Our result suggests that wildtype *ufo1* regulates *P1-wr* tissue-specific expression by histone modification as well as DNA methylation, which implicates that *Ufo1* might alter the chromatin structure of *P1-wr* leading to transcriptionally reactivation. "
(a) Dept. of Crop and Soil Sciences, Penn State University

SESSION P29 – EVOLUTION OF DEVELOPMENT & PHYSIOLOGY

P29001 "Polar Auxin Transport in the Moss *Polytrichum ohioense*: Developmental Regulation, Anatomical Connections and Evolutionary Implications"
Poli, DorothyBelle-presenter poli@roanoke.edu(a) Cooke, Todd J. (b)
http://faculty.roanoke.edu/poli/
"Bidirectional auxin transport has been most recently characterized at a molecular level in roots of higher plants. We characterized the bi-directional auxin transport in older moss sporophytes using a modified agar-block technique which accommodates the small cross-sectional area of the moss sporophyte and its subsequent tissue types. Because acropetal transport in sporophytes was significantly reduced from previous years, we investigated the fluxes and polarities of auxin transport occurring in different tissues at different developmental stages. When radiolabeled auxin was applied as an apical source and collected at the basal end, young seta exhibited basipetal auxin transport in the cortical sections and vascular regions, resulting in overall basipetal auxin transport in the whole seta. However, in older seta (early capsule stage) stronger basipetal transport was observed in the cortical region with acropetal backflow occurring in the vascular region resulting in a much decreased overall basipetal polarity in the whole seta. When the experiment was reversed to have a basal source and an apical sink, young seta had no net transport while older seta showed slight acropetal transport in the cortical region and whole seta. These experiments suggested that polar auxin transport is important in moss sporophyte development and have strong connection to metabolic transport in this species. "
(a) Roanoke College, Department of Biology (b) University of Maryland, Cell Biology and Molecular Genetics Department

P29002 Studying the Evolution of Uptake and Efflux of Auxin and the Polar Auxin Transport in Land Plant Gametophytes
Bader, Geoffrey A-presenter gabader@roanoke.edu(a) Poli, DorothyBelle (a)
"Previous research involving bryophyte sporophytes and auxin has already had an impact on understanding the role of auxin in the evolution of land plants (Poli et al., 2003). Bryophytes have been shown to exhibit many auxin controlled responses found in vascular plants, such as tropisms and apical dominance (Cooke et al., 2002). This research has indicated that auxin played a critical role in the development and shape in all land plant sporophytes. My research on basal plants during the gametophyte stage addresses a number of important issues. Paramount to the research is determining if gametophytes can influx IAA from the environment, and efflux it back out, in order to maintain equilibrium. It appears that both the hornwort *Anthoceros* and the liverwort *Riccia* exhibit simple diffusion of auxin. At this time, we are still analyzing polar auxin transport in the gametophytes. This research point is critical because the development of polar auxin transport could have led to the evolution of flat, thalloid plants

into upright and erect sporophytes that maintain a constant upward axis (Poli et al, 2003) and this research will determine if gametophytes use auxin in a similar manner as higher plant sporophytes."
(a) Roanoke College

P29003 A genomic approach to the molecular basis of evolutionary novelty in flower structure and development in Passifloraceae
Dornelas, Marcelo C.-presenter dornelas@unicamp.br(a) Cutri, Lucas (a) Aizza, Lilian C.B. (a) Fonseca, Tamara c (a) Tsai, Siu M (b) Martinelli, Adriana P. (b)
"Advances in developmental genetics and evolutionary developmental biology have recently shed light on the molecular mechanisms controlling the origins of evolutionary novelty and on the maintenance of naturally occurring developmental variation. The genus *Passiflora* provides a remarkable example of floral complexity and diversity. Comprising about 600 species, this genus exhibits several unique floral features, including multiple series of brightly colored coronal filaments, diverse operculum morphology, an androgynophore, and elaborate floral nectary structures. This extreme variation of flower morphologies allowed a wide range of interactions with pollinators to evolve. Our long-term goal is to uncover patterns of conservation and divergence of the floral transcriptome among *Passiflora* species, particularly to elucidate the role of gene duplications and shifting expression patterns in the origin and diversification of different floral forms and structures present in the genus. With this aim, we have obtained about 10,000 ESTs from flower cDNA libraries of two divergent *Passiflora* species: *P. suberosa* L. and *P. edulis* var. flavicarpa Deg. within the frame of the PASSIOMA Project. Among these ESTs we have identified members of MADS-box family of transcription factors, known to control the identity and development of floral organ in model species. We have studied the expression pattern of some of these genes by using RT-PCR and in situ hybridization. We hope that the analysis of natural interspecific variation, using a genomic approach, is likely to deliver interesting insights on the molecular basis of naturally occurring and evolutionary meaningful developmental modifications during *Passiflora* flower development. "
(a) Universidade Estadual de Campinas UNICAMP (b) Universidade de Sao Paulo USP

P29004 Unilateral Incongruity in Tomato: Role of Self-Incompatibility Factors
Bedinger, Patricia A.-presenter bedinger@colostate.edu(a) Covey, Paul A. (a) Kondo, Katsuhiko (b) Kumar, Aruna (b) Welch, Lilli (a) Frank, Eric (a) van der Knaap, Esther (c) Lopez Casado, Gloria (d) Rose, Jocelyn (d) McClure, Bruce (b)
"Self-Incompatibility (SI), wherein self pollen is rejected by styles, is widespread in plants and functions to prevent inbreeding. In gametophytic SI, RNases encoded at the *S*-locus (*S*-RNases), are the female SI determinants. Apart from specificity determinants, additional pistil factors are required for SI, including the asparagine-rich HT-family proteins. Interspecific pollen rejection is less well understood than intraspecific SI. Often interspecific pollinations are only successful in one direction; this phenomenon is known as unilateral incongruity or incompatibility (UI). In tomato, genetic studies of *Solanum pennellii* X *S. lycopersicum* and *S. habrochaites* X *S. lycopersicum* crosses have directly implicated the S-locus in UI, but the role of SI proteins in UI is complex. We examined the mode of pollen tube rejection and assessed the potential role of SI genes in crosses between wild tomato species. We find that there are at least two modes of interspecific pollen rejection; rapid (in the upper 15% of the style) and slow (in the lower half of the style). Neither mode necessarily requires high levels of S-RNase expression. Two asparagine-rich *HT*-family genes, *HT-A* and *HT-B*, are tightly linked and map to a UI QTL on Chromosome 12. While *HT-A* is functional in all wild tomato accessions tested, the *HT-B* gene contains a point mutation that should eliminate expression in all tested accessions of *S. habrochaites*, regardless of whether plants were self-compatible or self-incompatible. Proteomic analysis supports this conclusion. Therefore, neither *S*-RNases nor HT-B protein appear to be essential for UI pollen rejection in wild tomato relatives, but HT-A protein and *S*-locus encoded factors other than *S*-RNases may play important roles in UI."
(a) Colorado State University (b) University of Missouri-Columbia (c) Ohio State University (d) Cornell University

P29005 Ethylene Production in Plants: Is the ACC oxidase enzyme at work in *Selaginella moellendorffii*?
DeCarme, Ashley R.-presenter ardecarme@wm.edu(a) Engstrom, Eric M. (a) Givens, Chris S. (a)
"Ethylene is a plant hormone that plays important roles in development and stress responses. Recent research has attempted to show that basal plants do not produce ethylene using the same pathway as seed plants--a pathway that involves the enzyme ACC oxidase (ACO) in its final step--but conclusive data is lacking. From recently released genomic data, we believe that the ACO enzyme is used in the same capacity in basal and seed plant lineages. Our research will investigate the presence and function of the ACO gene in a fully sequenced basal plant, the lycophyte *Selaginella moellendorffii*. We will conduct phylogenic analysis of several newly identified putative ACO orthologs alongside ACO genes and other dioxygenase genes from a variety of plant families. Using genes grouped as ACOs, we will continue verification that all genes identified as putative ACO orthologs are translated. Finally, we will confirm functional identity of each putative ACO through a complementation assay with a mutant *A. thaliana* lacking functional ACO genes. Through these steps, we will be able to show whether or not *S. moellendorffii* contains a similarly functioning version of an enzyme central to the seed plant ethylene biosynthesis pathway and furthermore, likely uses the same pathway entirely. As related work is completed in other basal lineages, it will be possible to identify the evolutionary point(s) of origin of the ACO enzyme and related pathway in plants. In addition, similar though they may be, the millions of years of divergence between the lycophyte ACO and seed plant ACO have almost certainly led to differences that could potentially be exploited by crop scientists. Our preliminary characterization of *S. moellendorffii* ACO will serve as a basis for further research in this direction. "
(a) College of William and Mary

P29006 Regulatory evolution of stress responsive gene duplicates in *Arabidopsis thaliana*
Zou, Cheng (a) Lehti-Shiu, Melissa D (a) Mackaluso, Joshua D (a) Shiu, Shin-Han-presenter shius@msu.edu(a)
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"Stress sensing and response mechanisms in plants are expected to evolve rapidly due to the selection pressure imposed by highly variable environmental conditions. One potential source of innovation in stress responses is gene duplication. In this study, we examined the evolution of stress responses among duplicated genes in *Arabidopsis thaliana* by integrating phylogenomic and expression data. We found that accelerated evolution of stress responses occurred right after gene duplication and was followed by stasis for an extended period of time. When we considered duplicated gene pairs, partitioning of ancestral functions (one duplicate is responsive to a particular stress condition while the other is not) occurred significantly more frequently compared to cases of parallel retention and loss. Furthermore, the pattern of stress response partitioning is extremely asymmetric. Only one duplicate kept most if not all of the ancestral stress responses. We also found a significant positive correlation between stress response and cis-element asymmetries. Duplicates losing most of their stress responses are also the ones with the most cis-element losses in their promoter regions. Furthermore, duplicate copies inheriting few or no ancestral responses also tend to be the ones with stress response gains, indicating that neofunctionalization likely contributed to their retention. Our findings provide important insight into the patterns and mechanistic basis of evolutionary changes in plant stress responses and lay the foundation for testing the adaptive significance of stress regulatory changes under biotic and abiotic environments."
(a) Michigan State University

P29007 Accession-dependent genetic regulation of bolting time in the *Arabidopsis* mutants with increased leaf number

Yu, Si-in-presenter siin0311@sogang.ac.kr(a) Lee, Byeong-ha (a)

"Leaves are the major lateral organs that determine the plant architectures in herbaceous plants and mainly develop during vegetative stage by the activities of shoot apical meristem. There is a strong correlation between leaf number and bolting, a characteristic phenotype during the transition to reproductive phase in *Arabidopsis thaliana*. In order to study interactions between leaf number and bolting, we isolated a Landsberg *erecta*-derived mutant named *multifolia1* (*mfo1*) that produces increased number of leaves and bolts at the same time as the wild type. Through positional cloning and genetic complementation, *mfo1* was found to be an allele of a previously reported mutant, *altered meristem program1-1* (*amp1-1*). *amp1-1* is defective in a glutamate carboxypeptidase, develops more leaves, and bolts earlier than its background, Columbia accession. Despite the same phenotype of many leaves, the bolting time differences between *mfo1* and *amp1-1* in comparison to its own background accession suggest the existence of genetic factor(s) differently function in each accession in the presence of *mfo1*|*amp1* mutation."

(a) Department of Life Science, Sogang University

P29008 Evolution of *Cellulose Synthase-like D* Genes in Plants: Gene Duplication and Its Relationship with Functional Redundancy and Divergence

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"Gene duplication plays a very important role in evolution by providing raw genetic materials for evolutionary selection and allowing subsequent functional divergence and innovation. In plants, duplicate genes have often been reported to exhibit functional redundancy, though few were found to be functionally divergent. Our recent genetic and cell biology studies show that the *Arabidopsis CSLD2* (*Cellulose Synthase Like D2*) and *CSLD3* genes are redundant during female reproductive development but are functionally divergent in root hair formation. To provide explanation for these phenotypes, we performed phylogenetic and gene duplication studies using sequences of all *AtCSLD*s (excluding *AtCSLD6* which seems to be a pseudogene) and their homologs in *Populus*, rice, *Physcomitrella* and *Selaginella*. Our result indicates that the ancestor of land plants could possibly contain only two copies of the *CSLD* genes, one of which developed into the *CSLD5* lineage in flowering plants and the other of which formed the *CSLD1/2/3/4* clade in land plants. In addition, we found evidence that the formation of *AtCSLD2* and *AtCSLD3* resulted from the most recent genome-wide duplication in *Arabidopsis*, suggesting that these two genes could have retained similar functions. Moreover, our sliding-window dN/dS analysis shows that most regions of *AtCSLD2* and *AtCSLD3* genes have been under strong purifying selection pressure since they were duplicated. However, the region that encodes the N-terminus of AtCSLD3 has been under relatively relaxed selection pressure by showing high dN/dS value, suggesting that AtCSLD3 might have gained new function through more sequence changes at the N-terminus. Therefore, *AtCSLD3* might be able to play a more important role in root hair development than *AtCSLD2*."

(a) The Samuel Roberts Noble Foundation, Plant Biology Division

SESSION P30 – GENE REGULATION MECHANISMS

P30001 The involvement of protein phosphatase 2A in ABA signaling transduction pathway
Hu, Rongbin-presenter rongbin.hu@ttu.edu(a) Zhu, Yinfeng (a) Zhang, Hong (a)
"Protein phosphatase 2A (PP2A) plays important roles in signal transduction pathways in animal cells. It serves as a tumor suppresser in humans, which highlights its role in maintaining cell homeostasis in animal systems. The functions of PP2A in plants were shown to be associated with actions that involve plant hormones auxin, abscisic acid (ABA) and ethylene. Like animal PP2A, plant PP2A is also made of three subunits: the scaffolding subunit A, the regulatory subunit B and the catalytic subunit C. It appears that the B subunit is the one that should specify the function for a particular PP2A trimeric combination. In order to study the function of PP2A in plant hormone signaling pathways, we decided to explore how PP2A is involved in ABA signaling pathways. We analyzed the function of one B subunit by studying its knockout mutant and its overexpression plants. We found that overexpression of this B subunit gene in Arabidopsis could make transgenic plants more sensitive to ABA treatment, indicating that this B subunit mediates a positive response in ABA signaling pathway. Furthermore, the overexpression plants also display insensitive phenotype to salt treatment, whereas the knockout mutant is less sensitive to chilling temperature than wild-type and overexpression plants at seedling stage. Our work with this B subunit gene indicates that PP2A is indeed involved in ABA signaling pathway and in abiotic stress response in plants. "
(a) Texas Tech University

P30002 Expression of the guar mannan synthase promoter in transgenic alfalfa
Naoumkina, Marina A.-presenter manaoumkina@noble.org(a) Vaghchhipawala , Sanchita (a) Dixon, Richard A. (a)
"Guar (*Cyamopsis tetragonaloba* L. Taub), a drought-resistant annual legume, is one of the most important commercial sources for seed gums. The viscosity and thickening properties of guar gum have found broad applications in different industries. The main consumer of guar gum is the petroleum industry, which uses it as an additive to reduce fluid friction during the drilling of oil wells. Guar galactomannan has been preferred to other galactomannan preparations for industrial applications due to its low cost. The desire to increase the viscosity of the gum has led to development of chemical derivatives which, however, are significantly more expensive. The properties of the polymer can potentially be enhanced by genetic modification. The mechanism of galactomannan biosynthesis has been well studied in the endospermic legumes, where it is synthesized in the endosperm of seed by the co-action of two membrane-bound enzymes, mannan synthase (MS) and galactosyl transferase. Isolation of an endogenous endosperm specific promoter will be desirable for genetic engineering of galactomannan metabolism in guar to prevent off-target genetic and metabolic perturbations in the plant. A 1.5 kb guar MS promoter region has been isolated. Skn-1 and gibberellin-responsive regulatory elements were determined to be present in this sequence; these elements were required for endosperm specific expression in rice. *GFP* and *GUS* genes driven by the MS promoter were introduced into alfalfa (*Medicago sativa*) to test the issue specificity of the isolated promoter. Alfalfa has been chosen as a readily transformable legume that contains galactomannan in the seed endosperm. The resulting expression pattern will be described, and the implications discussed for galactomannan engineering. "
(a) The Samuel Roberts Noble Foundation

P30003 Cell-specific transcript accumulation in a model C_4 plant *Cleome gynandra*
Kajala, Kaisa-presenter kk325@cam.ac.uk(a) Hibberd, Julian M (a)
"C_4 photosynthesis concentrates CO_2 around RuBisCO and reduces the rate of photorespiration. The initial fixation of carbon, the decarboxylation reaction and the regeneration of the substrate are spatially distributed between two specialised cell types, mesophyll (M) and bundle sheath (BS), and to achieve this the expression of key genes is cell-specific. We are interested in the evolution of the regulation of cell-specific gene expression and are studying *Cleome gynandra* (Brown et al., 2005, TIPS 10:215-221; Marshall et al., 2007, Plant J 51:886-896; Voznesenskaya et al., 2007, Funct Plant Biol 32:247-267), the closest known C_4 relative of *Arabidopsis* (C_3). We have obtained cell-specific mRNAs from both M and BS cells by laser capture microdissection (LCM), and qPCRs have confirmed expected cell-specific accumulation patterns of transcripts of C_4 key genes. Illumina gene expression tag sequencing of cell-specific cDNAs and comparison to recent EST sequencing of *C. gynandra* (Brautigam, A., Kajala, K., *et al., in prep*) has allowed us to generate large-scale insight into cell-specific transcripts. Furthermore, we have identified elements in the untranslated regions (UTRs) that play a role in post-transcriptional regulation of certain cell-specific transcripts. Taking a comparative approach between *C. gynandra* and *Arabidopsis* allows us to identify which components have been altered during the evolution of C_4 photosynthesis in *C. gynandra*. "
(a) Department of Plant Sciences, University Of Cambridge

P30004 Regulation of epidermal cell patterning by GLABRA2 and single-repeat R3 MYB transcription factors in Arabidopsis
Wang, Shucai-presenter wangshucai@yahoo.com(a) Chen, Jin-Gui (a)
"Trichome and root hair cell patterning in Arabidopsis is controlled by several different types of transcription factors. It has been proposed that an R2R3 MYB-type transcription factor, GL1 or WER, a bHLH transcription factor (GL3 or EGL3), and a WD-repeat protein TTG1 form a complex to induce the expression of both *GL2*, encoding a homeodomain protein, and single-repeat R3 MYB genes, including *TRY*, *CPC*, *TCL1*, *ETC1*, *ETC2* and *ETC3*. We confirmed that a complex between GL1 or WER and GL3 or EGL3 is required and sufficient to induce the expression of *GL2* and a subset of single-repeat R3 MYB genes. However, available genetic evidence supports that the GL2 and single-repeat R3 MYB transcription factors have opposite roles in epidermal cell patterning. To further investigate the roles of GL2 and single-repeat R3 MYB transcription factors in epidermal cell patterning, we generated double and higher order mutants between *gl2* and single-repeat R3 MYB mutants, and examined trichome and root hair patterning. We found that in all mutants between *gl2* and single-repeat R3 MYB mutants, trichome number was dramatically reduced and trichome morphology changed, compared with single-repeat R3 MYB mutants. On the other hand, mutants between *gl2* and single-repeat R3 MYB gene mutants phenocopied that *gl2* single mutants in root hair patterning and mucilage formation. Taken together, these findings revealed distinct relationships between GL2 and single-repeat R3 MYBs in the regulation of trichome and root hair patterning in Arabidopsis. "
(a) Department of Botany, University of British Columbia, Vancouver, BC, V6T 1Z4

P30005 "The Arabidopsis Kelch domain-containing protein, AtKelch, is positively involved in ABA signal transduction pathway"
Zhu, Yinfeng-presenter yinfeng.zhu@ttu.edu(a) Shen, Guoxin (a) Zhang, Hong (a)
"In our effort to identify substrate proteins for a stress-related E3 ligase AtCHIP, we found a Kelch-domain containing protein, AtKelch, which might be a substrate protein of AtCHIP. The Kelch domain is made of up to 50 amino acid residues that were named after a Drosophila mutant protein. We studied AtKelch expression by fusing the promoter of *AtKelch* to the *<beta>-glucuronidase* gene and analyzed the GUS activity during Arabidopsis growth and development. It appears that AtKelch expression is both developmentally and spatially regulated. We further studied the function of AtKelch by analyzing *AtKelch*-antisense plants and *AtKelch*-overexpressing plants. The *AtKelch*-antisense plants displayed chlorotic phenotype, indicating that AtKelch is required for chloroplast function. Furthermore, these *AtKelch*-antisense plants displayed insensitive phenotype to ABA treatment. On the contrary, *AtKelch*-overexpression made Arabidopsis more sensitive to ABA treatment, indicating that AtKelch is positively involved

in ABA signal transduction pathway. Moreover, when *AtKelch* is overexpressed in ABA insensitive mutant *abi2-1*, the ABA-induced seed dormancy, suppression of root elongation, inhibition of vegetative growth and stomatal closure in *abi2-1* was partially rescued in comparing to wild-type plants, clearly indicating that AtKelch plays a critical role in ABA signal transduction pathway, and AtKelch might function upstream of ABI2 in Arabidopsis."
(a) Texas Tech University

P30006 Two alternatively spliced isoforms of the Arabidopsis thaliana SR45 protein have distinct roles during normal plant development
Zhang, Xiao-Ning-presenter xnzhang@umd.edu(a) Mount, Stephen M (a)
"The serine-arginine-rich (SR) proteins constitute a conserved family of pre-mRNA splicing factors. In Arabidopsis thaliana, they are encoded by 19 genes, most of which are themselves alternatively spliced. In the case of SR45, the use of alternative 3' splice sites 21 nucleotides apart generates two alternatively spliced isoforms. Isoform 1 (SR45.1) has an insertion of seven amino acids (TSPQRKTG in place of arginine) relative to isoform 2 (SR45.2). The biological implications of SR45 alternative splicing have been unclear. A previously described loss-of-function mutant affecting both isoforms, sr45-1, shows several developmental defects, including defects in petal development and root growth. We found that the SR45 promoter is highly active in regions with actively growing and dividing cells. We also tested the ability of each SR45 isoform to complement the sr45-1 mutant by overexpression of isoform-specific GFP fusion proteins. As expected, transgenic plants overexpressing either isoform displayed both nuclear speckles and GFP fluorescence throughout the nucleoplasm. We found that SR45.1-GFP complements the flower petal phenotype, but not the root growth phenotype. Conversely, SR45.2-GFP complements root growth but not floral morphology. Mutation of a predicted phosphorylation site within the alternatively spliced segment, SR45.1-S219A-GFP, does not affect complementation. However, a double mutation affecting both Serine 219 and the adjacent Threonine 218 (SR45.1-T218A-S219A-GFP) behaves like isoform 2, complementing the root but not the floral phenotype. In conclusion, our study provides evidence that the two alternatively spliced isoforms of SR45 have distinct biological functions."
(a) University of Maryland

P30007 Rejuvenation of mature shoots of *Sequoia sempervirens* in vitro : changes in DNA methylation and genome arrangement
Huang, Li-Chun -presenter bolch@gate.sinica.edu.tw(a) Huang, Hao-Jen (b) Murashige, Toshio (c)
"The complicated mechanisms of plant phase change that regulate cellular events are still poorly understood. Gene expression of genomewide DNA arrangements and methylation changes occur in many eukaryotes during development. Here, we examined the DNA from nucleus and mitochondria obtained from cultured Sequoia sempervirens shoots of the juvenile, adult and rejuvenlated tissues. Juvenile and rejuvenile individuals were characterized by a degree of nuclear DNA methylation of 6.5-7.3%, while mature shoots had 8.6% methylcytosine. In addition, there was a gradual increment in extent of mitochondrial DNA methylation as the degree of reinvigoration increased. The changes in nuclear DNA structure were demonstrated by Amplified Fragment Length of Polymorphisms (AFLP) and southern blot. We suggest that both indicators may be associated with the loss of physiological, biochemical and morphogenic abilities during phase change, through a number of molecular interactions, which are substantially discussed. "
(a) Institute of Plant Microbial Biology, Academia Sinica (b) Department of Biology, National Cheng-Kung University (c) Botany and Plant Sciences, University of California at Riverside

P30008 "An rbcL mRNA binding protein present in C4 and C3 plants regulates chloroplastic synthesis of Ribulose 1,5-bisphosphate (Rubisco) large subunit"
Berry, James O-presenter camjob@buffalo.edu(a) Patel, Minesh (b) Zielinski, Amy M (a) Bowman, Shaun M (a)
"In leaves of most C4 plants, ribulose 1,5 bisphosphate carboxylase (Rubisco) accumulates only in bundle sheath (bs) cells that surround the vascular centers, and not in mesophyll (mp) cells. We have shown previously that control of mRNA translation and stability mediate the C4 expression patterns of genes encoding the large and small Rubisco subunits (chloroplast rbcL and nuclear RbcS, respectively). The current focus of our research is the identification and characterization of mRNA/protein interactions associated with Rubisco gene expression in C4 plants. We hypothesize that common regulatory systems determine Rubisco gene expression in both C3 and C4 plants. For establishment of cell-type specific C4 gene expression patterns, some of these must have diverged between these two groups, through modification of pre-existing factors, or by acquisition of novel processes not present in C3 species. In support of this hypothesis we have recently identified plastid-localized mRNA binding activities specific to the 5-prime UTR of rbcL mRNA. Binding to rbcL mRNA occurs only in light, when rbcL is expressed, and at least one rbcL mRNA binding protein, p44, is present primarily in bs cell chloroplasts, where Rubisco is specifically localized in C4 leaves. In addition, analysis of Arabidopsis insertion mutants, biochemical analyses, and ongoing RNA silencing studies, indicate that p44 is essential for translation, but not stability, of rbcL mRNA in this C3 plant. The occurrence of highly conserved orthologs of this protein in other plant species, including C3 and C4 monocots and dicots, suggests an important conserved regulatory role in many plants, with modified function in C4 species."
(a) University at Buffalo (b) NC State University

P30009 Regulation of a Bean Proline-Rich Protein Gene Expression During Defense Response in Transgenic *Arabidopsis.*
Das, Songhita-presenter songhita@mail.utexas.edu(a,b) Mehdy, Mona (a,b)
http://www.icmb.utexas.edu/directory/details.asp?id=1698
"During the defense response to pathogen attack & wounding, plant cells modify their walls to produce an effective barrier to pathogen invasion. This involves both up-and down-regulation of cell wall protein genes. One down-regulated gene is the French bean proline-rich protein, PvPRP1.The PvPRP1 protein is hypothesized to be less cross linked due to low tyrosine content compared to other tyrosine-rich proline-rich proteins, and to not contribute to cell wall strengthening.*PvPRP1* mRNA half life is reduced in elicitor treated cells. The 3'-UTR of PvPRP1 has two AUUUA motifs. AUUUA motifs are often known to regulate mRNA half life. A 50-kD protein PRP-BP specifically binds to a 27bp region containing the first AUUUA motif in cellular extracts, and is hypothesized to contribute to *PvPRP1* mRNA down-regulation. To further study this mechanism, three different PvPRP1 constructs were introduced in *Arabidopsis* : (1) the full-length with 3'-UTR (2) a truncated 3'-UTR containing only the first AUUUA motif (3) a truncated 3'-UTR without AUUUA motifs. Jasmonate serves as a signal molecule for gene induction and repression. Earlier work on an *Arabidopsis* homologue of *PvPRP1*, *AGP31*, showed 30% decrease in mRNA within 8h to methyl jasmonate treatment. Currently, the above PvPRP1 transgenic lines are being treated with methyl jasmonate and are being analyzed by Q-PCR. The PRP-BP has also been cloned and transformed in Arabidopsis to study further mRNA regulation. My work will help in understanding mRNA destabilization and may lead to improved disease resistance of crops in future. "
(a) University of Texas, Austin (b) Institute of Cell and Molecular Biology, Section of Molecular, Cell and Developmental Biology

P30010 "Histone methylation and histone ubiquitylation in regulation of gene transcription, plant growth and development"
Shen, Wen-Hui-presenter wen-hui.shen@ibmp-ulp.u-strasbg.fr(a) Berr, Alexandre (a) Menard, Rozenn (a) Molitor, Anne (a) Gao, Juan

(a) Meyer, Denise (a)
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"Histone methylation and histone monoubiquitylation are two types of epigenetic memory marks in eukaryotes. Both types of modifications can act either in activation or in suppression of transcription. Reverse genetic analysis in Arabidopsis unravels critical roles for regulators of histone methylation and/or histone monoubiquitylation in several processes of plant growth and development. Our studies demonstrated that H3K36 di- and tri-methylation together with H2B ubiquitylation are involved in activation of expression of FLOWERING LOCUS C (FLC) and its homologue MAF genes. This regulatory pathway is essential for the control of flowering time. Our work on POLYCOMB group (PcG) genes showed that a PRC1-like complex containing LHP1, AtRING1a and AtRING1b acts in conjunction with the PRC2-catalyzed H3K27 methylation in suppression of Class I KNOX genes (STM, BP/KNAT1, KNAT2 and KNAT6). This regulatory pathway plays important roles in the maintenance of proper stem cell activity within the shoot apical meristem (SAM). We will show and discuss our recent data to highlight roles of histone methylation and histone ubquitylation in gene transcription, plant growth and development as well as in plant responses to environmental stimuli, including abiotic and biotic stresses. References: LIU, Z., et al. (2009) Plant J., accepted. XU, L., et al. (2009) Plant J. 57:279-288. XU, L. and SHEN, W.-H. (2008) Curr. Biol. 18:1966-1971. XU, L., et al. (2008) Mol. Cell. Biol. 28:1348-1360. LIU, S.M., et al. (2007) Plant J. 52:914-926. ZHU, Y., et al. (2006) Plant Cell 18:2879-2892. ZHAO, Z., et al. Nature Cell Biol. 7:1256-1260. DONG, A., et al. (2005) Plant Physiol. 138:1446-1456. "
(a) IBMP du CNRS

P30011 Calcium signaling and the role of a polyadenylation factor in plants response to environment
Li, Q. Quinn-presenter liq@muohio.edu(a) Xu, Ruqiang (a) Liu, Man (a) Addepalli, Balu (b) Hunt, Arthur G (b)
http://www.polyA.org
"Processing of mRNA is recognized as an important gene expression regulatory hub due to its role in dramatically increase of transcriptome and proteome complexity in higher eukaryotes. Among such processing events is the alternatively polyadenylation of mRNA that produce diverse transcript ends, many of which may result in different protein coding capacity. We have previously characterized an Arabidopsis polyadenylation factor, Cleavage and Polyadenylation Specificity Factor subunit 30 (CPSF30) that is part of the plant polyadenylation apparatus. A T-DNA insertion in the first exon of the gene for CPSF30 (*OXT6*) led to an increased tolerance to oxidative stress. Interestingly, we found that the CPSF30 is a calmodulin binding protein as well as an RNA binding protein, and its activity can be modulated by redox. Moreover, calmodulin inhibits the RNA binding activity of CPSF30, indicative of a role of calcium signaling. Further characterization of the *oxt6* mutant revealed that it also differently responds to other environmental stimuli, e.g. heat stress, hormones, flowering time, and even lateral root development. Early analysis of mRNA polyadenylation profile of *oxt6* demonstrates potential change of alternative polyadenylation profile. More interestingly, *OXT6* gene also encodes another larger protein (C30Y) that contains most of CPSF30, but has a C-terminal extension that contains a domain homologous to a protein implicated in mRNA splicing. Since C30Y also contains the calmodulin binding domain, its role in environmental and developmental responses would also be impacted by calcium signaling. Further analysis of the calmodulin binding domain mutants on both proteins and their responses to environmental stimuli are on going. "
(a) Miami University, Ohio (b) University of Kentucky

P30012 The RNA-Binding Protein ELF9 Directly Reduces *SOC1* Transcript Levels through Nonsense-Mediated mRNA Decay in Arabidopsis
Noh, Yoo-Sun-presenter ysnoh@snu.ac.kr(a) Song, Hae-Ryong (a) Song, Ju-Dong (a) Cho, Jung-Nam (a) Amasino, Richard M (c) Noh, Bosl (b)
"*SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) is under a complex transcriptional regulatory network that allows for the integration of multiple floral regulatory inputs from photoperiods, gibberellin, and *FLOWERING LOCUS C*. However, the posttranscriptional regulation of *SOC1* has not been explored. Here we report that EARLY FLOWERING 9 (ELF9), an Arabidopsis RNA-binding protein, directly targets the *SOC1* transcript and reduces *SOC1* mRNA levels, possibly through a nonsense-mediated mRNA decay (NMD) mechanism. The fully spliced *SOC1* transcript is up-regulated in *elf9* mutants as well as in mutants of NMD core components. Further, a partially spliced *SOC1* transcript containing a premature termination codon increases more significantly than the fully spliced transcript in *elf9* in an ecotype-dependent manner. A Myc-tagged ELF9 protein (MycELF9) directly binds to the partially spliced *SOC1* transcript. Previously known NMD target transcripts of Arabidopsis are also up-regulated in *elf9* and recognized directly by the MycELF9. *SOC1* transcript levels are also increased by the inhibition of translational activity of ribosome. Thus, the *SOC1* transcript is one of direct targets of ELF9, which appears to be involved in NMD-dependent mRNA quality control in Arabidopsis."
(a) Seoul National University (b) Gyeongsang National University (c) University of Wisconsin

P30013 Arabidopsis thaliana PRP40s are RNA polymerase II C-terminal domain-associating proteins
Kang, Chang Ho-presenter jacobgnu69@gnu.ac.kr(a,b) Feng, Yue (b) Vikram, Meenu (b) Jeong, In Sil (a) Lee, Jung Ro (a) Bahk, Jeong Dong (a) Yun, Dae-Jin (a) Koiwa, Hisahi (b) Lee, Sang Yeol (a)
"The carboxyl-terminal domain (CTD) of the largest subunit of RNA polymerase II functions as a scaffold for RNA processing machineries that recognize differentially phosphorylated conserved (YSPTSPS)n repeats. Evidence indicates that proteins that regulate the phosphorylation status of the CTD are determinants of growth, development, and stress responses of plants; however, little is known about the mechanisms that translate the CTD phosphoarray into physiological outputs. We report the bioinformatic identification of a family of three phospho-CTD-associated proteins (PCAPs) in Arabidopsis and the characterization of the AtPRP40 (Arabidopsis thaliana PRE-mRNA-PROCESSING PROTEIN 40) family as PCAPs. AtPRP40s-CTD/CTD-PO4 interactions were confirmed using the yeast two-hybrid assay and far-western blotting. WW domains at the N-terminus of AtPRP40b mediate the AtPRP40b-CTD/CTD-PO4 interaction. Although AtPRP40s interact with both phosphorylated and unphosphorylated CTD in vitro, there is a strong preference for the phosphorylated form in Arabidopsis cell extract. AtPRP40s are ubiquitously expressed and localize to the nucleus. These results establish that AtPRP40s are specific PCAPs, which is consistent with the predicted function of the AtPRP40 family in pre-mRNA splicing."
(a) Division of Applied Life Science (BK21 Program) and EB-NCRC, Gyeongsang National University, JinJu, GN660-701, Korea (b) VFIC, Dep. of Hort. Sci., and MEPS Program, Texas A&M University, College Station, TX 77843-2133, USA

P30014 A Reverse Genetics Approach Targeting the *Arabidopsis* MAP Kinase Kinases Reveals the Involvement of MKK7 and MKK9 in Meristem Development
Doczi, Robert-presenter robert.doczi@rhul.ac.uk(a) Hatzimasoura, Elizabeth (a) Helfer, Anne (a) Ditengou, Franck (c) Menke, Frank (d) Hirt , Heribert (b) Palme, Klaus (c) Bogre, Laszlo (a)
"Mitogen Activated Protein Kinase (MAP kinase) cascades, are conserved eukaryotic signalling modules that convert external signals into intracellular responses. In this work we analyzed the function of the group D MAPK kinases, MKK7 and MKK9. Promoter:GUS analyses demonstrate that expression of *MKK7* is confined to stipules, while *MKK9* is expressed at different sites of auxin maxima, implying a role for these kinases in developmental regulation. The expression of both genes somewhat increases in response to IAA and TIBA, and even more so in response to ACC.

Constitutive overexpression of *MKK7* gives rise to meristemless seedlings, whereas increased *MKK9* levels lead to dwarf plants with asymmetric meristems. We also generated *Arabidopsis* plants expressing *MKK7* and *MKK9* under the control of an inducible promoter system. Due to the severity of the phenotypes we chose to concentrate on inducible expression of *MKK7* and *MKK9*. Previous data indicate the involvement of MKK7 in polar auxin transport and in the activation of pathogen resistance and of MKK9 in ethylene signalling (Dai et al, Plant Cell 2006 18: 308-20; Zhang et al, Plant J. 2007 52:1066-7; Yoo et al, Nature. 2008 451: 789-95). Induction of *MKK7* and *MKK9* expression results in the collapse of both shoot and root apical meristems. Whole genome CATMA microarrays were performed on seedlings expressing *MKK7* and *MKK9* revealing significant transcriptional responses. The transcription profiles generated imply that a major role of MKK7 and MKK9 action is to modulate/arrest seedling growth in response to environmental stimuli. "
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P30016 A Conserved Palindromic Sequence is Hypothesized to be Involved in *Nostoc punctiforme* Akinete Gene Expression
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"The photosynthetic nitrogen-fixing cyanobacterium *Nostoc punctiforme* forms symbiotic association with terrestrial plants. From vegetative state can differentiate into three different cellular types: heterocysts, hormogonia, and akinetes. Akinetes are produced from vegetative cells in response to low light or phosphate limitation and can withstand long periods of desiccation and cold. DNA microarray analysis of a *zwf* mutant strain identified genes that were differentially expressed during akinete differentiation. A set of 19 genes were chosen based on the presence of a conserved palindrome in the upstream intergenic region. Rapid amplification of cDNA ends (RACE) was successfully used to map the +1 transcriptional start site(s) of most of the genes, however no conserved spacing of the palindrome in relation to the transcriptional start sites was yet observed. To confirm the DNA array results, three of the up-regulated genes were tested for cell-type specific gene expression in akinetes by electroporation of a GFP transcriptional reporter plasmid containing upstream intergenic regions. An electromobility shift assay was used to see if proteins in a crude extract interacted with the palindrome. Transcriptional reporter plasmids were confirmed to be un-mutated by sequence analysis. Epifluorescence microscopy confirmed akinete specific gene induction along filaments of the reporter strains following heterocyst and akinete induction. A conserved palindrome associated with genes regulated during akinete differentiation have been identified. Preliminary evidence indicates interaction with a protein. Reporter strains containing palindrome mutation or deletions are in progress to establish the requirement for this palindrome in cell-type specific gene expression."
(a) California State University, Northridge

P30017 Detection of protein-protein interactions in plants using the transrepressive activity of the EAR motif repression domain
Matsui, Kyoko-presenter k-matsui@aist.go.jp(a) Ohme-takagi, Masaru (a)
"The activities of many regulatory factors involve interactions with other proteins. We demonstrated previously that AtMYBL2, a protein with a single MYB domain, acts as a negative regulator of anthocyanin biosynthesis in *Arabidopsis*. AtMYBL2 is a repressor, which has repression domain named L2R at its carboxy-terminal end, and it suppressed the expression of DIHYDROFLAVONOL 4-REDUCTASE indirectly via interaction with TRANSPARENT TESTA8, a bHLH transcription factor. We attempted, next, to exploit such transacting regulation by the repression domain as a technique for detection of protein-protein interactions. Here, we demonstrate that the transrepressive activity of EAR-motif repression domain, namely, SRDX, can be used to detect protein-protein interactions and protein factors incorporated into transcriptional complex both in transient expression assay and in transgenic *Arabidopsis* plants. The transrepressive activity of SRDX using FOS and JUN was demonstrated as a model system, and then we used two MADS box plant proteins, PISTILLATA and APETALA3, which are known to form heterodimers, to demonstrate our method in a transient expression assay. Furthermore, when we fused TTG1, which is a WD40 protein and interacts with bHLH transcription factors, to SRDX, we observed a phenotype similar to that of ttg1 mutants in transgenic Arabidopsis. This phenotype might have been due to suppression of the expression of genes that are regulated by the bHLH transcription factor with which TTG1 interacted. Our results indicate that the transrepression mediated by SRDX can be used to detect and confirm protein-protein interactions in plants and should be useful in attempts to identify factors that form transcriptional protein complexes."
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P30018 Mitochondrial and chloroplast retrograde regulation pathways converge via abscisic acid signalling factors
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"Plant cells integrate signals from external sources and from within organelles to regulate gene expression of nuclear located genes, referred to as anterograde and retrograde signaling respectively. No components involved in plant mitochondrial retrograde signaling have been identified to date. Functional characterisation of the promoters of the alternative oxidase *AOX1a*, a marker for mitochondrial retrograde response, and the strongly co-expressed external NAD(P)H dehydrogenase *NDB2* from Arabidopsis thaliana identified two CGTG-containing regulatory elements involved in the stress inducibility of these promoters. Deletion of this element in both promoters abolished the response to rotenone, a specific inhibitor of complex 1 in the mitochondria. This element overlaps with a previously identified potential binding site for the transcription factor abcisic acid insensitive 4 (ABI4) in the *AOX1a* promoter. The *AOX1a* promoter was fully de-repressed in *abi4* mutants, and was unresponsive to rotenone, thus mitochondrial retrograde signaling was compromised in this mutant. Binding of the ABI4 transcription factor to this region of the *AOX1a* promoter was demonstrated by electro-mobility shift and yeast 1-hybrid assays. These results show that ABI4 plays a central role in mediating mitochondrial retrograde signals to induce the expression of *AOX1a*. Furthermore, these results link expression of components of the alternative respiratory chain to ABA signaling, identify at a molecular level components involved in the mitochondrial retrograde response and provide the first direct evidence of common factors between chloroplastic and mitochondrial retrograde signaling pathways as ABI4 has been previously shown to act downstream of at least two chloroplast retrograde signaling pathways"
(a) ARC CoE Plant Energy Biology, University of Western Australia

P30019 Sugar and Gibberellin Signaling Crosstalk through MYBS1 and MYBGA Interaction in Cereals
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"During cereal grain germination and seedling growth, the expression of α-amylase genes is regulated negatively by sugar in embryos and positively by gibberellin (GA) in aleurones/endosperms, through the action of the sugar response complex (SRC) and GA response complex (GARC) in their promoters, respectively. MYBGA and MYBS1 are two transcription factors that bind to the GA response element (GARE, an important cis-acting element of GARC) and the TA box (a cis-acting element shared by SRC and GARC), respectively. Our previous studies have shown that MYBGA and GARE interaction interferes glucose repression on *α-Amy8* expression, and MYBGA and MYBS1 cooperation are required for high-level *α-Amy8* expression in endosperms. In the present study, mechanisms of the GA interference on sugar signaling, through the MYBGA-MYBS1 interaction, in germinating cereal grains was further investigated. MYBS1 was fused with the GFP reporter protein, and cellular localization of the fusion protein was studied. By using rice aleurone transient expression, transgenic rice overexpression and knockout mutant assay approaches, nuclear localization of

MYBS1-GFP fusion protein was found to be promoted by glucose starvation but inhibited by glucose. Presence of GA or overexpression of MYBGA, which facilitates nuclear localization of MYBS, was able to override glucose inhibition of α-amylase gene transcription due to formation of a MYBGA-GARE and MYBS1-TA box complex. Our studies provide new insights into the mechanism by which MYBS1 and MYBGA interaction interferes sugar signaling at the early stage of cereal grain germination and seedling growth."
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P30020 Correlation between codon usage and translation efficiencies of synonymous codons in tobacco chloroplasts
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"The 20 amino acids, except for methionine and tryptophan, are codoed for by 2 to 6 codons called synonymous codons. Synonymous codons are not used with equal frequency in protein coding sequences, and are used differently by different organisms. It has been thought that the codon usage is correlated with the translation efficiency. Since the chloroplast genome lacks tRNA genes corresponding to the most frequent alanine and proline codons, the translational efficiency of synonymous codons in the chloroplast might be different from their usage in the genome. We devised an *in vitro* assay for relative translation rates of synonymous codons, and measured the translation efficiencies of 9 synonymous codon groups in tobacco chloroplasts. Our results indicate that translation efficiencies of synonymous codons are not always correlated with codon usage in tobacco chloroplasts. This raises an important question for the so-called codon optimization according to codon usage. Nakamura, M and Sugiura, M. (2007) Translation efficiencies of synonymous codons are not always correlated with codon usage in tobacco chloroplasts. Plant J. 49: 128-134. Nakamura, M and Sugiura, M. (2009) Selection of sysnonymous codons for better expression of recombinant proteins in tobacco chloroplasts. Plant Biotech. 26: 53-56."
(a) Grad. Sch. Natural Sci., Nagoya City Univ. (b) Sugiyama Human Res. Ctr., Sugiyama Jogakuen Univ.

P30022 Upstream elements of the Arabidopsis Response Regulator 18 (ARR18) in plant two component system
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http://www.zmbp.uni-tuebingen.de/PlantPhysiology/ResearchGroups/harter/index.html
"The Two Component System (TCS) is a Histidine-to Aspartate (H-D) phosphorely that allows the plant to transduce a signal by propagating certain stimuli. In Arabidopsis thaliana, the multi step TCS is composed of a hybrid histidine kinase (AHK) that autophosphorylates in response to an external signal, as well as a phophotransfer protein (AHP) that then receives the phosphate from the AHK and transfers it to the Response Regulator (ARR) which represents the last element in the phosphorely. Higher plants contain 11 B-type response regulators, all shares a common structural design, containing both the phospho-accepting receiver and GARP DNA-binding domains. Most likely all type-B family members serve as transcriptional regulators but in general little is known about the function of most of them. The purpose of our study is to analyse the role of B-type response regulator 18, but to completely understand the ARR18 function, it is also required to investigate the ARR18 upstream elements in the two component system. Here we report the successful identification of the putative interacting partners of ARR18 at the AHP level. These finding opens the possibilities of several signalling pathways. This interaction does not prove though that a phosphorely between the interacting AHPs and ARR18 is taking place. It remains therefore open whether interacting AHPs are phosphorylating ARR18 or whether the interaction with the ARR18 occurs to achieve other sort of regulation. This work is supported by the Deutscher Akademischer Austausch Dienst (DAAD). "
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P30023 Histone Deacetylase19 Is Required for the Repression of Salicylic Acid Biosynthesis and Salicylic Acid-mediated Defense Responses in Arabidopsis
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"Histone deacetylases remove acetyl groups from the N-terminal tails of histones, which lead to repressive chromatin structures or to the modification of transcription factor-binding surfaces. Therefore, the role of histone deacetylases is often closely associated with the repression of transcriptional activity. Histone deacetylase19 (HDA19), an Arabidopsis RPD3/HDA1-class histone deacetylase has been implicated in multiple developmental processes and defense responses. Here, we show that HDA19 is involved in the repression of salicylic acid (SA)-mediated defense responses in Arabidopsis. Loss of HDA19 activities increased SA content and the expression of a group of SA biosynthetic and pathogenesis-related (PR) genes, resulting in enhanced resistance to *Pseudomonas syringae*. A mutation in *SID2/ICS1* suppressed the increased expression of PR genes but not the SA biosynthetic genes in the *hda19* mutants. Finally, we could observe that the acetylation levels of histone H3 within *EDS1* and *PR* promoters are increased in the *hda19* mutants compared to in wild type. Taken together, these results indicate that HDA19 plays a key role in repressing defense responses prior to pathogen attack and modulating the activity of defense responses by preventing harmful over-stimulation of defense mechanisms through a histone deacetylation-mediated constitutive repression of *EDS1*- and *PR*-gene transcription."
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P30024 Association studies of stress-related and wood-forming genes in loblolly pine
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"As part of ADEPT2 (Allele Discovery of Economic Pine Traits) the expression of 92 genes involved in responses to water stress and diseases and 112 genes involved in wood formation and lignin biosynthesis was analyzed using real-time qPCR. Gene expression data was collected on 426 individuals from an association population of loblolly pine trees provided to us by North Carolina State University (NCSU). Clones were provided for biological replication. We are using the software Tassel to detect significant associations between our gene expression data and more than 5,000 SNPs that have been identified at UC-Davis to break down complex adaptive traits. Preliminary expression results for genes involved in lignin biosynthesis, cellulose synthesis, cell wall expansion, etc. revealed large differences among individuals. However, only modest differences in expression of disease- and drought-related genes were observed among individuals. Variation in gene expression levels between clones was also detected for disease- and drought-related genes, possibly due to low constitutive gene expression of these genes. We are further examining induced gene expression in response to drought, pitch canker disease, and drought and pitch canker combined on a panel of 24 individuals from the same population. Previous studies have found that expression of disease and drought-induced genes varies among trees. However, we know of no study that has examined differential gene expression in response to both drought and disease in loblolly pine trees. We hope to correlate gene expression levels with

phenotypic data collected by NCSU, the University of Florida, and our own lab and to identify coordinately regulated genes."
(a) Molecular & Environmental Plant Sciences, Texas A & M University (b) University of California-Davis (c) North Carolina State University

P30025 Comparison Of Consensus Binding-Site Sequences Of Arabidopsis AP2-Type Transcription Factors With Two AP2 DNA-Binding Domains.
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"Arabidopsis contains 14 AP2-type transcription factors (TFs) with dual AP2 domains, which include important regulators of development, such as APETALA2 (AP2), a class A homeotic TF for floral organ development, TARGET OF EAT1 (TOE1), a flowering repressor, AINTEGMENTA (ANT) involved in ovule development, BABY BOOMER (BBM) involved in embryo development, and PLETHORA1,2 (PLT1,2) for the establishment of root apical meristem. They also include WRINKLED1/ASML1 (WRI1) involved in the regulation of seed oil accumulation. Although the DNA binding site sequence of ANT has been described, direct targets have not been established for these AP2-type TFs. We have recently shown that WRI1 binds to a sequence designated AW-box conserved among many genes involved in fatty acid synthesis in plastids. We used random oligonucleotide selection method to identify and compare consensus binding site DNA sequences of 7 AP2-type TFs, and examined roles of each AP2 DNA-binding domains in the recognition of target site sequences. "
(a) Lab. Biochem., Grad. Sch. Bioagr. Sci., Nagoya Univ. (b) Toyota Genesis Res. Inst. (c) Utrecht Univ.

P30026 The bZIP63 transcription factor: from transcriptional regulation to functional analysis.
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" The members of the multi-gene plant bZIP transcription factor family play an important role in seed development, hormonal signalling and biotic and abiotic stress responses. The Arabidopsis genome contains more than seventy genes encoding bZIP factors, but a specific function has only been identified for some family members. The four highly homologous bZIP factors (bZIP9, 10, 25 and 63), comprising the C-group of the bZIP family, recognize the same *cis*-element and can heterodimerize with one another. Nevertheless, the analyses of gene transcriptional responses indicate member-specific functions in plant development. Unlike other group members, bZIP63 is low-expressed in seedlings grown in light but is upregulated when they are transferred to darkness. Further analyses suggest that bZIP63 transcription is not directly regulated by light but rather by the changes in internal sugar levels in relation to nitrogen availability. The metabolic profiling of two different lines overexpessing bZIP63 revealed the significantly altered levels of free amino acids, which had previously been reported to be induced by dark treatment (Miyashita and Good, 2008; Ishizaki et al., 2005). Furthermore, in agreement with data on the transcriptional activity of bZIP63 / bZIP53 (group-S) heterodimers (Weltmeier et al., 2006), there was almost no increase in proline level in sucrose-treated bZIP63-overexpressing seedlings compared to wild type. The obtained results will be discussed in relation to the bZIP63-dependent metabolic regulation of dark adaptation. "
(a) ZMBP, University of Tuebingen (b) Albrecht-von-Haller-Institute, University of Goettingen

P30027 Differential expression of genes regulating flavonoid biosynthesis in yellow seed coat phenotypes in canola (*Brassica napus* L.)
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http://www.dowagro.com
"The inherent association of the yellow seed coat (YSC) phenotype with low fiber and high oil content in canola (*B napus*) makes it a preferred trait for commercial development. Extensive research on the YSC trait in *Arabidopsis* has shown that mutation in genes regulating flavonoid biosynthetic pathway lead to the yellow seed coat color or *transparent testa* phenotype. To understand YSC trait regulation in canola, we have carried out two sets of microarray experiments using doubled haploid (DH) lines derived from a cross between a YSC line and a black seed coat color (BSC) line. The first set included YSC vs. BSC DH lines and the second set included analysis of low fiber vs. high fiber DH lines of the same cross. An experiment was carried out using RNA extracted from seed coats collected at 20- and 25- days after pollination. Genome-wide transcriptome analysis using 36,000 canola ESTs has revealed a similarity in regulation of seed coat color development between canola and *Arabidopsis* and highlighted the role of genes involved in flavonoid biosynthetic pathway. "
(a) Dow Agrosciences, Indianapolis, IN

P30028 Importance of specific phospholylation of SAMDC uORF protein in translational regulation
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"*S*-adenosylmethionine decarboxylase (SAMDC), a key enzyme for polyamines biosynthesis, was tightly regulated for homeostatic levels by translational inhibition of uORF in down-stream gene expression. Carnation *SAMDC* mRNA contains a functional uORF (54AAs). To explore the functional mechanism of uORFs, we used a *GUS* reporter gene driven with the 35S promoter and uORF region of *SAMDC* gene for *in vivo* assay. We produced transgenic tobacco plants with the point-mutated uORF peptides at several putative phosphorylation sites. Point-mutated proteins in all phosphorylation sites (Pno) or except Ser10 (P10) increased significantly the transcriptional efficiency of GUS, implied the phosphorylation at Ser28 and Ser54 was essential for transcriptional regulation at native level. Also, the translational efficiency was completely retained in point-mutated protein (P17) at Ser10, Ser28 and Ser54, implied that phosphorylation at Ser17, which is a putative site for protein kinase C, is a essential component for a translational inhibitor. Also, each phosphorylation of Ser28 (P28) and Ser54 (P58), which were putative sites for casein kinase II and cAMP/cGMP dependent kinase, respectively, decreased dramatically its down-stream translation. Therefore, it is suggested that specific kinase for those phosphorylation sites may regulate the homeostatic level at transcriptional and/or translational level in cellular response. Also, our results from transgenic plants with coexpression uORF peptide with uORF-GUS construct suggested that uORF protein might effectively act for functional translational inhibitor *in trans*. These results also suggested the possibility that uORF peptide may be involved in an *in vivo* modulation of *SAMDC* gene expression in response to some environmental signals."
(a) Sunchon National University

P30029 Regulation of Gene Expression and Metabolite Contents Induced by Nitrogen Supply in Rice
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"Nitrogen is important nutrient for plant growth and development. Here we show the result of our comprehensive analysis of nitrogen (nitrate ammonium) supply-induced modulations in gene expression and metabolite contents in rice. We found a number of genes that were up-regulated and down-regulated by nitrogen-supply and fluctuations in metabolite contents that were partly different in shoots and roots. We also found that two

MYB proteins (MYB-NR1 and MYB-NR2) were candidates for nitrogen-responsive transcription factors in rice. The corresponding genes, *MYB-NR1* and *MYB-NR2*, were rapidly induced by supply of nitrate but not ammonium in both shoots and roots. To reveal the effects of overexpression of these MYB proteins and to identify the target genes of the MYB proteins, we generated transgenic callus overexpressing *MYB-NR1* or *MYB-NR2*. Based on the results of microarray analysis using the callus, we selected candidates for the target genes of the MYB proteins. Transactivation assays using maize protoplasts revealed that MYB-NR2 can transactivate the promoters of genes for tryptophan synthase α subunit involved in tryptophan biosynthesis and for tryptophan decarboxylase catalyzing the first step in the tryptophan secondary metabolism. Furthermore, up-regulation of these putative target genes was found to occur subsequently to up-regulation of *MYB-NR2* by nitrate treatment in rice seedlings. Based on the results of measuring the contents of tryptophan and serotonin, one of the secondary metabolites of tryptophan, we will discuss the physiological function of MYB-NR2. This work was supported in parts by grants from the Program for Promotion of Basic Research Activities for Innovative Biosciences."
(a) Graduate School of Agricultural and Life Sciences, The University of Tokyo (b) National Institute of Agrobiological Sciences (c) Core Research of Evolutional Science & Technology, Japan Science and Technology Agency

P30030 Transcriptomic analysis of apple fruit ripening and texture attributes
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"Molecular events regulating cultivar-specific apple fruit ripening and sensory quality are largely unknown. Such knowledge is essential for genomic-assisted apple breeding and postharvest quality management. In this study, transcriptomic analysis, scanning electronic microscopic examination and systematic physiological characterization were performed on two apple cultivars, 'Pink Lady' and 'Honeycrisp', which are with distinct ripening behavior and texture attributes. Substantial differences of crispness and firmness in fruit cortex were observed. SEM images of fruit cortex tissues prepared from fruits with similar developmental stage suggest that the cell wall thickness may contribute to the observed firmness and crispness phenotype. A high-density long-oligo apple microarray consisting of a duplex 190,135 cross-hybridization-free 50-70-mer isothermal probes, and representing 23,997 unigenes was manufactured on a Nimblegen array platform. The developmental stage- and cultivar-specific expression profiling analysis and QPCR validation indicated that genes in several functional groups express differentially between cultivars and ripening stages. These groups include cell wall metabolism (both degradation and biosynthesis), hormonal metabolism and response (including ethylene, auxin, gibberellin and brassinosteroid) and transcription factors (such as NAC and WRKY families) as well as signal transduction and secondary metabolism related pathways. In many cases, genes in the same functional groups were regulated (up or down) similarly as fruit ripening progressed; however, different gene family members or possible allelotypes were differentially regulated between two cultivars, likely reflecting the high-level heterozygosity and allopolyploidy of apple genome."
(a) USDA ARS Tree Fruit Research Lab (b) Dept. Hort. and Landscape Architecture, WSU

P30031 "The R2R3 MYB Transcription Factor, *NtAn2*, Is a Key Regulator of Anthocyanin Biosynthesis in Tobacco (*Nicotiana tabacum* L.)"
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"Tobacco is a commonly used heterologous system for studying combinatorial regulation of the flavonoid biosynthetic pathway by the bHLH/MYB-transcription factor (TF) complex in plants. However, little is known about the endogenous tobacco bHLH and MYB TFs involved in the pathway. We isolated a gene (*NtAn2*) encoding a R2R3 MYB TF from developing tobacco flowers. *NtAn2* shares high sequence homology with other known flavonoid-related MYB TFs and is expressed in developing flowers but not in leaves. Constitutive ectopic expression of *NtAn2* induces whole-plant anthocyanin production in tobacco. In transgenic tobacco expressing *NtAn2*, both subsets of early and late flavonoid pathway genes are up-regulated. Suppression of *NtAn2* by RNAi resulted in a white-flowered phenotype and the inhibition of the late pathway genes. Yeast two-hybrid assays demonstrated the interaction between *NtAn2* and three heterologous bHLH TFs known to induce anthocyanin synthesis in reproductive tissues of tobacco. Bimolecular florescent complementation using split-YFP assays demonstrated that *NtAn2* interacts with maize bHLH TF *Lc* in tobacco cells and that the complex is localized to nuclei. Co-expression of *NtAn2* and *Lc* in tobacco protoplasts activated the promoters of key flavonoid pathway genes. These results suggest that *NtAn2* is a key gene controlling anthocyanin production in reproductive tissues of tobacco."
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P30032 The Anatomical Basis and Heritability of Figured Wood in a Genotype of *Populus*
Fan, Youran-presenter yfan@purdue.edu(a) Wiedenhoeft, Alex C. (b) Rupert, Kendal M. (a) Meilan, Richard (a)
"Figure, resulting from deviations in the size, type, and orientation of cells or cell assemblages, can greatly enhance the value of wood, but its occurrence is unpredictable, preventing large-scale commercial use. Here we report results from a hybrid male poplar genotype, *Populus x canescens* (hereafter referred to as: 'Grober'), whose figure has been shown to be under genetic control. The figure in Grober is a combination of curly and spiral grain, and appears most strikingly when logs are quarter-sawn. To evaluate the structural differences between wood from Grober and one of its straight-grained progenitors (*P. alba*), seven anatomical characters were measured in 11-month-old, field-grown trees. All stems for sections were sampled 5 cm above ground and controlled with respect to size, weight, moisture content, and diameter. Specific gravity was significantly different between those two species (p <0.001). *Populus alba* has taller rays (in both height and cell number) and more vessels per mm^2, whereas Grober has longer fibers and vessel elements, larger tangential vessel diameters, and more rays per unit of tangential distance (p <0.01 for all characters). Reciprocal grafts between straight-grained and figured trees demonstrated that figured wood is not the result of a graft-transmissible factor; scions retained their properties and were not affected by the rootstock on which they were grown. Three over-expression constructs were assembled using tubulin genes that were derived from hybrid aspen clone INRA 353-38 (*P. tremula x P. tremuloides*) and mutagenized at specific sites. Mutagenesis of their orthologs in *Arabidopsis thaliana* has resulted in the helical arrangement of cells in their stems. The function of these genes in *Populus* will be tested by transforming the mutagenized versions into INRA 353-38. The ultimate goal of this work is to determine the cytological and genetic bases for figured wood in poplar."
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P30033 The plant-specific Dof transcription factors in the moss *Physcomitrella patens*
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"Many studies with various seed plants including *Arabidopsis*, rice, potato and tobacco have suggested that Dof transcription factors play roles in diverse biological processes unique to plants[1]. However, the Dof transcription factors in lower plants have not been characterized yet, and the origin

of the Dof transcription factors is uncertain. Here, we show our search of the *Dof* genes in the genomes of the moss *Physcomitrella patens* and some algae to investigate the origin of the Dof transcription factors. We found 19 putative *Dof* genes in the moss (*PpDof1-19*) and one *Dof* gene in the green alga *Chlamydomonas reinhardtii* (*CrDof*), while there was no identifiable *Dof* gene in the red alga *Cyanidioschyzon merolae* and the diatom *Thalassiosira pseudonana*. Hence, it is suggested that the origin of the Dof transcription factors pre-dates the divergence of the green algae and the ancestors of terrestrial plants. Furthermore, phylogenetic analysis with *Arabidopsis*, rice, *P. patens* and *Chlamydomonas Dof* genes revealed that these *Dof* genes are classified into three groups, one of which includes *PpDof1-6*, *CrDof* and several angiosperm *Dof* genes[2]. Since intimate relationships between *PpDof1* and *2*, *PpDof3* and *4*, and *PpDof5* and *6* were verified by further characterization, we generated single knockout lines for each gene and double knockout lines in which both closely related *Dof* genes were disrupted. Phenotypic analysis of these lines revealed that the *PpDof1 PpDof2* double knock out line showed abnormal growth. The disruption of *PpDof1* and *PpDof2* genes affected the branching frequency and the gametophore formation, suggesting the involvement of *Dof* genes in growth regulation in the moss. [1]Yanagisawa, *Trends Plant Sci.*, 7, 555, (2002). [2]Shigyo *et al.*, *Plant Cell Physiol.*, 48, 179, (2007)."
(a) Graduate School of Agricultural and Life Sciences, The University of Tokyo (b) Core Research for Evolutional Science and Technology, Japan Science and Technology Agency

P30034 "Characterization of homologous chromosome pairing, synapse and recombination during autotetraploid Arabidopsis meiosis"

Peterson, Ross-presenter pete6029@umn.edu(a) Chen, Changbin (b)

"Whether in crop breeding or nature, polyploidy plants have displayed phenotypic advantages leaf mass, flower numbers, and bud size compared to diploid plants, therefore polyploidization was a widely used approach in crop breeding. Recent studies on Arabidopsis autotetraploids revealed a partially diploidized chromosome recombination during meiosis. The few investigations that have been done on the altered but conversed meiosis in polyploid plants indicate key differences between that of diploid meiosis, and raises new questions about the unknown mechanisms of meiosis in polyploid species. In this case in point, we examine two autotetrapolyploid of different ecotypes, Columbia and Lansberg, of the commonly used model plant Arabidopsis thaliana. The difference between these two ecotypes includes a mitochondrial DNA insertion that is believed to contribute to the plants fertility. After the resolution of the defected meiosis, the Columbia ecotype with the mitochondrial DNA insertion exhibits fertility defect to a lower degree than its Landsberg counterpart who lacks this insertion. The application of fluorescence in situ hybridization (FISH) with probes to label the telomere, centromere and mitochondrial DNA insertion were applied to differentiate among the two Arabidopsis ecotype and their hybrid tetraploids to demonstrate homologous chromosomes behaviors during meiotic recombination."
(a) University Of Minnesota (b) University of Minnesota

P30035 Coordination of protein import and synthesis in leaf chloroplasts

Sun, Chih-Wen-presenter cwsun@ntnu.edu.tw(a) Huang, Yen-Chiao (a) Chang, Hsin-Yen (a)

"Due to the large amount of proteins required for photosynthesis, chloroplasts in leaf cells have a greater need for protein import and protein synthesis than plastids in other organs. We previously reported that the Arabidopsis transcription factor CIA2 specifically up-regulates leaf expression of genes encoding protein translocons Toc33 and Toc75, which are essential for protein import into chloroplasts. Protein import efficiency was therefore reduced in *cia2*-mutant chloroplasts. To further understand the function of CIA2, gene expression profiles of the wild type and a *cia2* mutant were compared by microarray analysis. Interestingly, in addition to genes encoding protein translocon components, other genes down-regulated in *cia2* almost exclusively encode chloroplast ribosomal proteins. Isolated *cia2*-mutant chloroplasts showed reduced translation efficiency and steady-state accumulation of plastid-encoded proteins. When CIA2 was ectopically expressed in roots, expression of both the protein-translocon and ribosomal-protein genes increased. Further analyses *in vivo* revealed that CIA2 up-regulated these genes by binding directly to their promoter regions. We propose that CIA2 is an up-regulator responsible for the higher protein demands of leaf chloroplasts by coordinatedly increasing both protein import and protein translation efficiencies."
(a) National Taiwan Normal University

P30036 Mediation of plant stress responses via mRNA turnover

Walley, Justin W.-presenter jwwalley@ucdavis.edu(a) Dehesh, Katayoon (a)

"To maintain homeostasis in an ever-changing environment organisms have evolved mechanisms to reprogram gene expression. One mechanism that is central to overall regulation of gene expression is mRNA degradation, which is initiated by poly(A) tail shortening (deadenylation). The CCR4-CAF1 complex is the major enzyme complex that catalyzes mRNA deadenylation and is conserved among eukaryotes. However, the components and functions of this global regulatory complex have not been well characterized in plants. We identified two *CAF1-like* genes with altered transcript levels five minutes after wounding *Arabidopsis* leaves by microarray analysis, indicating that these genes may play a role in stress responses. Using a combination of qRT-PCR and luciferase reporter constructs we have shown that these *CAF1-like* genes respond rapidly and transiently to a range of abiotic and biotic stresses. Additionally, we have examined their role in stress tolerance. Analysis of T-DNA null mutants demonstrates that these two *CAF1-like* genes can have unique roles in mediating response to various abiotic stresses. Consistently, there is limited overlap between the transcriptional profiles of these *caf1* mutants. Further suggesting that these CAF1 homologs have distinct functions in mediating stress tolerance in *Arabidopsis*, possibly due to deadenylation of unique mRNA substrates. "
(a) University Of California, Davis

P30037 The role of cis and trans elements: heterologous plastid regulatory elements significantly decrease transgene expression

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"Plants have evolved mechanisms to coordinate plastid gene expression. Nuclear encoded RNA binding proteins (RBPs) imported from the cytosol exhibit species specificity for cis elements located in plastid 5' untranslated regions (UTRs). Plastid gene coding regions are 80-97% identical whereas the non-coding regions are 45-79%, including psbA, a commonly utilized regulatory element in genetic engineering strategies. Plastid transformation vectors were made for tobacco and lettuce, with endogenous or heterologous psbA promoters, 5'UTR and 3'UTR, regulating expression of the anthrax protective antigen (PA) or human proinsulin fused with CTB (Pins). Plants with endogenous psbA in tobacco (Nt/Nt) and lettuce (Ls/Ls) showed 14% and 11% PA. However, tobacco plants with lettuce psbA showed a 94% reduction in transgene expression. Similarly, Nt/Nt lines showed 60% CTB-Pins whereas transgene expression was reduced by 97% in lettuce when regulated by tobacco psbA. Transcript abundance showed 84% reduction in CTB-Pins monocistron in Ls/Nt lines compared to Nt/Nt lines. Similarly, pag transcripts were reduced by 65% in monocistrons and 88% in dicistrons in Nt/Ls lines when compared to Nt/Nt lines. In gel shift assays with Ls or Nt psbA UTR, the foreign UTRs did not effectively compete for binding RBPs. Northern blots suggest that RBPs stabilize the foreign gene transcript pool that is not associated with polyribosomes, provided the transcripts carry the endogenous psbA UTR sequence. There was no significant difference in turn over of these proteins when expressed using endogenous or heterologous regulatory sequences. The implications of these findings for plastid biotechnology will be

discussed."
(a) University Of Central Florida

P30038 "A cyanobacterial circadian input factor, Pex, alters its molecular size under diurnal light cycle"
Kuboi, Naoyuki-presenter kutsuna@yokohama-cu.ac.jp(a) Shinsuke, Kutsuna (a)
"In a cyanobacterium, *Synechococcus elongatus* PCC 7942, a circadian input protein Pex accumulates in dark transiently, and then down-regulates the transcription of the clock gene *kaiA*. Herein, we analyzed molecular size of a protein complex of Pex in vivo and in vitro. The extract of the cyanobacterial cells were fractionated from 13 to 43 kDa in molecular size by gel-filtration method. Then, Pex in the obtained fractions were analyzed by western blotting method. 17 kDa protein band was detected as Pex protein in 34 kDa complex, in addition to previously reported 13 kDa protein. The 17 kDa protein signal was absent in pex deficient mutant cell extract, therefore we determined the 17 kDa protein was Pex. The size of 17 kDa is consistent to the one deduced from the open reading frame (orf) of *pex*. The structural analysis of Pex protein indicated that it formed a homo dimer with its hydrophobic amino acids. Then we examined the importance of the amino acid in the kaiA DNA binding activity. Two mutants of the Pex protein on the hydrophobic amino acids were examined but it showed no significant DNA binding activity *in vitro*. We also examined whether the two Pex mutant molecules form a dimer *in vitro* and *in vivo* by the same method, however, in the both cyanobacterial cells and expressed- and purified-Pex proteins from *E. coli* exhibited the dimer. From these results, we conclude that Pex is translated as a 17 kDa protein and then forms 34 kDa dimer in the cell to bind onto kaiA promoter region. We also detected Pex with 15 kDa in size under light period, in which it was in a 30-kDa complex. Potential secondary translation initiation codon(s) exists in frame of orf in pex gene, might suggest alternative translational control in the gene under diurnal light condition. "
(a) International Graduate School of Arts and Sciences, Yokohama City University

P30039 "Circadian and growth phenotypes in a cyanobacteria lacking transcription factor, CmpR"
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"A circadian input gene, *pex*, responds to light-dark transition and delays the phase of the circadian rhythm in *Synechococcus elongatus* PCC 7942. To identify a regulator of the pex expression, we made random transposon insertion mutants of the bioluminescent reporter of *pex*. One of the mutants exhibited stronger bioluminescence than that of the others in our standard light condition. Northern blotting analysis of the *pex* mRNA confirmed that it was more abundant not only in light but also in dark condition. We also characterized the mutant showing slow colony formation and abnormal circadian rhythm monitored by bioluminescence reporter. Then it was concluded that the causative gene of the both mutant phenotypes was *cmpR*, encoding a LysR family transcriptionalp regulator of several representative bicarbonate transporter genes in the *Synechococcus* by plasmid rescue and complementation. We could analyze the mutant phase-delay phenotype by using bioluminescent reporter of the circadian clock operon *kaiBC*, because the shape of the mutant rhythm was not different from that of wild-type, except to difference on the phase. And we determined that the phase-delay in the mutant was more than 5-h. Thus, it is indicated that the defect in the mutant changes the normal resetting of the circadian oscillator and delayed the timing of the kaiBC operon and pex gene. Although, the phase-delay phenotype was partly suppressed in cmpR/pex double mutant, the defect in colony size was not suppressed in the cell. Then, we assayed whether CmpR and its putative binding motifs in *pex* promoter in vitro. While CmpR bound to a representative DNA motif in *psbAII* promoter, it showed no activity for *pex* promoter. "
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P30040 Calmodulin interaction with the TGA family of transcriptional activators
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http://www.life.uiuc.edu/zielinski
"TGA proteins are members of a family of DNA-binding proteins in plants that were initially described by their ability to bind activator sequence-1(as-1)and sequences resembling as-1, such as the LS7 and LS5 elements of the PR1 promoter. Experiments employing dominant-negative mutations, chromatin immunoprecipitations and gene knockouts demonstrated that TGA proteins play both positive and negative roles in regulating PR1 expression. We previously demonstrated that calmodulin (CaM) binds TGA3 in a Ca^{2+}-dependent manner; this interaction enhanced the apparent affinity of TGA3 for an as-1-like element in the AtCam3 promoter (Szymanski et al., 1996. Plant Cell 8: 1069). CaM interaction with TGA proteins has been confirmed by protein array and co-immunoprecipitation experiments (Popescu et al., 2007. PNAS 104: 4730). Analyses of the structures of the TGA proteins using the criteria of Yap et al. (2000. J Struct Funct Genomics 1: 8-14) to identify CaM-binding domains predict that TGA2, 3, 5, 6 and 7 interact with CaM in a domain comprising approximately 30 amino acids at their C-termini. TGA1 and 4, in contrast, lack comparable domains. This prediction was supported by experiments in which GFP fused with the C-terminal 64 amino acids of TGA3, 5, 6 or 7 were challenged with CaM in the presence or absence of Ca^{2+}. Binding experiments with GFP-TGA3 demonstrated interaction between the transcription factor and conventional CaM isoforms as well as the CaM-like protein CML9, but not the CaM-like protein CML8. Current experiments are aimed at defining more precisely the residues in TGA proteins involved in CaM-binding and determining whether interaction with CaM modulates TGA dimer formation, interaction with the PR1 LS5 and 7 regulatory elements or TGA interaction with NPR1."
(a) University Of Illinois

P30041 Analysis of expression of genes in sugar metabolism in root nodules of alfalfa
Grimes, Martha M-presenter mem4@nmsu.edu(a) Ortega, Jose L (a) Champa, Sengupta-Gopalan (a)
"The legume-Rhizobium symbiosis involves very complex interactions, which lead to the formation of a new organ, the root nodule. The bacteria residing in the nodule fixes atmospheric N2 into a form usable by the plant and in turn obtains carboxylic acid from the plant. The photosynthate is delivered to the nodule in the form of sucrose and the carbon derived from the metabolism of sucrose in the nodules is used for several physiological processes; including plant and bacterial respiration, N2-fixation and assimilation, and the biosynthesis of starch and cellulose. Sucrose-phosphate synthase (SPS) plays a key role in the synthesis of sucrose in photosynsthetic tissues. We have recently shown that nodules exhibit high SPS activity. The objective of this research project is to understand the role of SPS in the roots nodules in context of N2-fixation and N2 assimilation. The goal here is to analyze the expression pattern of genes encoding other key enzymes in sugar metabolism in the nodules under conditions where the symbiont can fix or not fix N2. We will present our data on the expression of genes encoding for enzymes in starch synthesis, starch breakdown, sucrose synthesis and sucrose hydrolysis and thus present a model focusing on sugar metabolism in the nodules. "
(a) New Mexico State University

P30042 Antisense transcripts of light-regulated rice genes
Terzaghi, William B. (a,b) Kocher, Neil-presenter neil.kocher@wilkes.edu(a) Grow, Casey (a) Mian, Naseem (a,b) Shah, Jay (a,b) SInghal, Sonia (b) Deng, Xing Wang (b)

"Natural antisense transcripts (NATs) are RNAs complementary to sense RNAs that are known to play roles in gene regulation. This study examined 21 genes with antisense partners which are involved in light-regulated pathways in Nipponbare rice (Oryza sativa japonica). Of the 21 genes with antisense partners, 17 were detected by RT-PCR in Nipponbare shoot and root cells. Low molecular weight RNA blots of the Os03g07300/ Os03g07310 gene pair revealed a root-specific small RNA (~40 nucleotides). Similarly, low molecular weight RNA blots of the Os12g05660/ Os12g05680 gene pair showed small RNAs derived from exon 13 of Os12g05680 that were not present in Os12g05660. RT-PCR of the Os12g17600 rbcS gene on chromosome 12 detected multiple small antisense fragments rather than one continuous RNA. Os11g02610/ncRNA769 and Os12g02530/ncRNA768, two RPT2 genes and antisense small RNAs involved in ribosomal RNA processing, were detected in the same tissue, raising the question of whether they coexist in the same cells. The results of this research will be used to determine whether NATs play regulatory functions in Nipponbare light responses."
(a) Wilkes University (b) Yale University

P30043 IIdentifying New Genes that Function in Establishment of Leaf Polarity
Liu, Tie -presenter tieliu@stanford.edu(a) Brenda, Reinhart (a) Kathryn , Barton (a)
"The establishment of adaxial-abaxial polarity is determined by differential gene expression. Among these regulator genes, members of class III homeodomain Leu zipper (Class III HD-Zip) family play important roles in influencing the activity of the meristem from which the lateral organs formed. The Arabidopsis thaliana genome contains five class III homeodomain-leucine zipper genes: REVOLUTA (REV), PHABULOSA (PHB), PHAVOLUTA (PHV), CORONA (CNA), and ATHB8. These proteins activity promote the development of upper (adaxial) leaf fates and meristem formation. In contrast with the adaxial expression patterns of Class III HD-Zip genes, KAN1 gene in Arabidopsis is expressed in abaxial domain of lateral organs and encodes a MYB transcription factor. To identify the genes whose expression is regulated by the REV and KAN1, microarray expression profilings were conducted on mRNA from inducible overexpression gene fusion of REV and KAN1 under glucocorticoid control. The differential expression of the genes identified by microarray was further confirmed by in situ hybridization analysis. "
(a) Carnegie Institution Of Washington

P30044 "Comparison of Stress-Induced, Differentially Expressed Genes to the Variance in VOC Emissions And Essential Oil Production in Pinus ponderosa and Copaifera langsdorfii"
Thornton, Brenda (a) Basu, Chhandak-presenter chhandak.basu@unco.edu(a) Guenther, Alex (b,a) Harley, Peter (b) Greenberg, James (b)
"Many plants produce secondary metabolites, known as terpenes, in response to pathogen attack and environmental stress. The terpenes emitted, in the form of volatile organic compounds (VOCs), are a dominant source of oxygenated compounds in the atmosphere, and have a significant impact on atmospheric chemistry. Plants can also store terpenes in the form of essential oils, which serve as primary deterrents to pathogens. These terpenes can be extracted and used commercially as perfumes, aromatherapy, medicine and biofuels. As technology to separate essential oils and measure VOC emissions has improved, a lot of research has been done to characterize their chemical structure and thermodynamic properties; however, little research has focused on the genetic regulation of terpenes in response to environmental conditions. Our research focuses on the differential expression of stress-induced genes in Pinus ponderosa and Copaifera langsdorfii when exposed to environmental conditions known to be associated with an increase in emissions of VOCs and production of essential oils."
(a) University of Northern Colorado (b) National Center for Atmospheric Research

P30045 Functional study of remorins from soybean
Son, Seung Min-presenter linewind@snu.ac.kr(a) Bae, Ju Hee (a) Im, Jong Hee (a) Oh, Chang Jae (a) Lee, Hyoungseok (c) Kim, Ho Bang (b) An, Chung Sun (a)
"Remorin, a plant specific protein including two conserved N-terminal proline rich and C-terminal coiled-coil regions, has been found in detergent-resistant membrane fractions, lipid rafts. And it is supposed to play roles in defensive or environmental responses by regulating protein sorting and signal transduction. But the molecular and biochemical function of the remorin remains largely unknown. In this study, five different cDNA clones showing high similarity with remorin genes in TIGR soybean EST database were isolated by RT-PCR using root or nodule cDNA pool and named *GmREM1-4* and *GmNOD-REM1*. Transcription levels of *GmREM3* and *GmREM4* were similar in all organs tested, but those of *GmREM1* and *GmREM2* were enhanced in root and stem. *In situ* hybridization revealed that transcripts of nodule-specific *GmNOD-REM1* were strongly detected in the infected cells of root nodule. Subcellular localization analysis using Arabidopsis protoplast suggested that GmNOD-REM1 might be targeted to the nucleus unlike previously reported remorins. To investigate the biochemical functions, *E.coli* expressed GmREMs were purified and their oligomeric status were analyzed under various conditions. To better understand the possible roles of remorin, screenings to clarify the signaling pathway and kinases asssociated with remorins are in progress. "
(a) School of Biological Sciences, Seoul National University (b) Institute of Biosciences and Biotechnology, Myongji University (c) Polar Biocenter, Korea Polar Research Institute (KOPRI)

P30046 MicroRNA172 Regulates Developmental Transitions via *FLOWERING LOCUS T* and *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* Genes in *Arabidopsis*
Jung, Jaehoon-presenter hanbari2@snu.ac.kr(a) Seo, Pil Joon (a) Lee, Sangmin (a) Kim , Ok-kyoung (a) Park, Chung-Mo (a)
"Plants undergo a series of distinct developmental transitions during their growth, beginning with seed germination and continuing through vegetative phase change, reproductive phase change, flowering, and seed production for the next generation. Environmental cues, including photoperiod and ambient temperature, and intrinsic developmental programs, such as gibberellic acid, regulate the developmental transitions through a complex network of signaling pathways. Here, we demonstrate that microRNA172 (miR172) regulates developmental phase transitions via *FLOWERING LOCUS T* (*FT*) and *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) genes in *Arabidopsis*. The miR172 signals promote reproductive phase change by inducing *FT*. The miR172-mediated *FT* signals also regulate vegetative phase change by modulating a subset of SPL transcription factor genes. Accordingly, while expression of the *SPL* genes was significantly induced in the transgenic plants overproducing miR172 (35S:*172*), the SPL transcript levels were reduced in the 35S:*172* X *ft-10* plants to a level comparable to those observed in the *ft-10* mutant. Furthermore, the *FT* regulation of the *SPL* genes was independent of miR156. These observations indicate that *FT* plays dual roles in the miR172 pathway: it promotes floral induction through the flowering pathway, and it also regulates vegetative phase change by inducing the *SPL* genes."
(a) Seoul National University

P30047 Arabidopsis SR protein atRSp31: identification of RNA targets and its role in stress response
Kalyna, Mariya-presenter mariya.kalyna@univie.ac.at(a) Maronova, Monika (a) Simpson, Craig G (b) Brown, John W (b) Barta, Andrea (a)

"SR proteins are a family of evolutionary conserved splicing factors that play crucial roles both in constitutive and alternative splicing (AS). The genome of Arabidopsis thaliana encodes nineteen SR proteins and their exact function is unclear. A phenotypic study of plants overexpressing the plant specific SR protein atRSp31 revealed that it promotes senescence and is a negative regulator of stress responses, including oxidative stress, salinity, abscisic acid and sugar treatments. To characterize the function of atRSp31, we aimed to identify RNA targets using plants overexpressing atRSp31 and atRSp31 mutant plants. Corroborating the data from the phenotypic study, microarray analysis of atRSp31 overexpressing plants pointed out genes involved in response to diverse biotic and abiotic stimuli, genes participating in ROS metabolism, carbohydrate metabolism, aging and senescence. These data were complemented by results of the recently established AS RT-PCR panel, a system of monitoring changes in AS in multiple genes in Arabidopsis (Simpson et al., 2008). Using this system in a study of about 90 genes we analysed the effect of overexpression of atRSp31 on AS patterns, and significant changes were observed in ten of them. Interestingly, three genes involved in DNA repair were modulated by atRSp31. Overexpression of atRSp31 caused a two-fold increase in usage of a proximal 3 splice site in the DNA repair endonuclease RAD1/UVH1. This splicing event leads to the production a truncated protein, which happens to be the same as in a rad1/uvh1 mutant. Phenotypic analysis now shows that atRSp31 overexpression and the rad1/uvh1 mutant lines both show hypersensitivity to the UV-C, while an atRSp31 mutant is more resistant to UV-C irradiation. "
(a) Max F. Perutz Laboratories, Medical University of Vienna, Dr. Bohrgasse 9/3, A-1030 Vienna, Austria (b) Programme of Genetics, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK

P30048 Finding RNA targets of splicing factors by genomic SELEX in *Arabidopsis Thaliana*
Bannikova, Olga-presenter olga.bannikova@univie.ac.at(a) Chen, Doris (a) Barta, Andrea (a)
"Serine/arginine-rich (SR) proteins are key players both in constitutive and in alternative splicing. The Arabidopsis genome contains 19 SR genes several of which have no orthologues in metazoan. Here we focus on two plant specific members of RS family proteins atRSZp33 and atRSp31.They encode proteins with an RNA-binding motif, presence or absence of two zinc knuckles, arginine/serine (RS) domain and a C-terminal tail rich in serine/proline dipeptides. Arabidopsis encodes conserved multidomain cyclophilin protein atCyp59 which has domain organization similar to RS proteins. It consists of PPIase domain at the N terminus, followed by an RNA recognition motif (RRM), a Zn-knuckle and a C-terminal domain enriched in charged amino acids and serines or RS/RD dipeptide repeats. We have previously shown that Cyp59 likely connects splicing and transcription as it binds to SR proteins as well as to the CTD of RNA polymerase II. The RRM domain shows evolutionarily the highest conservation from S. pombe to humans. Therefore, we set out to find RNA target sequences which bind to the RRM of atCyp59, atRSZp33, atRSp31. One method we used is a Genomic SELEX using recombinant GST-tagged proteins. The RNA library was constructed by random priming of sheared Arabidopsis DNA with a direct and reverse primer and selection of fragment with the desired length (200-300 nt. After ten rounds of selection we were able to obtained 6.5% of RNA is bound to the protein of interest. Now we are carrying out the 454 deep sequencing of this high affinity pool. Then the sequences will be analyzed. "
(a) Max F. Perutz Laboratories, Medical University of Vienna

P30049 Tyrosyl-DNA phosphodiesterase I is required for maintenance of organ size during *Arabidopsis* development
Kim, Sang-Gu-presenter kimsg@snu.ac.kr(a) Lee, So-Young (a) Kim, Hoyeun (a) Hwang, Hyun-Ju (a) Jeong, Young-Min (a) Woo, Je-Chang (b)
"Although many proteins and cellular processes that are required for plant development have been identified, the molecular framework by which they involve in the stable maintenance of organ sizes is still elusive in plants. Here, we report the functional nuclear protein, a homolog of human Tdp1, for organ size maintenance during Arabidopsis development. The loss-of-function *TDP1* mutation displays developmental defects and dwarfish phenotype in Arabidopsis. And this phenotype is substantially caused by decreased cell numbers without any changes of individual cell sizes. The *tdp1* plants exhibit hypersensitivities to camptothecin (CPT), a potent topoisomerase I inhibitor, and show rigorous cell death in cotyledons and rosette leaves, suggesting the failure of DNA damage repair in *tdp1* mutants. Our recombinant AtTDP1 protein certainly hydrolyzes the 3 prime-phosphotyrosyl DNA substrates related to repairing of in vivo topoisomerase I-DNA induced damages. Taken together, we suggest that AtTDP1 protein may play a pivotal role in organ size maintenance during Arabidopsis development"
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SESSION P31 – GENOME EVOLUTION

P31001 Evolutionary genomics of supersized plant mitochondrial genomes
Alverson, Andrew J-presenter alversoa@indiana.edu(a) Rice, Danny W (a) Stern, David B (b) Palmer, Jeffrey D (a)
"Angiosperm mitochondrial (mt) genomes vary in size by at least one order of magnitude, and much of this variation occurs within a single family, the Cucurbitaceae, whose mt genomes vary from an estimated 330 kb to 2400 kb in size. We fully sequenced the mt genomes of *Citrullus lanatus* (379,236 nt) and *Cucurbita pepo* (982,833 nt), the two 'smallest' characterized cucurbit mt genomes. The size and sequence complexity of the *Citrullus* mt genome is similar to previously sequenced plant mt genomes. The larger *Cucurbita* mt genome actually contains slightly less coding sequence than *Citrullus*. Expansion of the *Cucurbita* mt genome reflects unprecedented increases in the acquisition and proliferation of small (<100 nt) repeated sequences, which account for >40% of the entire genome. Both genomes contain transposable elements and protein coding genes of nuclear origin, and >11% of the *Cucurbita* mt genome resembles plastid DNA that might have been resident in the nuclear genome before entering the mt genome. Draft sequences of the *Cucumis melo* (~2.6 Mb) and *Cucumis sativus* (~1.6 Mb) mt genomes validate early size estimates and indicate major, recent size increases in the mt genomes of cucurbits, recommending them as an excellent model system for studying the large and variably sized mt genomes of plants. "
(a) Indiana University (b) Boyce Thompson Institute for Plant Research

P31002 Rates and patterns of plastid genome evolution in the grasses
Jansen, Robert K-presenter jansen@mail.utexas.edu(a) Guisinger, Mary M. (a) Chumley, Timothy W. (a,e) Kuehl, Jennifer V. (b) Boore, Jeffrey L. (c,d)
http://www.biosci.utexas.edu/ib/faculty/jansen.htm
"Plastid genomes of grasses (Poaceae) are unusual both in their organization and rates of sequence evolution. There has been a recent surge in the availability of their plastid genome sequences, but a comprehensive comparative analysis of genome evolution has not been performed that includes any related families in the Poales. We report on the genome sequence of Typha latifolia (Typhaceae, Poales), and we present comparisons of genome organization and sequence evolution within Poales. Our results confirm that grass plastid genomes exhibit a rapid acceleration in both genomic rearrangements and nucleotide substitutions. Poales have multiple structural rearrangements, including three inversions, three genes losses

(accD, ycf1, ycf2), intron losses in two genes (clpP, rpoC1), and expansion of the inverted repeat at both the large and small single copy regions. Most of these rearrangements are restricted to the Poaceae, and the IR expansion into the SSC correlates with phylogenetic relationships within the family. Comparisons of 73 protein-coding genes for 47 angiosperms including nine genera of Poaceae confirm that the branch leading to Poaceae has significantly accelerated rates of change relative to other Poales, monocots, and other angiosperms. Furthermore, rates of sequence evolution within grasses are lower, indicating a deceleration during diversification of the family. Overall there is a strong correlation between accelerated rates of genomic rearrangements and nucleotide substitutions in Poaceae, a phenomenon that has been noted recently throughout angiosperms. The cause of the correlation is unknown, but faulty DNA repair mechanisms have been suggested in other systems, including bacterial and animal mitochondrial genomes."
(a) Univerisity of Texas at Austin (b) DOE Joint Genome Institute and Lawrence Berkeley National Laboratory (c) Genome Project Solutions (d) University of California Berkeley (e) Central Washington University

P31004 "Developing objective, *a priori* criteria for investigating genomic evidence of endosymbiotic gene transfer."
Stiller, John W-presenter stillerj@ecu.edu(a) Huang, Jinling (a) Shroeder, Brian (b)
http://www.ecu.edu/cs-cas/biology/stiller_john.cfm
"Complete sequences of eukaryotic genomes provide large, complex data sets for comparative analyses. These data can be examined for cumulative phylogenetic signal indicating genealogical relatedness, or for concerted conflicts that could reflect historical or cryptic endosymbioses. In this context, putatively algal genes found in nonphotosynthetic protists have been cited as evidence of past endosymbiotic gene transfer (EGT) from a lost plastid. Such *a posteriori* results from genome-level data mining are difficult to interpret, however, because they do not address whether the amount of aberrant phylogenetic signal found is significantly greater than what is expected from null or alternative models. We therefore developed tests of *a priori* predictions of a prominent example of presumed EGT, that is, putatively algal genes in oomycete genomes as evidence in support of the chromalveolate hypothesis. In genome level analyses of signal from similarity scores, contingency tests on genes unrelated to plastid function provide extraordinarily significant support for EGT from red algae into plastid-containing diatoms, but indicate that none of that support is shared between diatoms and non-photosynthetic oomycetes. Our results argue strongly against *a posteriori* interpretations of genome-level data, and in favor of explicit tests of the different assumptions inherent in competing *a priori* hypotheses. Moreover, we uncovered a strong association between overall sequence similarities found in genomes and their relative sizes in numbers of genes. We further explore this relationship as a potential null model against which *a priori* hypotheses of EGT can be tested, and as a method for objectively uncovering possible cryptic endosymbiotic gene contributions."
(a) East Carolina University (b) Oakwood School

P31005 Possible role for aneuploidy in the evolution of *Arabidopsis*
Matsushita, Starr C.-presenter smatsushita@ups.edu(a) Wright, Kirsten (a) Madlung, Andreas (a)
"Aneuploidy occurs when chromosomes are either lost or gained from parent to offspring. Organisms that are affected by aneuploidy often show either infertility or reproductive isolation from the progenitors. In rare cases, infertility due to aneuploidy can be overcome by genome duplication, restoring fertility by providing each chromosome with a pairing partner during meiosis. Genome duplication following aneuploidy could therefore lead to the evolution of new chromosomal combinations (cytotypes) of a species and, depending on how much chromosomal information is lost or duplicated in these events, might ultimately lead to speciation. To test this hypothesis we used a hybrid of *Arabidopsis thaliana* (diploid - 10 chromosomes) and *Arabidopsis suecica* (tetraploid containing 10 chromosomes from *A. thaliana* and 16 chromosomes from *A. arenosa*) that had undergone spontaneous genome duplication. Using fluorescent in-situ hybridization (FISH), we determined the degree of aneuploidy in its offspring and investigated if genetically separate sibling populations have been formed. Our data show that despite genome duplication and high fertility, the F3 offspring show a high degree of mitotic aneuploidy in their cells. At this point in their evolution none of the 6 sibling lines have shown any novel or established cytotypes from their progenitor."
(a) Department of Biological Sciences, University of Puget Sound

P31007 Phylogenetic analysis for cytoplasmic male sterility and fertility restoration genes revealed their coevolution relationship in *Oryza* species
Li, Shaoqing (a) Tan, Yanping (a) Xie, Hongwei (a) Zhu, Yingguo-presenter zhuyg@public.wh.hb.cn(a)
"Although characterization of genes associated with cytoplasmic male sterility (CMS) and fertility restoration (Rf) has been well documented, the studies of phylogenetic or evolution relationship between nuclear Rf and CMS factors in mitochondria in Oryza species has been usually ignored. Here, 41 accessions from seven Oryza species with AA genome were employed and analyzed for the status of Rf genes for Honglian (HL, gametophytic type) and Wild-abortive (WA, sporophytic type) CMS, and for the phylogenetic relation of the mitochondria and Rf candidates on chromosome 10. The 41 investigated accessions were categorized into five groups according to the restoring ability for HL- and WA-CMS. The phylogenetic tree based on restriction fragment length polymorphism (RFLP) patterns of CMS-associated mitochondrial genes shows that these 41 Oryza lines fall into five distinct groups. A phylogenetic tree based on PCR profiles of nuclear Rf candidates on chromosome 10 were also established, and five groups were distinctively grouped. The accessions in each subgroup between the two phylogenetic trees are well parallel to each other. Furthermore, according to the distribution of Rf genes for HL- and WA-CMS, the accessions with similar fertility restoring pattern were always clustered in the same subgroup of the two phylogenetic trees. Therefore, we conclude that genetic diversity of CMS-associated mitochondrial genes is compatible to that related to nuclear Rf genes, CMS and Rf seems existing a coevolutionary relationship in the Oryza species. Key Words: cytoplasmic male sterility (CMS), coevolution, fertility restorer (Rf), Oryza species, phylogenetic analysis "
(a) Key Laboratory of MOE for Plant Developmental Biology

P31008 Evolution of the non-recombining region of the papaya Y chromosome
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"The sex chromosomes of papaya (*Carica papaya*) offer an opportunity to investigate the evolution of incipient sex chromosomes due to their recent origin. We sequenced two X/Y gene pairs and analyzed the levels of polymorphism and divergence between X and Y chromosomes. Both X- and Y-linked copies have reduced polymorphism compared with autosomal alleles; however, this reduction is significant only for the X chromosome. We hypothesize that the reduced variation on the X is due to background selection. We also found autosomal diversity is reduced in cultivar papaya relative to wild varieties, consistent with a domestication bottleneck. Finally, divergence analyses support the recent and independent emergence of sex chromosomes in papaya, with an estimated age of 2 million years ago. "
(a) Miami University

P31009 The molecular mechanism for generating diversity in flowering time of cultivated rice
Takahashi, Yasuyuki-presenter y-takaha@bs.naist.jp(a) Teshima, Kosuke M. (b) Yokoi, Shuji (a) Innan, Hideki (b) Shimamoto, Ko (a)
"Rice (*Oryza sativa* L.) has evolved during the last 8000 years of domestication and breeding. One major reason for the spread of rice cultivation to a wide range of geographical regions is the diversification of flowering time. Rice is a facultative short day plant, and molecular genetic studies have identified the major genes involved in short day flowering. However, the molecular mechanisms promoting the diversity of flowering time in cultivated rice are not known. We used a core collection of 64 rice cultivars from around the world and studied the expression levels and polymorphisms of 6 genes in the short day flowering pathway. The RNA levels of *Heading date 3a* (*Hd3a*), encoding a floral activator, are highly correlated with flowering time and there is a high degree of polymorphism in the Hd1 protein, which is a major regulator of *Hd3a* expression. Functional and non-functional alleles of *Hd1* are associated with early and late flowering, respectively, suggesting that *Hd1* is one of the major determinants of variation in flowering time of cultivated rice. We also found that the type of *Hd3a* promoter and the level of *Ehd1* expression contribute to the diversity in flowering time and *Hd3a* expression level. The contributions of these three factors were evaluated by a statistical analysis, using a simple linear model, whose results supported our experimental observations. These results in rice differ considerably from those in *Arabidopsis thaliana* in which diversity in flowering time is mainly associated with variations in vernalization responses. It appears that crops and plant species can adopt unique strategies to generate variations in flowering time and that the strategy used is one of the most important characters in the life of plants."
(a) Nara Institute of Science and Technology (b) The Graduate University for Advanced Studies

P31010 Population structure and genetic differentiation among populations of *Cornus canadensis*
Simis, Molly-presenter msimis1@mix.wvu.edu(a) DiFazio, Stephen (a) Goldstein, Mike (b)
"The base of Ice Mountain, Hampshire Co., WV, is a cold-producing slope and is home to a rare population of the boreal forest herb *Cornus canadensis* (bunchberry). *C. canadensis* is one of three herbaceous dwarf dogwood species. The presence of *C. canadensis* is rare because it normally grows at high latitudes or high elevations, and the base of Ice Mountain is only 210 meters at a latitude of approximately 39.4^{o}N. Samples from Ice Mountain and several core populations have been analyzed in order to determine the degree of genetic differentiation between and among populations. Samples from locations including three sites in Alaska and one site in Minnesota have been analyzed. Amplified Fragment Length Polymorphism (AFLP) with neutral markers has been used to assay genetic variation. The population genetics software Arlequin has been used to perform an Analysis of Molecular Variance (AMOVA) and estimate Fst, the genetic differentiation among subpopulations. I hypothesize that pairwise Fst will be elevated between Ice Mountain and the core populations because of its distinct history as a glacial refugium."
(a) West Virginia University (b) USDA Forest Service

P31011 Duplication and Divergence of *GrpE* genes in Arabidopsis
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"GrpE stimulates the exchange of nucleotide and the release of protein substrate from Hsp70 in the Hsp70/DnaK chaperone machine. In Arabidopsis, two GrpE homologues each can be found in mitochondria and plastids. In each organelle, these GrpE proteins are coupled with two isoforms of Hsp70. We traced the duplication history of these genes with the web-based program (http://wolfe.gen.tcd.ie/athal/dup) and found that the mitochondrial *GrpE* pair, which is located in duplicated blocks in the genome, is likely caused by a recent polyploidy event (24-40 million years ago), while the plastid pair likely arose from an ancient duplication event. In contrast, the mitochondrial *Hsp70* duplication might be the result of an ancient event, while the plastid *Hsp70* duplication likely results from a recent one. Retention of two copies of mitochondrial *GrpE* is common in many higher plant species, suggesting that duplication and divergence of the co-chaperone complies with stress tolerance needs. Transcriptomic data from the public domain show that the expression of mitochondrial *GrpE* genes, *Mge1* (At5g55200) and *Mge2* (At4g26780) is differentially regulated by UV-B and heat stress, respectively. The mitochondrial *Hsp70*, *mtHsc70-1* (At4g37910) and *mtHsc70-2* (At5g09590) also showed similar expression patterns to *Mge1* and *Mge2*, respectively, in response to stress, suggesting functional divergence of the Hsp70 machinery in mitochondria. We confirmed that Mge2 is a heat-inducible protein by RT-PCR and immunoblot and that Mge2-YFP fusion protein is localized in mitochondria. By studying its T-DNA knockout lines we further found that Mge2 is dispensable for growth and development under normal conditions, but necessary for thermotolerance."
(a) Agricultural Biotech. Research Center, Academia Sinica

P31012 Identification of an *Arabidopsis thaliana* 18S rDNA variant with 270 bp deletion
Ayalew, Mentewab-presenter mayalew@spelman.edu(a) Jacobsen, Megan (b) Flowers, Rebekah (a)
"In *Arabidopsis thaliana* , there are approximately 1200-1500 rRNA genes encoding the 18S, 5.8S, and 25S rRNAs. These rRNAs clusters are found on chromosomes 2 and 4 and are considered to be virtually identical in sequence complexity. However, because of difficulties in sequencing repeat regions, the degree of sequence variation is not known. To explore sequence variation in the Arabidopsis 18S gene we cloned and sequenced 47 copies of 18S genes. While most sequences had limited polymorphism, 3 clones showed 270 bp deletions. To rule out amplification and cloning artifacts, primers were designed that specifically amplify the deleted variant. PCR using *Arabidopsis* genomic DNA as template corroborated the presence of a deleted variant. The deleted region maps to a critical region, Helix 18, which is necessary for codon-anticodon recognition and stabilization. We therefore investigated whether the newly identified variant was expressed. RT-PCR was conducted on RNA extracts from leaves, roots, stems, flowers and the deleted variant was found to be expressed. Our results suggest that the deleted 18S variant may have a specific role."
(a) Spelman College (b) Emory School of Public Health

P31013 Comparison of Ancient Mu Inserts in Maize and Teosinte
Restrepo, Christian D-presenter civic88@ufl.edu(a) Hunter, Charles T III (a) Latshaw, Susan P (a) Ibekwe, Emeka I (a) McCarty , Donald R (a) Koch , Karen E (a)
"Conservation and/or proliferation of transposable elements during the evolution and domestication of maize can tell us much about those processes and about differences between maize inbred lines. Mu transposons are found throughout maize germplasms and have been an invaluable genetic tool for identifying gene function via forward and reverse genetics. When a Mu element is found close to, or within a gene, changes in that gene's expression typically occur. Maize inbreds theoretically lack Mu activator elements (MuDR transposase), thus their complement of Mu elements are expected to be stable and distinct. Through bioinformatic and PCR analyses we found that three inbreds (B73, W22, and Mo17), together with five teosinte parviglumis inbreds (courtesy of J. Doebley), not only contain stable inserts from classic members of the Mu family (Mu's 1-9), but also the group of more diverse Mu's (Mu's 10 and 12). Phylogenic and sequence analysis showed that the Mu 10 element is very similar to Mu 9 (MuDR), and that both contain MudrA-and MudrB-like domains. Also of note is that Mu 12 inserts in B73 are approximately as abundant as all the Mu 1-9 elements together. Preliminary data from Mu-anchored 454 sequencing has indicated distinct profiles of ancestral Mu-inserts and different evolutionary histories for diverse maize and teosinte inbreds. Analyses of these insert sites could shed light on the specific physiological and genetic characteristics as well as the differences of the maize inbreds and the mutations they carry. "
(a) University of Florida

P31014 The expression of young gene duplicates in developmental tissues of *Arabidopsis thaliana*.
Owens, Sarah M-presenter owenssm@muohio.edu(a) Harberson, Nicholas A (a) Moore, Richard C (a)
"Gene and genome duplication can lead to increased morphological complexity by giving rise to new genes and gene functions. The classic model suggests that new gene duplicates are functionally redundant and that a new function can only arise through mutation. There is now evidence that suggests functional redundancy does not always follow gene duplication. A new function can be created upon duplication, not only through the creation of new genes, but also through the creation of new expression domains. I am looking for evidence of functional divergence, in the form of divergent developmental expression patterns, between 18 pairs of unlinked recent gene duplicates in *Arabidopsis thaliana*. These duplicates are specific to the *A. thaliana* lineage and have a silent site divergence rate of less than 4%, indicating they emerged less than 2 million years ago. Although the duplicate genes maintain all coding regions, the average extent of the duplicated region is 2917 bp, including an average of only 726 bp of upstream promoter region. The relatively limited extent of the duplicated promoter region suggests that these duplicate loci may have lost cis-regulatory elements or gained new ones, leading to divergent expression patterns. Differential expression will be evaluated between rosette leaves, cauline leaves, stems, young flower buds, open flowers, roots, and siliques. I will compare the expression data I obtain from my experiments in *A. thaliana* to the single copy ortholog in the sister species, *Arabidopsis lyrata*, to polarize any changes in gene expression between duplicates; that is, whether there have been gains or losses in expression relative to the ancestral expression pattern of the single copy gene."
(a) Miami University Botany Department

P31015 "A new-mutant resource in maize: stable, single-gene knockouts from the transposon-mutagenic UniformMu population"
McCarty, Donald R. (a) Collins, Joe M.-presenter jcoll86@ufl.edu(a) Wu, Shan (a) Guan, Jiahn-Chou (a) Liu, Funnce (a) Campbell, Darwin (b) Sen, Taner (b) Lawrence, Carolyn (b) Sachs, Marty (c) Koch, Karen E. (a) Avigne, Wayne T. (a) Latshaw, Susan P. (a) Hunter, Charles T. III (a) Suzuki, Masaharu (a) O'brien, Brent A. (a) Restrepo, Christain D. (a) Eveland, Andrea L. (a) Ibekwe, Emeka I. (a)
"A new resource for identifying single-gene knockouts in maize is available through MaizeGDB (www.maizegdb.org). The UniformMu Reverse Genetics Project at UF has produced 2,727 stable, sequence-indexed Mu-insertion mutants. This collection may be BLAST-searched via Genbank (NCBI) or at uniformmu.uf-genomics.org. Each insert is carried by a specific, defined line that is identified by the Mu-insertion sites. Genome location and annotation for each insertion can be obtained at MaizeGDB and a direct link to the Maize Genetics Cooperation Stock Center enables seed requests. The Mu-insertions have been sequence-validated by interception of at least two axes in a DNA grid of 24x24 pooled samples. Germinal inheritance was verified by 454-sequencing a subset of the 2,727 lines (including 690 Mu-insertions) in F2 progenitors. PCR analysis (with sequenced products) of 85 lines further validated the mutants, with 100% of the subset carrying the sequence-indexed Mu-insertion. One to two F3 sibling ears contribute seeds to each line, and each line carries an average of 4.7 unique inserts. Lines that segregate for visible-kernel or seedling mutants carry greater insert numbers. Approximately 1,200 of the mutants were identified in genes annotated in rice and *Arabidopsis* genomes; including 123 mutants in specific enzymes, 68 in transporters, and 64 in DNA- or RNA-binding proteins."
(a) Plant Mol & Cell Bio Program, University of Florida (b) Corn Insects and Crop Genetics Research Unit, USDA-ARS (c) Maize Genetics Cooperation Stock Center, USDA-ARS

P31016 Graphic representation of maize centromere 2 using custom annotation software
Wolfgruber, Thomas K-presenter tomwolf@hawaii.edu(a) Sharma, Anupma (a) Schneider, Kevin (a) Presting, Gernot G (a)
"DNA sequences in the centromeres of *Zea mays* (maize) are not well understood, but are known to consist primarily of two DNA repeats - the satellite sequence CentC and centromeric retrotransposons of maize (CRM). The CRM1 subfamily was recently shown to contain two parental and five recombinant subgroups. To study the distribution of sequences underlying a functional maize centromere, we developed software to automatically display centromeric elements, including CRM subfamilies and recombinants, CentC repeats, and other repetitive and genic sequences of the maize centromere 2. The resulting images provide a bird's eye view of centromere evolution."
(a) Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa

P31017 Identification and characterization of centromeric retrotransposons in maize
Sharma, Anupma-presenter anupma@hawaii.edu(a) Wolfgruber, Thomas K (a) Presting, Gernot G (a)
"Centromeric retrotransposons (CR) are located almost exclusively at the centromeres of plant chromosomes. Centromeric retrotransposons contain a putative chromointegrase domain that is thought to regulate their integration specificity. Previously, we determined the orthologous relationships between four CR subfamilies of maize and rice and discovered that two sequence variants of the CRM1 subfamily recombine to generate subgroups that differ in abundance and activity. To understand the evolution of CRs, we analyze the Zea mays inbred B73 genomic sequence to identify novel CRM subfamilies and determine their structure, phylogenetic relationships, relative abundance, insertion times and distribution at the maize centromeres."
(a) Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa

P31018 Delineation of the Functional Maize Centromeres by Chromatin Immunoprecipitation
Schneider, Kevin L-presenter kevinls@hawaii.edu(a) Wolfgruber, Thomas K (a) Lee, Hye-Ran (b) Sharma, Anupma (a) Jiang, Jiming (b) Presting, Gernot G (a)
"Maize centromeres consist of repetitive DNA sequences, including the satellite repeat CentC and centromeric retrotransposons of maize (CRM). CENH3, a centromere-specific histone, is located predominantly at the functional centromeres. To delineate the centromeres in *Zea mays* inbred B73 we employed chromatin immunoprecipitation (ChIP) with the anti-CENH3 antibody to enrich for centromeric DNA, followed by pyrosequencing and mapping the reads to the maize genome. As expected, CRM and CentC sequences are enriched in ChIP reads as compared to the whole genome. Identification of the functional centromeric regions is the first step towards gaining insights into the function and evolution of centromeric sequences."
(a) Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa (b) Department of Horticulture, University of Wisconsin-Madison

SESSION P32 – HEAVY METALS & PHYTOREMEDIATION

P32001 A broccoli COQ5 methyltransferase involved in ubiquinone biosynthesis mediates selenium volatilization
zhou, Xin (b) Yang, Yong (a) Thannhauser, Theodore W (a) Kochian, Leon V (a) Li, Li-presenter ll37@cornell.edu(a,b)
"Biological selenium volatilization, which converts selenium into volatile compounds, provides an important means for the cleanup of selenium

polluted environments. To identify novel genes whose products are involved in plant selenium volatilization, a broccoli cDNA encoding COQ5 methyltransferase (BoCOQ5-2) in ubiquinone biosynthetic pathway was isolated. Its function was authenticated by complementing a yeast *coq5* mutant and by detecting increased cellular ubiquinone levels in *BoCOQ5-2* transformed bacteria. Proteomic analysis of differentially expressed proteins between bacteria expressing *BoCOQ5-2* and those containing the empty vector further supported its functional role in ubiquinone biosynthesis. BoCOQ5-2 was found to specifically promote selenium volatilization but not sulfur emission in both bacteria and transgenic *Arabidopsis* plants. Bacteria expressing *BoCOQ5-2* produced an over 160-fold increase in volatile selenium compounds when they were exposed to selenate. Consequently, the *BoCOQ5-2* transformed bacteria had dramatically enhanced tolerance to selenate and selenite, and contained reduced levels of total selenium in the cells. Transgenic *Arabidopsis* expressing *BoCOQ5-2* volatilized three times more Se than the vector only control plants when treated with selenite and exhibited significant tolerance to selenium. BoCOQ5-2 represents the first plant enzyme that is not known to be directly involved in sulfur/selenium metabolism, yet mediates selenium volatilization. This discovery opens up new prospective regarding our understanding of the complete metabolism of selenium and could lead to ways to modify selenium accumulator plants with increased efficiency in the phytoremediation of selenium contaminated environments."
(a) Robert W. Holley Center for Ag & Health, USDA-ARS (b) Cornell University

P32003 Distinctive plant response to cadmium and arsenate due to *AtPCS1* and *CePCS* overexpression
Wojas, Sylwia-presenter sylwiawojas@biol.uw.edu.pl(a) Antosiewicz, Danuta M (a) Clemens, Stephan (b)
"Phytochelatins (PCs), small heavy-metal complexing peptides, synthesized from glutathione by phytochelatin synthase (PCS), play an important role in cadmium and arsenate tolerance. Previously, different authors presented contradictory results on the effects of *PCS* overexpression in various plant species. *PCS* overexpressing plants were shown to be more tolerant to As; however, their response to Cd ranged from Cd-hypersensitivity to increased Cd-tolerance. Our study on a model plant species tobacco, transformed with either of two different *PCS* genes: *AtPCS1* and *CePCS*, investigates the mechanisms underlying the contrasting responses to Cd and As due to PCS overexpression. We demonstrated previously that plants overexpressing *AtPCS1* were Cd-hypersensitive, whereas CePCS transformants: more Cd-tolerant. The expression of *AtPCS1* and *CePCS* did not influence the expression level of tobacco *NtPCS1*. The significant increase in PCS activity in *AtPCS1* expressing tobacco was accompanied by decreased Cd-detoxification capacity and higher oxidative stress level, which possibly explains the increase in Cd-sensitivity. The interrelationship between the rate of PCs biosynthesis, and their transport to vacuole/degradation, is probably different in *AtPCS1* and *CePCS* expressing plants, which might be an additional factor contributing to the observed difference in Cd-tolerance. Moreover, we demonstrated that expression of both *PCS* genes resulted in an increase of As-tolerance, with CePCS transformants most tolerant. These results suggest possible differences between the metabolic pathways/ regulation mechanisms associated with As and Cd detoxification by phytochelatins. "
(a) Univerisity of Warsaw, Faculty of Biology, Institute of Experimental Plant Biology (b) University of Bayreuth, Department of Plant Physiology

P32004 Expression of the wheat transcription factor TaCRF1 confers cadmium resistance in yeast and rice
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"Cadmium (Cd) is a widespread soil pollutant in industrial and agricultural areas. We isolated a novel gene from a wheat root cDNA library, which conferred a strong Cd tolerance when expressed in yeast (*Saccharomyces cerevisiae*) and rice (*Oryza sativa*). The gene, which we called TaCRF1 (*Triticum aestivum Cadmium Resistance Factor1*), encodes a protein with a structure similar to class A of heat shock transcription factors (Hsf). A green fluorescent protein (GFP) fusion protein of TaCRF1 (TaCRF1-GFP) located in the nucleus. Among the two very close homologs in rice, OsHsfA4b and OsHsfA4d, only OsHsfA4b could confer Cd tolerance. A detailed analysis to identify the region conferring Cd tolerance revealed that the DNA binding domain of TaCRF1 played a major role. Within this region, Ala-31 and Leu-42 were essential for Cd tolerance. A search for the target of TaCRF1 revealed that Cd resistance mechanism of TaCRF1 requires metallothionein in yeast and rice. TaCRF1 may have a function in Cd tolerance in wheat, since its transcript level increases upon Cd treatment."
(a) POSTECH-UZH Global Research Laboratory, Division of Molecular Life Sciences, Pohang University of Science and Technology, Pohang, KOREA (b) Division of Molecular and Life Science, and Biotechnology Research Center, Pohang University of Science and Technology, Pohang, KOREA (c) Institute of Plant Biology, University of Zurich, Zollikerstrasse 107, 8008 Zurich, Switzerland

P32005 Phytoremediation of a Variety of Organic Pollutants
Doty, Sharon L-presenter sldoty@u.washington.edu(a) Lee, Keum Y (a) Kang, Jun W (a) Strand, Stuart E (a)
http:// http://faculty.washington.edu/sldoty/
"Halogenated hydrocarbons, such as trichloroethylene (TCE), are serious environmental contaminants of soil, groundwater, and air. Using microarrays of poplar and Arabidopsis, we have determined a number of genes that are differentially regulated in response to TCE. Many of these genes have homology to genes involved in the three phases of pollutant detoxification in plants. Chlorpyrifos is one of the commonly used organophosphorus insecticides and causes serious environmental and human health problems. To evaluate potential for degradation of chlorpyrifos by plants, several plant species including aspen, cottonwood, and willow were investigated. Chlorpyrifos was taken up by plant roots and degraded into TCP which is non-toxic. To our knowledge, this work represents the first report for phytoremediation of chlorpyrifos. In order to develop plants more capable of tolerating the stressful environment of contaminated sites, we introduced a construct to overexpress a chaperone protein that confers general stress tolerance including salt, heat, cold, and drought. The poplar hybrid clone 717-1B4 (Populus tremula x P. alba) was transformed with Agrobacterium tumefaciens. Increased tolerance to general stress and the presence of pollutants is being investigated. "
(a) University of Washington

P32006 Engineering Transgenic Grasses for in situ Treatment of RDX and TNT
Strand, Stuart E-presenter sstrand@u.washington.edu(a) Bruce, Neil C (b) Rylott, Liz (b) Doty, Sharon (a) Zhang, Gengyun (a)
"Phytoremediation provides a low cost and aesthetic way for pollutant remediation. TNT and RDX are toxic contaminants that are common pollutants at manufacture plants and military training ranges. TNT and RDX are phytotoxic, mutagenic and have a serious impact on the environment and human health. Recent advances have shown that transgenic Arabidopsis and tobacco, transformed with the bacterial nfsI and xplA genes, can take up and detoxify explosives from hydroponic solution and contaminated soil. Perennial grass species, due to their ability to grow and cover ground totally year round and their wide geographic distribution, are excellent candidates for phytoremediation. In order to test the performance of these genes in perennial grasses, the nfsI and xplA genes were transformed into creeping bentgrass with a biolistic co-transformation method. From 8 dishes of transformed embryogenic calli that induced from nodes of a single creeping bentgrass strain, 84 hygromycin resistant callus lines were obtained. The integration of the nfsI and xplA genes were confirmed by PCR assay. Results of TNT and RDX stress tests with culture medium supplemented with TNT or RDX indicated that 12 transgenic lines showed clear TNT toxicity tolerance at 0.15 mM TNT level and 7 transgenic lines showed increased growth using RDX as the sole nitrogen source at 165 ppm. Data from hydroponic culture and soil flow through experiments

indicated that transgenic lines with xplA gene significantly removed more RDX from medium than control plants. "
(a) Univ Washington (b) University of York

P32007 "The Adaptation Mechanism to Copper Deficient Environments via a Master Regulator, SPL7, in *Arabidopsis*"
Yamasaki, Hiroaki-presenter doyasa@pmg.bot.kyoto-u.ac.jp(a) Shikanai, Toshiharu (a)
"Copper, one of essential micronutrients for most living organisms, is mainly utilized as a cofactor of proteins involved in photosynthesis and scavenging of reactive oxygen species in higher plants. Most abundant copper protein is plastocyanin (PC), localized to thylakoid lumen of chloroplasts, and is essential for photosynthesis in higher plants. Another major copper protein, copper/zinc superoxide dismutase (CSD), localized to cytosol (CSD1) and chloroplast stroma (CSD2), is involved in the scavenging of reactive oxygen species. In copper deficient conditions, the expression of CSD1 and CSD2 is down-regulated and their function is compensated by iron superoxide dismutase (FSD) specifically expressed in low copper conditions. Previously we demonstrated that a microRNA, *miR398* was involved in this down-regulation of CSD1 and CSD2 (Yamasaki et al., 2007). *miR398* is expressed in low copper conditions and is involved in the degradation of *CSD1* and *CSD2* mRNA directly. Consequently, limited copper is preferentially transferred to indispensable copper protein like PC. In this study, we identified SPL7 (*SQUAMOSA promoter-binding protein-like 7*) as a transcription activator for *miR398*. SPL7 is analogous to Crr1 functioning in copper homeostasis in Chlamydomonas. SPL7 recognized and directly bound to a GTAC core motif, an essential element for copper response in Chlamydomonas, in the promoter region of *miR398* and activated the transcription of *miR398* in copper deficient conditions. In addition, SPL7 up-regulated multiple microRNAs involved in the degradation of copper proteins, and also FSD, some copper transporters and copper chaperone in low copper conditions. Taken together, we propose that SPL7 is a master regulatory factor involved in copper homeostasis in *Arabidopsis*. "
(a) Graduate School of Science, Kyoto University

P32008 Investigating the molecular basis for metal transporter gene hyperexpression that is the hallmark of metal hyperaccumulating plant species
Milner, Matthew-presenter mjm269@cornell.edu(b,a) Kochian, Leon (b)
"Metal hyperaccumulating plant species are plants that are endemic to metalliferous soils and are able to tolerate and accumulate metals in their above ground tissues to very high concentrations. One such hyperaccumulator, Thlaspi caerulescens, has been widely studied for its remarkable properties to tolerate toxic levels of zinc (Zn) and cadmium (Cd) in the soil, and accumulate these metals to very high levels in the shoot. Thlaspi caerulescens has the ability to accumulate as much as 3% Zn and 1% Cd in the shoots of the plant with no signs of toxicity. Early molecular investigations in our lab into how this plant functions as a metal hyperaccumulator led to the identification of TcZNT1 as a gene encoding a high affinity Zn transporter and low affinity Cd transporter which is expressed at very high levels in the roots of T. caerulescens. Our lab and other labs have subsequently found that high expression of TcZNT1 and other metal transporters is a hallmark of metal hyperaccumulators. To begin to understand the molecular basis of this hyperexpression, we have identified two different members of the E2F family of transcription factors that complement a yeast mutant defective in Zn uptake through disruption of the yeast transcription factor that regulates expression of the yeast high affinity Zn transporter. We have also found that only one of these E2Fs is able to bind with high affinity directly to a putative E2F-binding motif in the promoter of TcZNT1 in T. caerulescens, possibly regulating its expression. Further characterization of the interactions between ZNT1 and the E2F family members is being conducted to more fully elucidate the role these transcription factors may play both in plant Zn homeostasis, and the hyperaccumulation of heavy metals."
(a) Cornell University (b) Robert W. Holley Center for Agriculture and Health

P32009 Improvement of plant abilities to degrade aromatic xenobiotics by introduction of bacterial genes for dioxygenases.
Demnerova, Katerina-presenter demnerok@vscht.cz(a) Novakova, Martina (a) Mackova, Martina (a) Macek, Tomas (b) Sylvestre, Michel (c)
"The aim of this work was to construct genetically modified plants with increased capabilities to degrade organic pollutants such as polychlorinated biphenyls, toluene, TCE and other. Phytoremediation using transgenic plants can thus provide a useful, cheap and effective method for decontamination of the environment. For this purposes several bacterial genes were chosen to clone into plants of *Nicotiana tab*acum and *Linum ussitatissimum*. Genes of bacterial dioxygenases *bphC* gene and *todC1C2* genes were chosen for plant transformation *BphC* gene encodes 2,3-dihydroxybiphenyl-1,2-dioxygenase which is able to open the aromatic ring of dihydroxybiphenyl. This gene was cloned in fusion with gene for beta-glucuronidase (GUS), luciferase (LUC) and with histidine tail. The *todC1C2* genes were chosen to clone into plants to produce oxygenase ISP_{TOL} (with histidine tail), a component of bacterial toluene dioxygenase that can oxidize toluene and other organic pollutants.Several genetic constructs were designed and prepared and the possible expression of desired proteins in tobacco plants was studied by transient expression via agrobacterial infiltration. Expressed oxygenases His/BphC, BphC/GUS, BphC/LUC and His/ISP_{TOL} were then detected by Western blot or histochemically. The next step involved preparation of transgenic plants. *BphC* gene was transferred into plant genome of *Nicotiana tabacum* by agrobacterial infection. The presence of transgenic DNA and expressed proteins was studied using several techniques. *TodC1C2* genes were transferred into plant genome of *Nicotiana tabacum* and *Linum ussitatissimum*. Acknowledgement: This work was supported by grants MSMT 1M06030 and MSM 6046137305 "
(a) Institute of Chemical Technology Prague, Czech Republic (b) Institute of Organic Chemistry and Biochemistry, CAS, Czech Republic (c) INRS-Quebec, Pointe-Claire, H9R 1G6, Quebec, Canada

P32010 "Utility of hybrid poplars and willows in the phytoremediation of a contaminated groundwater plume containing 1,4-dioxane."
Lefebvre, Daniel-presenter lefebvre@queensu.ca(a) Silva, Anthony (a) Columbus, Melanie (a)
"The employment of poplar and willow trees for the phytoremediation of an old industrial dump site is an environmentally friendly process that is relatively inexpensive to implement by comparison to conventional remediation practices. However, the efficacy of phytovolatilization can be difficult to assess in the field. In this study the ability of various lines of native and hybrid poplars and willows to phytovolatilize the organic pollutant 1,4-dioxane at concentrations of up to 5 mg/L in a groundwater plume was assessed. To complicate matters, the chemical plume also contained high concentrations of ethylene glycol. By characterizing the transpiration rates over the course of a growing season and using a novel trapping technique the rate of contaminant phytovolatilization was quantified for each of the tree lines present. Comparisons between the tree lines effectiveness at removal of the contaminant are made. These results will allow for more effective future planning of phytoremediation sites containing 1,4-dioxane and similar contaminants as well as help in developing processes to monitor release of contaminants into the atmosphere. "
(a) Department of Biology, Queen's University

P32012 Agrobacterium-mediated transformation of flax (Linum usitatissimum L.) with heavy metal binding proteins genes
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http://www.agritec.cz
"Flax (Linum usitatissimum L.) as an industrial crop utilized mainly for technical purposes is a good candidate for heavy metal phytoextraction from polluted soils. Despite of the existing variation within commercial flax varieties and germplasm resources in the uptake of Cd, Pb, and Zn and their transport to the above-ground plant parts, the accumulation is not high enough to meet requirements of large-scale phytoextraction technology. The alternative approach to laborious extensive screening of germplasm resources for improving heavy metal tolerance/accumulation is the introduction of foreign genes responsible for heavy metal binding and detoxication. The hypocotyl segments of germinated seedlings of flax cv. Jitka and linseed breeding line AGT-0917 were cocultivated with Agrobacterium tumefaciens strain EHA105 containing binary vectors with two genes of interest (GOI), respectively: (1) pBI-alpha-MT with alpha-domain of metal-binding mammalian metallothionein alpha-mt and (2) pBI-CP with short synthetic metal-binding peptide cp. Both GOI were translationally fused to uidA reporter gene and both vectors contained nptII selectable marker gene under the control of 35S CaMV promoter. The expression of beta-glucuronidase (uidA/gus) gene served for early determination of transformed shoots regenerated directly from epidermal/subepidermal cells of cultured hypocotyls. Putative, kanamycin selection surviving T0 transformants were proved by PCR for GOI insertion. Segregating T1 progeny of several tenths of T0 transformants covering various transformation events is recently analysed for gene copy number, gene expression and phenotypic behaviour under Cd treatment. "
(a) AGRITEC Plant Research Ltd.

P32013 Studies on Fe and Zn cross-homeostasis system in response to Zn excess in *Arabidopsis halleri* and *Arabidopsis thaliana*
shanmugam, varanavasiappan-presenter varanavasiappan@rediffmail.com(a,b) Lo, Jing-Chi (a,c) Liang, Hong-ming (a) Yeh, Kuo-Chen (a,b)
"In plants, heavy metals such as Cu, Zn, Mn, Fe, Co and Ni function as micronutrients at minimum quantity for the growth and development. However, they are consider toxic, when acquired in excess quantities. Plants have developed different tolerance strategies to grow on soils rich in heavy metals. *Arabidopsis halleri* is well known for its metal tolerance and hyper-accumulation for Zn. We aimed to investigate a cross-homeostasis system of Fe and Zn in dealing with Zn excess in *A. halleri* and Zn non-hyperaccumulator *Arabidopsis thaliana*. *A. halleri* shows low expression of the Fe acquisition and deficiency response-related genes *IRT1* and *IRT2* as compared to *A. thaliana*. In *A. thaliana*, lowering the expression of *IRT1* and *IRT2* through the addition of excess Fe to the medium increases Zn tolerance. Excess Zn induces significant Fe deficiency in *A. thaliana* and reduces Fe accumulation in shoots, which triggers the expression of *IRT1* and *IRT2* in roots. By contrast, the accumulation of Fe in both shoots and roots of *A. halleri* was stable under various Zn treatments. We also observed the IRT1 protein accumulation and root surface ferric chelate reductase activity are low in *A. halleri* as compared to *A. thaliana* both under Fe deficiency and Zn excess conditions. We conclude that a fine-tuned Fe homeostasis mechanism in *A. halleri* maintains efficient Fe usage, with Fe concentration unaffected by excess Zn in shoots."
(a) Agricultural Biotechnology Research Center, Academia Sinica (b) Molecular and Biological Agricultural Sciences, Taiwan International Graduate Program, Academia Sinica and National Chung Hsing University (c) Institute of Plant Biology, National Taiwan University

P32014 Two novel cadmium-resistance associated proteins DvCRP1 and DvCRP2 from *Dunaliella viridis* dramatically increase cadmium tolerance and accumulation in yeast and plant
Meng , Xiangzong-presenter xzmeng@sibs.ac.cn(b) Xu , Zhengkai (a,b) Song, Rentao (a)
"*Dunaliella* is a genus of wall-less unicellular eukaryotic green alga with prominent resistance to salt and heavy metal stresses. It is valuable to exploit the stress-tolerant genes of *Dunaliella* for improving the stress-resistant ability of plants.Yeast has been used as model system to isolate and analyze plant genes by functional complementation studies. In this study, we constructed a yeast expression library of *D. viridis*. By functional complementation of the yap1-deficient *Saccharomyces cerevisiae* strain with a cadmium (Cd^{2+})-sensitive phenotype, we isolated two *D. viridis* cDNA (designated *DvCRP1* and *DvCRP2*) encoding two completely novel proteins with no homology to present public protein databases. Both *DvCRP1* and *DvCRP2* can dramatically increase the tolerance and accumulation of cadmium and copper in yeast. Sequence analysis revealed the existence of putative cysteine-rich heavy metal-binding motifs in DvCRP1 and DvCRP2. The functional domain and motifs of DvCRP1 and DvCRP2 were defined by deletion and site-directed mutation analysis in yeast. GFP fusion localization showed that both DvCRP1 and DvCRP2 were localized in secret vesicules of yeast. To utilize *DvCRP1* and *DvCRP2* for phytoremediation of cadmium and copper contamination, two genes were overexpressed in *Chlamydomonas* and tobacco. Transgenic *Chlamydomonas* and tobacco expressing *DvCRP1* or *DvCRP2* showed enhanced cadmium tolerance. The cloning of these two novel cadmium resistance genes from *Dunaliella viridis* provides new opportunity for genetic engineering of heavy metal high tolerance and accumulation plants for phytoremediation."
(a) School of Life Sciences, Shanghai University (b) Institute of Plant Physiology & Ecology, Chinese Academy of Sciences

P32015 Phytochelatin synthase is regulated by Cd-dependent protein phosphorylation at a threonine residue near its catalytic site
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"Phytochelatin synthase (PCS) uses glutathione as its substrate to catalyze the synthesis of heavy metal-binding peptides, known as phytochelatins. PCS has been described as a constitutive enzyme that may be controlled by post-translational modifications. In vitro experiments demonstrate that PCS activity is increased following phosphorylation by casein kinase 2 (CK2), and it decreases following treatment with alkaline phosphatase. Site-directed mutagenesis experiments indicate that Thr 49 is the site for phosphorylation. The mutant AtPCS1(T49A) cannot be phosphorylated, and its activity is significantly lower than that of the wild-type enzyme. In the modeled three-dimensional structure of AtPCS1, Arg 183 is close to Thr 49. The mutant AtPCS1(R183A) can be phosphorylated, but it shows much lower catalytic activity than the wild-type protein. We propose that Arg 183 interacts with the phosphorylated Thr 49 to give the active site of PCS a distinctive shape. Furthermore, the phosphorylation of AtPCS1 by CK2 is dependent on Cd and is inhibited by GSH. The N-terminal catalytic domain of AtPCS1 was expressed (AtPCS1-N), and its catalytic activity was even more sensitive to Cd or phosphorylation status than to that of AtPCS1. However, AtPCS1-N phosphorylation is not controlled by Cd or GSH, indicating a role for the C-domain in Cd-binding and regulation of phosphorylation. In addition, kinetic studies showed that AtPCS1 activity has a sigmoidal dependence on GSH, and it is estimated to be a dimer, whereas AtPCS1-N was present only in monomeric form. The C-terminal domain may thus participate in the formation of the quaternary structure of AtPCS1, and it may play a regulatory role in protein phosphorylation, forming a competent active site that can accommodate its substrates."
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P32016 Endophytic phytoremediation- solution for a cleaner environment
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"Cottonwood (*Populus* sp.) and willow (*Salix* sp.) are important colonizing trees with rapid growth, extensive root systems, high water uptake, and the ability to grow in nutrient-poor environments. Through our previous research, we identified a variety of endophytes (microbes living inside the

plant) within poplar and willow trees that increase plant growth by producing hormones and providing fixed nitrogen. Endophytes have been shown in other systems to increase the success of phytoremediation projects. In this work, we hypothesize that remediation of trichloroethylene (TCE), a common environmental contaminant and a suspected carcinogen, can be optimized using specific species of fast-growing trees such as poplar and willow if appropriate endophytes are present. Currently we are screening a variety of willow and poplar species to identify those with the highest level of TCE removal. Then TCE uptake in the presence and absence of endophytes will be compared to identify those capable of TCE degradation. With necessary genetic manipulations to increase the degradation of the pollutant, the data generated will be used to test the best plant/endophyte partnerships for enhanced TCE removal from TCE-contaminated sites and develop a green technology for the removal of hazardous pollutants for a cleaner environment. "

(a) College of Forest Resources, University Of Washington

P32017 Sympastic Cd uptake in roots using stable isotopes ^{113}Cd and ^{114}Cd and characteristic of xylem loading process in eggplant and a related plant with different Cd accumulation in the shoots

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"Approximately 7 % of 381 samples of eggplant were found to contain cadmium (Cd) concentrations above Codex limit (0.05 mg Cd kg fw^{-1}) in Japan. Therefore, new technologies for reducing the Cd levels in eggplant are urgently required in Japan. Recently, novel finding was reported that Cd concentration in eggplant (*Solanum melongena*) fruits can be drastically reduced by grafting them with *Solanum torvum* rootstock. However, its mechanisms have not been elucidated so far. We thus characterized physiological mechanisms of different Cd accumulation in shoots of *S. melongena* and *S. torvum* by examining sympastic Cd uptake in roots using new method of stable isotopes Cd and translocation from roots to shoots via xylem loading process. In concentration dependent experiment in roots, sympastic ^{113}Cd uptake in roots increased with the increase of Cd concentration in the medium, but saturated at high ^{113}Cd concentration. Based on the saturated curve, Km value was almost same in both plants, but Vmax value was 1.5-fold higher in *S. melongena* than in *S. torvum*. On the other hand, concentration dependent experiment in xylem loading process also revealed saturated curve at high Cd concentration. Based on the saturated curve, Km value was approximately 7-fold higher in *S. torvum* than in *S. melongena*. Additionally, we tried to estimate a Km value for Cd concentration in roots. Even when evaluating the Km values for Cd concentration in roots, the Km value was approximately 4-fold higher in *S. torvum* than that in *S. melongena*. These results suggest that xylem loading process is a critical factor in determining Cd accumulation in shoots of both plants. Acknowledgment This work was supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN). "

(a) National Institute for Agro-Environmental Sciences

P32018 Enhancing the plant uptake of pyrene by DNA components

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"The ability of DNA component (DC) to bind with various types of PAHs has been well known. In our previous paper, such property has been exploited to employ aqueous DNA as a washing agent for PAHs contaminated soil. In this paper we will discuss the effect of DC on phytoremediation of PAH-contaminated soil. Seeds of zucchini were germinated and grown for 14 days in a moist perlite bed. For the hydroponic system, the seedlings were transferred to flasks containing hydroponic solutions of water, 1000 mg L-1 DC. For soil system, they were transferred to plastic pots filled with 300 grams of the prepared PAH-spiked soil. The plants were grown in a chamber equipped with an incandescent light source with intensity above 10,000 lux. A sequence of 16/8 hour light/darkness was simulated using an automated circuit controller. After the specified cultivation period, the plant roots and shoots were separately harvested, weighed, and then thoroughly washed with tap water. They were cut into small pieces and then stored in glass bottles in a freezer until analysis. The pyrene contents of the roots and shoots were analyzed following. In hydroponic cultivation of Zucchini in DC solutions, the yield for the DC was almost comparable with the water control. A significantly higher uptake of pyrene in the DC system was also apparent. Specifically, the shoot concentration was two-folds higher with DC. In the contaminated soil cultivation, plant growth was not inhibited by DC amendment. Shoot concentration of pyrene was close to three-times higher with DC compare with no DC cultivation. These preliminary results thus show the potential of DC for improving PAHs release and plant uptake from soil."

(a) National Institute of Advanced Industrial Science and Technology

P32019 Nickel modulates function of mitochondria in the hyperaccumulator *Alyssum murale* roots.

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" Plant metal hyperaccumulator species are widely used as models to unravel the heavy metal tolerance and hyperaccumulation mechanisms. *Alyssum murale* is capable of tolerating and hyperaccumulating Ni for up to 3% of its total aerial biomass. The ability of hyperaccumulator plants to withstand and accumulate high concentrations of Ni is attributed to extraordinary process utilized by plants for tolerance and detoxification. We do not yet understand how Ni hyperaccumulation is achieved in plants or how hypaerccumulators tolerate such high levels of Ni in their tissues. Several studies have indicated *in planta* complexation of Ni with amino acids and their vacuolar/trichome localization. These studies still do not give a lead in terms of how Ni is moved to aerial plant parts or how non-toxic form(s) of Ni are translocated in planta. In our study, detectable levels of Ni were found in the mitochondria. In addition, differential organic acid root-secretion patterns in response to Ni were found in hyperaccumulators and non-accumulators *Alyssum* plants. We investigated the effect of Ni on the function of tricarboxylic acid (TCA) cycle in mitochondria of hyperaccumulators and nonaccumulator *Alyssum* plants. Knowledge of the effects of Ni on the mitochondrial biochemical functions should aid in the understanding of the mechanisms by which organic ligands may play a role in the hyperaccumulation and possible translocation of Ni within plants. "

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P32020 Expression of an Arabidopsis CAX1 mutant showing enhanced metal tolerance in tobacco

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"Heavy metal contamination represents a serious hazard to our health through direct contact or by its entry into the food chain. Phytoremediation is the use of plants to remove heavy metals from the soil; it is non-aggressive and economically viable with the added advantage of being environmentally compatible. An ideal plant to utilize as a heavy metal bioremediator would have to grow fast, have an extensive root system, a large above ground biomass for metal accumulation, be easy to harvest, and metal tolerant (Baker 2001). While these traits are not found in a single plant it may be possible to introduce genes into non-metal tolerant plants to meet most of the above mentioned criteria. In plants, yeast, and bacteria, cation/proton exchangers (CAXs) have been shown to translocate calcium and other metal ions utilizing the proton gradient. Recently, a single mutation in a conserved histidine (H338N), in the C2 sensor domain of the tonoplast located CAX1, was demonstrated in yeast to confer both improved metal tolerance and increased transport of both cadmium and zinc, with a reduction in the transport of calcium (Shigaki et al., 2005). Introduction of this mutant gene CAX1cd into tobacco was carried out to determine if the presence of the altered gene could induce greater metal tolerance and tonoplast metal transport in the plant. Following exposure of plants to zinc for 3 weeks, we measured phenotypic changes, zinc

accumulation and vacuolar transport of zinc in transformed plants expressing the WT form of CAX1, as well as the CAX1cd mutant and the empty vector. These results will be presented and discussed. This work was supported by a CONACyT Grant, 49735, to BJB and a National Science Foundation grant NSF 0344350 to KDH. "
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P32021 Molecular analysis of genes related to arsenic uptake and detoxification in sensitive and tolerant varieties of shrub willow (*Salix* spp.)
DeLeon, Alyssa M.-presenter amdeleon@syr.edu(a) Pulley, Emily E. (a) Smart, Lawrence B. (a)
"As a fast-growing perennial, shrub willow (*Salix* spp.) is a strong candidate for the remediation of arsenic-contaminated soils in temperate climates. Arsenate (AsO_4^{3-}) is physiologically similar to phosphate (PO_4^{3-}) and is thought to enter cells via high-affinity phosphate transporters (encoded by *PHT1* genes). Arsenic detoxification may be mediated by phytochelatins, whose glutathione precursors are synthesized by γ-glutamylcysteine synthetase (encoded by *γ-ECS*). Upon exposure to 250 µ;M AsO_4^{3-}, an As-sensitive clone (*Salix eriocephala*; clone ID 00X-026-082) and an As-tolerant clone (*S. viminalis x S. miyabeana*; clone ID 99202-011) wilt within 24 h and 6 d, respectively. The goal of this study was to determine whether As tolerance is related to the expression of *PHT1;4/7* and/or *γ-ECS*. Both clones were treated with +P/-As, -P/-As, +P/+As, and -P/+As solutions for 48 h (following 3 weeks of growth from cuttings in either +P or -P hydroponic culture). Mean transpiration was highest for the +P/-As plants, followed by the +P/+As, -P/-As and -P/+As plants, respectively. This trend suggests that the effects of AsO_4^{3-} are more dramatic in the absence of PO_4^{3-}. Root samples were harvested at 0, 4, 12, and 48 h, and RNA was isolated via a hot borate method. Semi-quantitative RT-PCR indicated that 99202-011 *PHT1;4/7* expression did not vary by treatment. *γ-ECS* expression appeared to be upregulated in the 4 h and 12 h -P/+As treatments, suggesting that this gene may play a role in 99202-011 As tolerance."
(a) Department of Environmental and Forest Biology, State University of New York, College of Environmental Science and Forestry

P32022 Uptake of silver by plants and plant cells
Yang, Ray Y. K.-presenter ryang@mail.wvu.edu(a) Fridley, Brooke A. (a) Cho, Eung H. (a)
"Over the years we have studied the uptake of silver ion by plant seedlings and plant cells. This involves the uptakes of the ion by the seedlings of alfalfa (Medicago sativa) and mung bean (Vigna radiate), as well as by the suspended cells of Stevia (S. rebaudiana). In this paper, the data collected, and the methods used to obtain them, will be presented, and their implications in phytomining and nanotechnology discussed. "
(a) Chemical Engineering Department, West Virginia University

SESSION P33 – HERBICIDE PHYSIOLOGY

P33001 Over-production of EPSPS in Palmer amaranth (Amaranthus palmeri) is responsible for the glyphosate resistance
Wang, Dafu-presenter dafu.wang@monsanto.com(a) Gaines, Todd (b) Sammons, Douglas (a) Westra, Phil (b) Kretzmer, Keith (a) Norris, Ashleigh (a) Ostrander, Elizabeth (a) Duncan, Bill (a)
"Glyphosate is a major broad spectrum herbicide used globally in crop production. Glyphosate resistant crops (soybean, cotton, corn etc) have been commercialized since 1995. Today these crops are planted in excess of 100 M acres in USA. The first glyphosate-resistant weeds were discovered in row crop production in USA in 2000. Glyphosate-resistant weeds challenge the weed management for these crops and in particular the production of cotton in southern Georgia with the discovery of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) in 2005. The mechanism of glyphosate resistance in Palmer amaranth was examined in three categories: target site, exclusion and metabolism in order to identify the major contributor. Our results exclude differences in whole plant uptake, translocation and metabolism of glyphosate but we have found major differences in the target enzyme EPSPS. Crude extracts of EPSPS for R and S plants were equally sensitive to glyphosate indicating the absence of potential target site resistance due to active site amino acid substitutions. However, a dramatic increase (ΔCt of 7-9) in expression of EPSPS cDNA by Real-Time PCR and an increase (up to 17-fold) in EPSPS protein by Western blot assay were shown in the glyphosate-resistant Palmer relative to susceptible plants. The specific activity of EPSPS in leaf crude extracts of glyphosate-resistant Palmer is about 24-fold of that in susceptible Palmer in the absence of glyphosate. The glyphosate-resistant Palmer at 2.5 mM glyphosate still retains 4-fold more EPSPS activity than sensitive plants without glyphosate. This is the first evidences showing that the mechanism of resistance correlates to the excessive level of EPSPS expression resulting from the EPSPS gene amplification in Palmer amaranth."
(a) Monsanto (b) Colorado State University

P33002 "No evidence of inhibition of germination or radicle elongation in *Amaranthus hypochondriacus* by Herbarumin I, a reported phytotoxin from plant pathogen *Phoma herbarum* "
Blauth, Susan L-presenter susan_blauth@redlands.edu(a) Soulsby, David P (a) Kard, Megan (a) Baron, Lynn (a) Kennedy, Tyler (a)
"The phytotoxicity of fungus *Phoma herbarum* Westend has been attributed to herbarumin I, II and III. The most toxic of these three phytotoxins has been found to be herbarumin I, with an IC50 of 5.43 x 10-5M as determined by measuring inhibition of radicle elongation in *Amaranthus hypochondriacus*. In order to determine the structure-activity relationships of herbarumin I toxicity, we relied upon a previously reported synthesis that provided versatility for the construction of herbarumin I and analogs. In addition, we explored the use of *Arabidopsis thaliana* as a model to test phytotoxicity in order to utilize available mutant seed stocks and genome sequence information for future studies. Herbarumin I was successfully synthesized and fully charcterized by GC-MS, NMR and optical rotation. We were also able to consistently measure IC50 of radicle elongation in both *A. thaliana* and *A. hypochondriacus* using other phytotoxins such as 2,6-dichlorobenzonitirile. However, we were unable to observe a significant effect on germination or radicle elongation in either *A. thaliana* or *A. hypochondriacus*. Further tests will be conducted before concluding previously observed phytotoxic effects of herbarumin I may be due to other compounds that co-isolated during their extraction."
(a) University of Redlands

SESSION P34 – HORMONE BIOLOGY

P34001 Isoprenylcysteine methylation and demethylation regulate abscisic acid signaling in *Arabidopsis*
Huizinga, David H-presenter dhuizing@iupui.edu(b) Omosegbon, Olutope (b) Omery, Bilal (b) Crowell, Dring N (a)
"Isoprenylated proteins bear an isoprenylcysteine methyl ester at the carboxyl terminus. Although isoprenylated proteins have been implicated in meristem development and negative regulation of abscisic acid (ABA) signaling, the functional role of the terminal methyl group has not been described. Here, we show that transgenic *Arabidopsis thaliana* plants overproducing isoprenylcysteine methyltransferase (ICMT) exhibit ABA

insensitivity in stomatal closure and seed germination assays, establishing ICMT as a negative regulator of ABA signaling. In contrast, transgenic plants overproducing isoprenylcysteine methylesterase (ICME) exhibit ABA hypersensitivity in stomatal closure and seed germination assays. Thus, ICME is a positive regulator of ABA signaling. To test the hypothesis that ABA signaling is under feedback control at the level of isoprenylcysteine methylation, we examined the effect of ABA on *ICMT* and *ICME* gene expression. Interestingly, ABA induced *ICME* gene expression, establishing a positive feedback loop whereby ABA promotes ABA responsiveness of plant cells via induction of *ICME* expression, which presumably results in the demethylation and inactivation of isoprenylated negative regulators of ABA signaling. These results suggest strategies for metabolic engineering of crop species for drought tolerance by targeted alterations in isoprenylcysteine methylation."
(a) Idaho State University (b) Indiana University-Purdue University Indianapolis

P34002 Auxin response under cold stress: underlying molecular mechanism
Shibasaki, Kyohei-presenter u0407003@iwate-u.ac.jp(a) Uemura, Matsuo (a) Tsurumi, Seiji (b) Rahman, Abidur (a)
"Plants respond to environmental stresses in multiple ways including changing the hormonal responses. The plant hormone auxin controls every aspect of growth and development. However, little is known about the effect of temperature stress on auxin response. To understand the mechanistic basis of cold temperature stress and auxin response, we characterized the root growth of *Arabidopsis thaliana* at 23 degC after pre-incubating the seedlings at 4 degC. The time course assay revealed that 8-12hr pre-incubation at 4 degC inhibited the root growth and reduced the gravity response approximately fifty percent compared to that of untreated controls. The auxin-signaling mutant *axr1* and *tir1*, which show a reduced gravity response, responded to cold treatment like wild-type, indicating that auxin transport rather than auxin signaling mechanism is affected by cold stress. Consistently, the expression analyses of auxin responsive marker *IAA2-GUS* and direct transport assay further confirmed that cold treatment inhibits root basipetal auxin transport. Additionally, trafficking of auxin efflux carrier PIN2, which plays an important role in basipetal auxin transport, was dramatically reduced in cold stressed seedlings. Likewise, the endosomal movement and golgi trafficking were also affected by cold treatment as visualized by live imaging of an endosomal marker ARA7, and the golgi trafficking marker NAG. Surprisingly, cold induced inhibition of protein trafficking was independent of change in cellular actin organization. Taken together these results, we suggest that the inhibition of auxin response by cold stress is a part of a global effect of cold stress on cellular protein trafficking machineries"
(a) Iwate University (b) Kobe University

P34003 Auxin characterization in loss-of-function mutants of the CHS interacting proteins Nap1 and Lap1
Watkinson, Jonathan I.-presenter jowatki2@vt.edu(a) Thomas, Lauren M. (a) Griffiths, Sophia G. (a)
"The plant hormone, auxin, regulates various aspects of plant growth and development. Its actions are controlled, in part, by directed polar transport from sites of synthesis to sites of action. Previous research has provided evidence that this transport can be modulated by flavonoids, specialized metabolites with functions in other aspects of plant growth, reproduction, and defense. We have identified two proteins that may contribute to the regulation of auxin flux through physical interaction with chalcone synthase (CHS), the first committed enzyme in the flavonoid biosynthetic pathway: LAP1 a soluble, leucine aminopeptidase, and NAP1, a nuclear-localized, alpha-helical protein. Preliminary data indicate that roots of *nap1* and *lap1* loss-of-function mutants show different responses to gravity. *nap1* and *lap1* mutants were crossed with plants carrying the GH3 auxin-responsive promoter fused to the GUS reporter. GUS expression is lower in root tips of both mutant backgrounds, indicating that there is reduced auxin in the root tip, indicative of reduced auxin transport. We are testing the hypothesis that LAP1 has a role in controlling the levels of auxin conjugates in plants by screening *lap1* seedlings for changes in free and conjugated auxin levels using HPLC-MS. Plants overexpressing Nap1 show a loss of negative gravitropism in inflorescence stems. This could be due to either reduced auxin flux or reduced lignin content, therefore we are analyzing lignin content in cross sections of inflorescences from these lines. Together, these experiments should provide new insights into the connection between auxin and flavonoids in the regulation of plant growth and development."
(a) Roanoke College

P34004 The Arabidopsis *1-Aminocyclopropane-1-Carboxylic Acid Synthase* (*ACS*) Gene Family; Functional Genomic Analysis
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"1-AMINOCYCLOPROPANE-1-CARBOXYLATE SYNTHASE (ACS) catalyzes the rate-limiting step in the ethylene biosynthetic pathway in plants. The Arabidopsis genome encodes 9 ACS polypeptides. We hypothesize that each member of the *ACS* gene family may have a distinct biological function. Here, we report various approaches for elucidating the role of each isozyme. Firstly, biochemical characterization of the various ACS polypeptides revealed that they are biochemically diverse. We think that the biochemical diversity defines a distinct biological function of each isozyme, which in turn defines its tissue specific expression. Second, functional intermolecular complementation experiments in *E.coli* show that all isozymes can form heterodimers. However, functional heterodimers are detected only among members that belong to the same phylogenetic branch. We propose that functional heterodimerization enhances the biochemical diversity of the *ACS* gene family; the non-functional heterodimers may have a regulatory role. Thirdly, analysis of promoter-*GUS* fusions reveals unique and overlapping expression patterns during plant development. This raises the prospect that functional ACS heterodimers may be formed in planta. Lastly, we have identified T-DNA insertion lines for 7 among the 9 *ACS* genes; high order mutations were constructed. The analysis reveals that ethylene is a repressor of flowering. The data also suggest that ethylene production is regulated by a combinatorial control mechanism among the various ACS isozymes during plant growth and development. These observations provide molecular insight into the unique and overlapping functions of the *ACS* gene family members in *Arabidopsis*."
(a) Plant Gene Expression Center (b) University of California at Riverside (c) North Carolina State University (d) Salk Institute for Biological Studies

P34005 Study on expression of AhNCED1 protein in peanut during development stages under water stress
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"We previously isolated and characterized a dehydration-inducible NCED gene, namely AhNCED1, from the dehydrated peanut (Arachis hygogaea L.) leaves. The expression of AhNCED1 gene exhibited typical and significant responses to water stress, NaCl stress and ABA application. Here, we study on expression of AhNCED1 protein in peanut during development stages under water stress. There were strongly expression in kernel and young leaf. The expression in roots at four-leaf stage was enhanced after 30% PEG for 1h, while the expression in stems and leaves were enhanced at 3h. In maturation stage, the expression in leaves was enhanced at 1h after water stress, while the expression in stems and roots were enhanced at 10h and 3h. There also were strong expression of AhNCED1 in pods at 24h and 48h. These results suggest that roots were the most responsive organ to water stress in four-leaf stage, and leaves had more sensitive to water stress than other organs. The endogenous ABA level in the roots in four-leaf stage and leaves maturation stage exposed to water stress increased 236.52% and 278.31%, respectively. We suggest that the site of stress perception and ABA biosynthesis in peanut during developmental stage was different. Leaf-sourced ABA in four-leaf stage may be one of the leaf-to-root signals in response to water-stress and Root-sourced ABA may be one of the root-to-leaf signals in response to water-stress in maturation stage. We aimed to reveal the relationships between the site of water-stress perception and ABA production in peanut during different developmental stage.

* This study were supported by the National Natural Science Fund (30771297), Natural Science Program of Guangdong Province (06025049) "
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P34006 The primary signaling outputs of brassinosteroids are regulated by abscisic acid signaling
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"Unlike animals, plants are sessile and need to constantly regulate their developmental and physiological processes to respond to various internal and external stimuli. Many phytohormones play essential roles in coordinately regulating these processes. Brassinosteroids (BRs) mainly play roles in promoting plant growth and development, while abscisic acid (ABA) is a hormone induced by many stress signals. Although it is known that BRs and ABA co-regulate hundreds of genes expression, whether their interaction is through modification or interaction of their primary signaling cascades or through independent signaling pathways remains a big mystery. In this study, we used biochemical and molecular markers of BR signaling and found that exogenous ABA rapidly inhibits BR signaling outputs as indicated by the phosphorylation status of BES1 and BR-responsive gene expression. Experiments using a bri1 null-allele, bri1-116, and analysis of subcellular localization of BKI1-YFP further reveal that the BR receptor complex is not required for this effect of ABA on BR signaling outputs. However, when the BR downstream component BIN2 is inhibited by LiCl, ABA fails to inhibit BR signaling outputs. Furthermore, using a set of ABA insensitive mutants, we found that the effect of ABA on the BR primary signaling pathway is dependent on the ABA early signaling components, ABI1 and ABI2. We propose that the signaling cascades of ABA and BRs primarily crosstalk after BR perception, but before the downstream transcriptional factor BES1, which also explains why a large proportion of BR-responsive genes are also regulated by ABA. Our studies provide significant insight into the molecular mechanisms by which BRs interact with ABA."
(a) State Key Laboratory of Genetic Engineering, and Institute of Plant Biology, School of Life Sciences, Fudan University

P34007 Physiological differences of three types of callus from seedling hypocotyls of *Eucalyptus urophylla*
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"Hypocotyls excised from 8 day-old seedlings were used as explants to establish a regeneration protocol for *E. urophylla*. SPCa medium was used as basic medium to eucalyptus regeneration by adding different combination of plant growth regulator. The explants could begin to dedifferentiate and form white callus at all medium tested during the first month. Another month later, these callus turned to 3 types of callus, brown, white and reseda. Half of callus cultivated on SPCa medium supplemented with 2,4-D and 6-BA turned to brown and the other kept white. The great mass of callus cultivated on SPCa medium supplemented with IAA and TDZ became brown. Half of callus cultivated on SPCa medium supplemented with N - phenyl-N-[6-(2-chlorobenzothiazol) yl] urea (PBU) and NAA became brown but the other showed reseda. Only the reseda callus could form adventitious buds when these callus incubated on SPCa medium supplemented with 6-BA and NAA. Several physiological indexes had been compared in order to seek the relationship between physiological indexes and morphogenesis. The POD activity of reseda callus was the highest. The PPO activity and the contents of H_2O_2 , MDA and soluble protein of brown callus were the highest. The activities of SOD and CAT did not showed obvious difference. The results indicated that PBU showed high efficiency on induction of adventitious buds of *E. urophylla*. And the POD activity could act as an indicator of morphogenesis associated the increase in the POD activity with the meristematic tissue formation. Key words: *Eucalyptus urophylla*; plant egeneration; Physiological Index; "
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P34008 The smoke clears: genetic and physiological characterization of karrikin responses in Arabidopsis thaliana reveals interplay with light signaling
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"Karrikins were recently identified in smoke as a novel family of germination stimulants which are active at low concentrations in a broad variety of plant species. Karrikins (KAR) can enhance germination of primary dormant (PD) *Arabidopsis thaliana* seed, but do not replace germination requirements for either light or gibberellic acid (GA) synthesis. To identify transcriptional markers of KAR_1 response that occur prior to germination, we performed microarray analysis of PD wildtype and GA-deficient *ga1* mutant seed at 24 h imbibition. Although remarkably few genes exhibited strong (> 2 fold) transcriptional changes for either genotype, we observed substantial overlap between the KAR_1-induced gene sets which was independent of GA synthesis or germination fates. qRT-PCR analysis of KAR_1-responsive genes during PD seed imbibition revealed four transcript pattern classes. Several transcripts were found to be upregulated by KAR_1 independently of light, and were specifically responsive to active karrikins versus other germination stimulants. KAR_1 also induces a cluster of genes involved in light signal transduction and flavonoid biosynthesis, suggesting an unanticipated role for KAR_1 in enhancing light-mediated processes. Consistent with this observation, KAR_1 promotes germination and inhibits hypocotyl elongation under low fluence light. PIL5 and SPT are bHLH transcription factors respectively implicated in light and cold regulation of seed germination. We demonstrate that *spt-1* germination and hypocotyl elongation is completely insensitive to KAR_1, while *pil5-1* retains KAR_1 response. A set of karrikin-insensitive mutants have now been identified through three forward genetic screens in Arabidopsis, providing the next steps toward dissection of the karrikin response pathway. "
(a) The University Of Western Australia (b) Kings Park Botanic Gardens

P34009 Crosstalk between auxin and ethylene signaling pathways in Arabidopsis
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"Auxin and ethylene regulate overlapping but distinct components of plant growth and development. For example, auxin affects gravitropism, phototropism, lateral root development, and vascularization, whereas ethylene affects plant senescence, growth, and stress responses. Mutations in auxin response genes result in resistance to auxin, and mutations in ethylene pathway genes result in resistance to ethylene. Additionally, however, many auxin-resistant mutants are also ethylene resistant, and many ethylene-resistant mutants are auxin resistant. The goal of this research is to elucidate the interactions between auxin and ethylene signaling pathways. We are characterizing auxin and ethylene mutant responses to various concentrations of the ethylene precursor ACC and indole-3-acetic acid (IAA) and examining double mutants between auxin- and ethylene-resistant mutants to determine if auxin and ethylene resistance is additive. We are using northern analyses of auxin and ethylene response transcripts in ethylene and auxin response mutants to uncover the molecular defects in these mutants. Further, we are examining GUS activity in ethylene and auxin mutants carrying *DR5:GUS* and *HS:AXR3NT-GUS* reporters to determine mutant effects on auxin-responsive transcription and Aux/IAA protein stability. Together, these analyses will illuminate the molecular underpinnings of the extensive crosstalk between auxin and ethylene signaling pathways. (This work was supported by the NSF and by an ASPB Summer Undergraduate Research Fellowship to ERB.) "
(a) Rice University

P34010 Auxin-induced *WUS* expression is essential for embryonic stem cell renewal during somatic embryogenesis in *Arabidopsis*
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"Somatic embryogenesis requires auxin and establishment of the shoot apical meristem (SAM). *WUSCHEL* (*WUS*) is critical for stem cell fate determination in the SAM of higher plants. However, regulation of *WUS* expression by auxin during somatic embryogenesis is poorly understood. Here, we show that expression of several regulatory genes important in zygotic embryogenesis were up-regulated during somatic embryogenesis of Arabidopsis. Interestingly, *WUS* expression was induced within the embryonic callus at the time when somatic embryos could not be identified morphologically nor molecularly. Proper *WUS* expression, regulated by a defined critical level of exogenous auxin, is essential for somatic embryo induction. Furthermore, it was found that auxin gradients were established in specific regions which could then give rise to somatic embryos. The establishment of auxin gradients was correlated with the induced *WUS* expression. Moreover, the auxin gradients seem to activate PIN-FORMED 1(PIN1) polar localization within the embryonic callus. Polarized PIN1 is likely responsible for the observed auxin polar transport and auxin accumulation in the SAM and somatic embryo. Suppression of *WUS* and *PIN1* indicated that both genes are necessary for embryo induction through their regulation of downstream gene expression. Our results reveal that establishment of auxin gradients and PIN1-mediated auxin polar transport are essential for *WUS* induction and somatic embryogenesis. This study sheds new light on how auxin regulates stem cell formation during somatic embryogenesis."
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P34011 Mechanism of gibberellin perception and DELLA protein recognition by the Arabidopsis gibberellin receptor
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"Gibberellin (GA) is one of crucial plant hormone regulating plant growth and development. GA response is negatively controlled by a GRAS family transcriptional regulator, DELLA protein. Once plant cells receive GA molecules, the DELLA proteins are rapidly degraded by ubiquitin-proteasome pathway. GA perception and following DELLA protein recognition by the GA receptor GID1 cause an enhancement of interaction between DELLA and SLY1 that is a component of SCF E3 ubiquitin ligase. Subsequent ubiquitination and destruction of the DELLA proteins trigger the transcriptional reprogramming of GA response genes. However, it is unknown how the GID1 only perceive active GA molecules and recognize the DELLA protein in GA dependent manner. Here, we determined crystal structures of the Arabidopsis GID1 in complex with GA and a signal receiver domain of DELLA protein at 1.8 angstrom resolution. GA molecule is binding with a pocket of GID1 core domain and the GA binding pocket is sealed by the GID1 N-terminal extension. Highly conserved DELLA, VHYNP, and LExLE motifs of the DELLA protein mainly interact with the GID1 N-terminal extension. These results suggest that GA molecule induces a formation of the DELLA protein binding surface in GID1 via conformational changes of the GID1 N-terminal extension."
(a) Nara Institute of Science and Technology (b) Duke University

P34012 Brassinosteroid signal transduction from cell surface receptor kinases to nuclear transcription factors
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"Brassinosteroid (BR) regulates gene expression and development through a receptor kinase-mediated signaling pathway in plants. Although many components of BR signaling pathway have been identified and studied, a major gap still exists in the BR signaling pathway. In particular, how upstream signaling regulates the GSK3-like kinase (BIN2) remains unclear. Here we close the last gap of the BR signaling pathway by demonstrating the molecular function of BSU1 upstream of BIN2 and downstream of BSK1. We show that BSU1 inactivates BIN2 by dephosphorylating a phospho-tyrosine residue that is required for its kinase activity. Both in vitro BSU1 treatment and in vivo BR treatment cause tyrosine dephosphorylation of wild type BIN2 but not of mutant bin2-1, which causes BR-insensitive phenotypes. Quadruple loss-of-function mutant of BSU1 family displays an extreme dwarf phenotype similar to BR-deficient mutants, indicating that BSU1-mediated BIN2 dephosphorylation is essential for BR-dependent plant growth. In addition, we show that BSK1 directly interacts with BSU1 in a BRI1-phosphorylation-dependent manner. This study reveals a mechanism of GSK3 regulation in plants and demonstrates a fully connected BR signaling pathway from the BRI1 receptor kinase to BIN2 and its substrates BZR transcription factors."
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P34013 *Arabidopsis* ATP-binding cassette transporters promote efflux of the auxin precursor indole-3-butyric acid
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"Plants have developed numerous mechanisms to store hormones in inactive but readily available states, enabling rapid responses to environmental changes. The phytohormone auxin has a number of storage precursors, including indole-3-butyric acid (IBA), which is apparently shortened to active indole-3-acetic acid (IAA) in peroxisomes by a process similar to fatty acid β oxidation. Whereas metabolism of auxin precursors is beginning to be understood, the biological significance of the various precursors is virtually unknown. We found that mutation of *PDR8/PEN3/ABCG36*, encoding a plasma membrane localized ATP-binding cassette transporter, specifically restores IBA, but not IAA, responsiveness to auxin signaling mutants. Moreover, both *pdr8* and *pdr9/abcg37* mutants confer hypersensitivity to a subset of auxins. Further, we found that *pdr8* and *pdr9* mutants display defects in efflux of the auxin precursor IBA. *pdr8* also displays developmental defects in root hair and cotyledon expansion that reveal previously unknown roles for IBA-derived IAA in plant growth and development. Our results imply that limiting IBA accumulation via PDR8- or PDR9-promoted efflux contributes to auxin homeostasis."
(a) Rice University

P34014 Receptor kinases involved in brassinosteroid signal transduction phosphorylate protein translation initiation factors
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"Brassinosteroids (BRs) are essential plant hormones that regulate multiple aspects of plant growth and development and require two receptor kinases, BRASSINOSTEROID INSENSITIVE 1 (BRI1) and BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1), for hormone perception and signal transduction. We previously isolated a putative cytoplasmic substrate of BRI1 with homology to the mammalian TGF-beta receptor interacting protein (TRIP-1). TRIP-1 (also known as eIF3i) is a dual function protein that regulates TGF-beta signaling in mammals and also plays a critical role in the eIF3 protein translation initiation complex in animals, yeast and plants. Arabidopsis BRI1 interacts with TRIP-1 *in planta* and phosphorylates TRIP-1 on multiple Ser/Thr residues *in vitro*. Initiation is the rate-limiting step in eukaryotic protein translation and is often regulated by phosphorylation of specific initiation factor subunits in response to various signals. A proteomic screen for novel BRI1 and BAK1 interactors identified four additional eIF subunits as putative kinase domain substrates for BRI1 and/or BAK1. Moreover, *in vitro* kinase assays confirmed that these eIF subunits were phosphorylated by both BRI1 and BAK1. We postulate that BR-dependent phosphorylation of TRIP-1 and other eIF subunits by BRI1 and/or BAK1

may affect initiation factor activity and thus impact protein translation, providing a novel mechanism for BR regulation of plant growth. We are studying the intersection of BR signal transduction and protein translation initiation by using a variety of mass spectrometry approaches to identify *in vivo* phosphorylation sites of eIF subunits, followed by analysis of their functional significance with respect to BR signaling and the assembly and activity of the translation initiation complex."
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P34015 Transcriptional network and mechanism for Brassinosteroid regulated Gene Expression and responses in Arabidopsis
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"Plant steroid hormone brassinosteroids (BRs) play important roles throughout growth and development. BRs signal through membrane receptor BRI1 to regulate the activities of BES1/BZR1 family transcription factors. To understand how BES1 regulates gene expression and BR responses, we have used Chromatin Immunoprecipitation and genomic tilling arrays (ChIP-on-chip) and identified about 1600 BES1 direct target genes. At the chromatin level, BES1 binding sites were largely free of the trimethyl histone 3 lysine 27 (H3K27me3), but partially overlap with H3K9me3. The result suggests involvement of histone modifications and chromatin structure in BES1-regulated transcription, which is consistent with our recent finding that BES1 recruits two histone demethylases to activate gene expression (PNAS 2008 105:7618). BES1 target genes are involved in multiple aspects of BR responses, including cell elongation, self-regulation of BR signaling and crosstalk with other signaling pathways. Transcription factors were highly enriched in BES1 target genes and the expression of which was strongly correlated with each other as shown in a gene regulatory network (GRN), revealed by ARACNe (Algorithm for the Reconstruction of Accurate Cellular Networks). The result demonstrates that BES1 functions through a transcriptional network to mediate BR responses. Finally, we found that BES1 activates the expression of MYB30 transcription factor gene and subsequently cooperates with MYB30 protein to further activate downstream targets, thereby providing a novel mechanism to amplify BR signal (Li et al. Plant J, in press). Our results, therefore, provide significant insights into the network and mechanism of BR-regulated gene expression and control of plant growth. Supported by grants from NSF and USDA."
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P34016 Indole-3-acetaldoxime dependent auxin biosynthesis in *Arabidopsis*
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"Auxins are hormones that regulate many aspects of plant growth and development. Indole-3-acetaldoxime (IAOx) has been proposed to be a key intermediate in the synthesis of indole acetic acid (IAA) and several other indolic compounds. Genetic studies of IAA biosynthesis in Arabidopsis have suggested that 2 distinct pathways involving the *CYP79B* or *YUCCA (YUC)* genes may contribute to IAOx synthesis and that several pathways are also involved in the conversion of IAOx to IAA. We present the biochemical dissection of IAOx biosynthesis and metabolism in plants by analyzing IAA biosynthesis intermediates. We demonstrated that the majority of IAOx is produced by *CYP79B* genes in *Arabidopsis* because IAOx production was abolished in *CYP79B*-deficient mutants. IAOx was not detected from rice, maize, and tobacco, which do not have apparent *CYP79B* orthologues. IAOx levels were not altered in the *yuc1 yuc2 yuc4 yuc6* quadruple mutants, suggesting that the *YUC* gene family probably does not contribute to IAOx synthesis. We determined the pathway for conversion of IAOx to IAA by identifying 2 intermediates, indole-3-acetamide (IAM) and indole-3-acetonitrile (IAN), in *Arabidopsis*. When $^{13}C_6$-labeled IAOx was fed to *CYP79B*-deficient mutants, $^{13}C_6$-atoms were efficiently incorporated to IAM, IAN, and IAA. This biochemical evidence indicates that IAOx-dependent IAA biosynthesis, which involves IAM and IAN as intermediates, is not a common but a species-specific pathway in plants; thus IAA biosynthesis may differ among plant species."
(a) RIKEN Plant Science Center (b) Tokyo Metropolitan University (c) Forestry and Forest Product Research Institute (d) University of California at San Diego

P34017 New cytokinins for tree biotechnology
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"The wild service tree (*Sorbus torminalis* (L.) Crantz) is one of a scarce species among forest trees and it is considered to be endangered. However, it is rated as one of the most valuable hardwoods with a great potential for wider use in forestry and forest ecology, and also for its importance in the timber industry. Wych elm (*Ulmus glabra*, Huds.), a native species in Europe, are also highly valuated trees for their great strength, tightly twisted grain, durability and for their cold and salt tolerance. However, during the years 1970-80, the second epidemic of Dutch elm disease destroyed most elm populations in Europe. For long-term sustainability of tree genetic resources, micropropagation technologies are proven to be useful (Mala et al. 2005). However, some difficulties during standardized micropropagation procedures still remain to be solved. The investigations of mechanisms of action of cytokinins and their different derivatives could substantially contribute to rationalization of micropropagation. Here, the influence of three different aromatic cytokinin derivatives (6-benzylaminopurine, meta-topolin and 6-(3-methoxybenzylamino)purine-9-βundefined-D-ribofuranoside (MeOBAPR)) on in-vitro multiplication, rhizogenesis and early senescence inhibition of the selected tree species were compared. Special attention has been paid to endogenous concentrations of cytokinins, auxins and ethylene, produced by the explants, grown on different cytokinins. Their importance as well as different roles in the process will be discussed. The work was supported by Czech Ministry of Education (MSM 6198959216). "
(a) Institute of Forest and Game Management (b) Palacky University & IEB AS CR

P34018 Plant Natriuretic Peptide: a hormone with paracrine and autocrine properties that interacts with ABA
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"Higher plants contain biologically active proteins called immunoreactive Plant Natriuretic Peptides (irPNP) as they are recognized by anti-human atrial natriuretic peptide rabbit serum (anti-hANP). We have identified and isolated two Arabidopsis thaliana irPNP encoding genes called AtPNP-A and AtPNP-B which are members of the expansin superfamily. AtPNP-A-promoter::GUS construct transformations reveal that AtPNP-A is mainly expressed in mesophyll cells in the leaves; so we investigated AtPNP-A expression using mesophyll protoplasts. We confirm that AtPNP is secreted from mesophyll protoplasts using AtPNP-A-GFP reporter constructs and together with our previous finding that PNP occurs in vascular tissue, it indicates movement of PNPs. Using transient reporter assays, we show that AtPNP-A expression is enhanced by heat, osmotica and salt; whereas native AtPNP-A is enhanced by osmotica and transiently by salt. AtPNP-A itself (and its second messenger cGMP) enhances its own expression but inhibits it at high concentrations indicating paracrine and autocrine effects. ABA also enhances AtPNP-A expression but AtPNP-A does not modulate expression of RD29A (an ABA responsive gene). However, AtPNP-A is able to reduce and delay the effect of ABA on guard cell closure. Together these results

show PNP appears to modulate its own expression by a feedback system that can be influenced by environmental stimuli and the presence of ABA."
(a) Monash University (b) University of the Western Cape (c) Deakin University

P34019 Cytokinins modulate auxin-induced organogenesis in plants via regulation of the auxin efflux
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"Postembryonic *de novo* organogenesis represents an important competence evolved in plants. Auxin and cytokinin (CK) are important regulators of the developmental fate of pluripotent plant cells. However, the molecular mechanism of their interaction in control of plant organogenesis is largely unknown. We employed the hypocotyl explants-based *in vitro* system to study the mechanism underlying *de novo* organogenesis. We show that auxin, but not CK, is able to induce organogenic response in hypocotyl explants. The auxin-induced organogenesis is accompanied with endogenous CKs production and tissue-specific activation of CK signalling, documented by measurement of CKs content, *ARR5::GUS* expression and qRT PCR of cytokinin primary response genes. CK modulates auxin-induced organogenesis via regulation of intercellular auxin distribution, visualised by DR5rev-driven reporters. The CK-mediated modulation of organogenesis could be simulated by the inhibitor of polar auxin transport. CK reduces auxin efflux from cultured tobacco cells and regulates the expression of auxin efflux carriers from the PIN family in hypocotyl explants. Finally, we found that endogenous cytokinins are necessary to maintain intercellular auxin distribution and *PIN* expression in *Arabidopsis* roots. Bases on these findings, we propose a model in which auxin acts as a trigger of the organogenic processes, whose output is modulated by the endogenously produced CKs. Important mechanism of this CK action is effect on auxin distribution via regulation of expression of auxin efflux carriers."
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P34020 Reevaluation of the role of the indole pyruvate decarboxylase (IPyA) pathway in auxin biosynthesis in plants
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" Auxins are critical regulatory signal molecules for regulation of plant growth and development. The indole pyruvate decarboxylase (IPyA) pathway has long been proposed as the main auxin biosynthesis pathway where tryptophan is converted into indole-3-pyruvic acid by a tryptophan transaminase or transferase, followed by enzymatic decarboxylation to indole-3-acetaldehyde (IAAld), which is further oxidized by an indole-3-acetaldehyde oxidase to IAA. Indole-3-pyruvate has been shown to be present in tomato and Arabidopsis and recently TAA1 (Tryptophan Aminotransferase of Arabidopsis) has been reported to potentially catalyze the formation of indole-3-pyruvic acid from L-tryptophan. Identifying an IPDC gene is critical to confirm IPyA pathway, or even to clarify the relationships among the whole array of enzymes involved in proposed auxin biosynthetic pathways. By sequence analysis, including motif identification, we have shown that there are five decarboxylase candidates for an IPDC in Arabidopsis. We have cloned each of these genes, obtained T-DNA insertion lines and produced overexpression lines for each of these genes. Because of the inherent instability of indolepyruvate, a functional assay for IPDC is not straight forward and conventional methods are subject to significant errors. Thus, we have developed a GC-MS based enzyme assay for IPDC. Using these methods that easily identify microbial IPDC activity, we were unable to show IPDC activity from fully functional cloned plant PDCs and related enzymes, nor were we able to demonstrate IPDC activity in protein extracts from wild type plants. Currently we are analyzing the overexpression lines and plants expressing a bacterial bifuctional enzyme. Supported, in part, by NSF 2010 grant MCB0725149. "
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P34021 Redirection of Trp metabolism in tobacco by ectopic expression of an Arabidopsis inodolic glucosinolate biosynthetic gene
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"Indole-3-acetaldoxime (IAOx) is a branch point compound of Trp metabolism in indole glucosinolate (IG) producing species such as Arabidopsis, serving as a precursor to IGs, the defense compound camalexin and indole-3-acetic acid (IAA). We synthesized both [$^{2}H_{5}$] and [$^{13}C_{10}{}^{15}N_{2}$]IAOx in order to quantify endogenous IAOx in Arabidopsis and tobacco, a non-glucosinolate producing species. Labeled and endogenous IAOx separate into E and Z isomers on LC (confirmed by ^{1}H NMR spectroscopy). Plant extracts contain both isomers in an approximately 2:1 ratio of E:Z. The products of the *in vitro* reaction in which Trp was incubated with AtCYP79B2 that had been expressed in *E. coli* showed a 3:1 ratio of E:Z isomers. These data indicate that the major product of the Arabidopsis CYP79B2 enzyme is the E isomer. Transgenic tobacco lines expressing AtCYP79B2 had significantly higher levels (compared to control lines) of IAOx (50-fold) as well as indole-3-acetonitrile (IAN, 20-fold) and IAA (2-fold). IAN is proposed to be a metabolite of IAOx or an enzymatic breakdown product of IGs induced upon tissue damage. Since tobacco does not produce detectable IGs, these data are consistent with IAN being a metabolite of IAOx.This work was supported by a grants from the NSF (MCB 0517420 and DBI 0077769) to JN and NSF MCB 0517506 to JLC. "
(a) University of New England (b) University of Massachusetts (c) Al-Azhar University (d) Boston University (e) National Agricultural Research Center for Tohoku Region

P34022 Arabidopsis Ubc13 promotes K63-linked polyubiquitination and is required for auxin-mediated root development
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"Among ubiquitin conjugating enzymes (E2s or Ubcs), Ubc13 is unique in that it can promote K63-linked polyubiquitination, which, unlike the conventional K48-linked polyubiquitination that leads to target protein degradation, is thought to serve as a signal. In yeast and mammalian cells, Ubc13 has been shown to function in DNA damage tolerance and NF-κB activation as well as some other less defined pathways. The essential role of Ubc13 in mammals is implied by the mouse embryonic lethality from *Ubc13* deletion, which hampers its further genetic analysis. Arabidopsis contains two highly conserved *UBC13* genes. We created and surprisingly found that mutant plants with both *UBC13* gene disruption are still viable and fertile. Nevertheless the homozygous double mutant plants display a number of altered phenotypes compared with wild-type or single mutants. In particular, the *ubc13* null mutant develops shorter and distorted roots with no hairs and caps, and the number of lateral roots is also reduced significantly. In addition, unlike wild-type and single mutants, the *ubc13* null mutant roots do not respond to N-acetylaspartate treatment, suggesting that the auxin-mediated root development pathway is compromised. Consistent with the above observations, microarray analysis indicates that the expression of a number of auxin-responsive genes is significantly altered in the *ubc13* null mutant. Genome-wide search for Ubc13-interacting ubiquitin ligases (E3s) identifies Rglg2 as one of the putative cognate E3s for Ubc13. Based on the above data, we propose that the specific E2-E3 interaction between Ubc13 and Rglg2 promotes K63-linked polyubiquitination of a yet unidentified target protein that is required to mediate auxin-induced transcriptional regulation of auxin-responsive genes."
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P34023 Post-translational modifications and important motifs controlling DELLA protein activity
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http://fds.duke.edu/db/aas/Biology/staff/zentella
"DELLA proteins are central negative regulators of the gibberellin (GA) signaling pathway. DELLAs act as transcriptional regulators, which likely modulate downstream gene expression through interaction with other transcription factors. Inactivation of DELLAs is essential for the promotion of plant growth and development. Recent advances have shown that the GA receptor, GID1, binds DELLA in the presence of GA. This triggers DELLA poly-ubiquitination and degradation via the proteasome. The N-terminal region of DELLA contains the GID1 binding domain, whereas its C-terminal GRAS domain is required for the ubiquitin E3 ligase SCF^{SLY1} recognition. Although the main DELLA-inactivating mechanism has been elucidated, its repressive mode of action is less clear. We have identified a handful of direct DELLA targets. ChIP results indicate that DELLAs associate with their target gene promoters. Here we address in more detail how DELLA proteins function and their regulation. There is evidence suggesting that DELLA activity may be modulated by phosphorylation and O-GlcNAcylation. Using a variety of biochemical and genetic approaches we aim to understand the role of specific post-translational modifications within DELLAs. We will present evidence that DELLAs are phosphorylated and O-GlcNAcylated in planta. We plan to identify the modified residues and their biological function. In addition, we are analyzing transgenic plants expressing mutant DELLAs in order to understand the function of different motifs in the protein."
(a) Duke University (b) University of Minnesota, St. Paul (c) University of Virginia, Charlottesville

P34024 The role of an Arabidopsis *WRKY* gene in plant response to high salinity stress during seed germination and vegetative growth
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"Salt stress severely restricts plant growth and limits crop yield worldwide. Approximately 50% of all irrigated lands are salt-afflicted, and the percentage is expected to increase due to global warming and human activity. With more than 70 members in Arabidopsis and about one hundred in the rice, maize and sorghum, the *WRKY* gene family plays key roles in plant responses to both biotic and abiotic stresses. Via particle bombardment-mediated transient expression studies, we have shown that *OsWRKY72* and *OsWRKY77*, function as activators, while *OsWRKY24* as a repressor, of abscisic acid (ABA) signaling in cereal aleurone cells. To address the roles of the homologues of these genes in dicotyledonous plants, we have analyzed dozens of Arabidopsis mutants. One of these mutants was found to be more resistant to salt stress than the wildtype. The germination rates of mutant seeds were significantly higher than those of the wildtype seeds in the MS media containing 200 mM NaCl. In addition, seedling root lengths were examined under different concentrations of salt every 2 days for a total of 10 days. Under the test condition, the mutant seedlings grew significantly longer roots than wildtype in 150 mM NaCl. To address the molecular foundation of this difference, we examined the expression patterns of key genes that are known to be involved in plant responses to abiotic stresses. Screening for proteins interacting with several WRKY proteins are also ongoing. A model will be presented to explain the possible mechanisms by which WRKY proteins modulate plant responses to high salinity stress during seed germination and early vegetative growth."
(a) University of Nevada

P34025 "Relieving DELLA Repression of Stem Elongation and Flowering, Evidence for a Proteolysis Independent Mechanism for GA signaling"
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"GA stimulates germination, stem elongation, and flowering by lifting DELLA protein repression of these responses via both proteolysis dependent and independent pathways. There are five members of the DELLA protein family in Arabidopsis with partially overlapping functions. GA biosynthesis lifts DELLA repression by triggering DELLA proteolysis via the ubiquitin-proteasome pathway. Perception of GA by the GA receptors GIBBERELLIN INSENSITIVE DWARF1 (GID1a, b, and c) enables GID1/GA to recognize and bind the DELLA protein. It appears that the SLY1 protein binds and ubiquitinates DELLA only when it is in the GID1/GA/DELLA complex. Polyubiquitinaiton by the SCF-SLY1 E3 ubiquitin ligase then targets DELLA for proteolysis. If DELLA proteolysis were the only mechanism for DELLA inactivation, then the level of DELLA protein should correlate with the degree of dwarfism and other GA phenotypes. In contrast, *sly1* mutants accumulate more DELLA protein but display less severe dwarf and germination phenotypes than the GA biosynthesis mutant *ga1-3* or the *gid1abc* triple mutant. Interestingly, *GID1* overexpression rescued the *sly1* dwarf and infertility phenotypes without decreasing the accumulation of the DELLA protein REPRESSOR OF GA1-3 (RGA). *GID1* rescue of *sly1* mutants appeared to be dependent on the level of GID1 protein, GA, and the presence of a functional DELLA motif. Since DELLA shows increasing protein interaction with GID1 with increasing GA levels in vivo, it appears that GA-bound GID1 can block DELLA repressor activity by direct protein interaction with the DELLA domain. Thus, a SLY1 independent mechanism for GA signaling may function without DELLA degradation."
(a) USDA-ARS, Wheat Genetic Unit (b) Washington State University, Dept of Crop and Soil Science

P34026 Auxin and Ethylene Differentially Induce the Phenylpropanoid Pathway
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"Phenylpropanoid biosynthesis is a tightly regulated and important metabolic pathway. The hormones auxin and ethylene enhance accumulation of flavonol intermediates of this pathway. Flavonols have been implicated as negative regulators of auxin transport, suggesting a mechanisms by which auxin regulates its own transport. We have examined the expression of genes encoding key pathway enzymes, such as chalcone synthase (*CHS*), the first enzyme in flavonoid biosynthesis and flavonoid 3'-hydroxylase (*F3'H*), which controls the relative abundance of kaempferol (K) and quercetin (Q). CHS is upregulated by IAA and ACC, consistent with enhanced carbon flow into the pathway, while F3'H is only induced by IAA, suggesting differential metabolite channeling. We find that IAA increases both K and Q accumulation in roots by confocal microscopic imaging of DPBA fluorescence, a dye which binds flavonols with distinct florescence patterns for Q and K. In contrast, ACC induces K accumulation significantly more than Q, consistent with predictions of metabolite accumulation from the gene expression studies. ACC induced gene expression is absent in the ethylene insensitive mutants, *ein2* and *etr1*, but is normal in the auxin receptor mutant, *tir1*. In contrast, IAA induced gene expression is lost in *tir1* and unaffected in *ein2* and *etr1*. We have also examined the kinetics of induction of these and the other pathway enzymes and upstream transcription factors and are building framework models describing the temporal pattern and genetic controls of this pathway. These results demonstrate that hormonal controls of the phenylpropanoid pathway can channel intermediates to allow unique metabolite accumulation patterns that modulate growth and developmental responses. Support from USDA NRIGC 2006-03406."
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P34027 AtRIC1 modulates lateral root development and seed germination
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"RICs, which contain a CRIB motif, are effectors of ROP small G protein. ROPs mediate a broad range of extracellular stimuli, including hormones

such as ABA and auxin, via interaction with effectors. RIC1 interacts with activated ROP and mediate the development of tobacco pollen tube and morphogenesis Arabidopsis leaf pavement cell. However, the role of RIC1 in the diverse development processes is not well known. We found that RIC1 is highly expressed in root, especially lateral root initiation sites and root hairs using RIC1 promoter-GUS plants. In normal 1/2 MS medium, *ric1* knockout plants have more lateral roots than wild-type. ABI3 (ABA insensitive 3), a transcription factor which plays important roles both in auxin as well as ABA signaling, has been reported to modulate lateral root development. ABI3 transcript level was higher in root of *ric1* knockout than that of wild-type. ABI3 is involved in not only lateral root development but also seed germination. *ric1* knockout mutant plants were hypersensitive to ABA in seed germination. These results suggest that RIC1 participates in ABA-signaling by suppressing ABI3 during lateral root development and seed germination. In further studies of RIC1 signaling, we will investigate which of the many ROPs interact with RIC1 to regulate lateral root development and seed germination."
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P34028 A novel G-protein coupled ABA perception system in Arabidopsis
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"Abscisic acid (ABA) is a vital plant hormone. ABA defines a plants response under abiotic stress conditions such as drought, in addition to regulating different aspects of normal plant growth and development. The identification of proteins that first sense ABA in plants, i.e. ABA receptors, and elucidation of their perception mechanisms is central to our understanding of the diverse processes regulated by ABA. Heterotrimeric G proteins mediate many aspects of ABA signaling in plants however, the ABA-perception mechanisms linked to G proteins remain elusive. Towards this, we have identified two highly homologous proteins in Arabidopsis, GTG1 and GTG2, as ABA receptors. Biochemical and molecular-genetic analyses confirmed that the Arabidopsis GTG proteins are a class of membrane-localized ABA receptors, and novel GTP-binding proteins. The GTG proteins bind ABA in a stereo-specific manner. Mutant plants lacking the two *GTG* genes exhibit hyposensitivity to ABA in all classic responses. The GTG proteins physically and functionally interact with the sole canonical Gα-protein of Arabidopsis, GPA1. Interaction of GTG1 and GTG2 proteins with GPA1 blocks their GTPase activity, consistent with ABA-hypersensitive phenotypes of *gpa1* mutants. The identification of GTG proteins is significant since they are not only a class of plant ABA receptors, but also connote a paradigm shift in G-protein signaling, combining the functions of both GPCRs and G-proteins to perceive and execute the first steps of a hormonal signaling pathway. "
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P34029 Highly sensitive and high-throughput analysis of plant hormones using MS-probe modification and liquid chromatography-tandem mass spectrometry: an application for hormone profiling in *Oryza sativa*
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http://labs.psc.riken.jp/brt/English/index.html
"We have developed a highly sensitive and high throughput method for the simultaneous analysis of 43 molecular species of cytokinins, auxins, ABA, and gibberellins (GAs). This method consists of an automatic liquid handling system for solid phase extraction and ultra-performance liquid chromatography (UPLC) coupled with a tandem quadrupole mass spectrometer (qMS/MS) equipped with an electrospray interface (ESI, UPLC-ESI-qMS/MS). In order to improve the detection limit of negatively charged compounds, such as GAs, we chemically derivatized fractions containing auxin, ABA, and GAs with bromocholine that has a quaternary ammonium functional group. This modification, that we call MS-probe, makes these hormone-derivatives have a positive ion charge and permits all compounds to be measured in the positive ion mode with UPLC-ESI-qMS/MS in a single run. Consequently, quantification limits of GAs increased up to 50-fold. Our current method needs less than 100 mg (fresh weight) of plant tissues to determine phytohormone profiles, and enables us to analyze simultaneously more than 180 plant samples. Application of this method to plant hormone profiling enabled us to draw organ-distribution maps of hormone species in rice and also to identify interactions among the 4 major hormones in the rice GA-signaling mutants, *gid1-3*, *gid2-1*, and *slr1*. Combining the results of hormone profiling data with transcriptome data in the GA signaling mutants allows us to analyze relationships between changes in gene expression and hormone metabolism."
(a) RIKEN Plant Science Center (b) Keio University (c) Kobe University (d) Nagoya University

P34030 "Role of BT2 in mediating multiple responses to nutrients, hormones and stresses in *Arabidopsis thaliana*"
Mandadi, Kranthi Kiran-presenter kmandadi@mail.bio.tamu.edu(a,b) Misra, Anjali (a,b) Ren, Shuxin (a,c) McKnight, Thomas D (a,b)
"Diurnal changes in light strongly affect several metabolic and physiological processes in plants such as nutrient status, resource allocation, growth and development. We previously described BT2, a BTB/POZ domain containing protein, as an activator of telomerase in mature *Arabidopsis thaliana* leaves. In the current study we investigate and present evidence of its surprising, yet fundamental, roles in mediating diverse hormone, stress and metabolic responses in plants. Steady-state expression of *BT2* mRNA was regulated diurnally and is under the control of the circadian clock, with a maximum expression in the dark. Sugar and nitrate status of plants modulated *BT2* expression by repressing and inducing it, respectively. Reverse genetic analysis using BT2 loss-of-function and BT2 over-expressing lines revealed that BT2 suppresses sugar and ABA-mediated responses during germination. Furthermore, genetic experiments performed using auxin accumulating mutant, *yucca*, suggest that BT2 positively regulated certain auxin responses during post-germination development. In addition to mediating nutrient and hormone responses, expression of *BT2* mRNA is responsive to a variety of biotic and abiotic stresses, suggesting that BT2 occupies a central location in a functional network that detects and responds to multiple inputs. Also, preliminary results of characterization of other members of BT family (BT1, 3, 4, 5) indicate certain redundancy to BT2 function in mediating nutrient and hormone responses."
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P34031 Affinity chromatography ligands based on N^9-substituted cytokinin derivatives
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"The search for phytohormone-binding proteins is not an easy task, since numerous proteins such as enzymes of the hormonal metabolism and transport, storage proteins as well as others that participate in the synthesis, modification and phytohormones destruction can bind hormones. Cytokinins belong to a class of plant hormones that play important roles in many aspects of plant growth and development.[1] Very broad group of natural cytokinins is based structurally on adenine moiety: the members of the group are therefore adenine derivatives with N^6 atom substituted by a side chain or a further substituted benzyl ring. So far, we have prepared and characterized a number of N^6-isopentenyladenine and N^6-benzyladenine derivatives substituted accurately in N^9 atom of the adenine moiety with the appropriate spacer-arm or suitable functional group and we designed potentially biospecific ligands for affinity chromatography of cytokinin derivatives. Moreover, we studied the stability, toxicity and biological activity of

newly prepared derivatives in the extensive number of bioassays such as tobacco callus assay, wheat senescence assay and *Amaranthus* assay as well as *in vitro* receptor assay in order to evaluate the biologic effects of newly prepared substances.[2,3] 1. Davies, P.J. (2004) Plant Hormones, Biosynthesis, Signal Transduction, Action! Kluwer Academic Publishers, The Netherlands. 2.Zatloukal M. et al., Bioorg. Med. Chem. 16, 9268 (2008) 3. Spichal L. et al., Plant Cell Physiol. 45, 1299 (2004) "
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P34032 Heterologous Expression of 1-Aminocyclopropane-1-Carboxylic Acid (ACC) N-Malonyltransferase from Mung Bean Hypocotyls
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"In the ethylene biosynthetic pathway, ACC N-malonyltransferase can irreversibly malonylate ACC into MACC, other than oxidation into ethylene, by transferring the malonyl group from malonyl CoA to ACC. Therefore, it was regarded as a vital enzyme which could regulate the rate of ethylene formation. However, its purification showed slow progress using traditional liquid chromatography methods due to its low abundance in plant. In 1997, my lab had successfully immunopurified a 40-kD of ACC N-malonyltransferase from mung bean hypocotyls. Later, its corresponding cDNA sequence (clone 11a) had also been cloned. Even though cDNA of 40-kD ACC N-malonyltransferase could be revealed from clone 11a, its ACC N-malonyltransferase activity needs to be confirmed by expression. Furthermore, protein encoded by clone 11a was found to share a high homology with cysteine protease. Hence, the relationship among protein 11a, ACC N-malonyltransferase and cysteine protease awaits clarification. In this study, three fragments of clone 11a were respectively subcloned into a vector, pYES2_YFP, to analyze the expression and activity of recombinant proteins in yeast strain INVSc1. After 24 hours induction, laser confocal microscopy analysis suggested that three recombinant proteins had been expressed in the yeast cells. However, native PAGE analysis indicated that only 11a without signal sequence had expressed the expected recombinant proteins. Then, immunopurification using an anti-YFP antibody column had successfully obtained the recombinant protein of 80 kD. However, neither ACC N-malonyltransferase activity nor cysteine proteinase activity could be detected. Because no functional enzyme was observed in yeast expression so far, the identity of clone 11a needs to be further investigated."
(a) The University of Hong Kong

P34033 Analysis of Dominant Negative Mutant Ethylene Receptors ERS1 and ETR2 from Zea mays
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"Ethylene is a gaseous hormone affecting plant development and responses to adverse environmental conditions. The binding of ethylene to its receptors leads to the induction of ethylene responses. Five ethylene receptors, ETR1, ERS1, EIN4, ETR2, and ERS2, have been described in Arabidopsis. Dominant negative mutants of ETR1 that confer a state of ethylene insensitivity have been isolated. One such mutant, etr1-1, has a Cys to Tyr mutation at residue 65 and results in a strong ethylene insensitive phenotype. In contrast to Arabidopsis, only two receptors, ZmERS1 and ZmETR2, are expressed in maize. To investigate the extent to which receptor function is conserved between monocots and dicots, the Cys to Tyr mutation was introduced into the corresponding position in each type of maize ethylene receptor and the mutant receptors were expressed in Arabidopsis. Expression of Zmetr2 or Zmers1 conferred a level of ethylene insensitivity similar to that observed in the etr1-1 or ein2-5 mutants as determined by the lack of triple response in dark-grown seedlings grown in the presence of ACC, the precursor to ethylene. Ethylene insensitivity was conferred when the transgenic plant is either hemizygous or homozygous. Light-grown mutant seedlings exhibited fully expanded cotyledons and emergence of true leaves when grown in the presence of ACC whereas growth of wild-type seedlings was severely repressed. Leaves of adult mutant plants were larger than wild-type and exhibited delayed senescence. Expression of the N-terminal portion of the mutant Zmers1 was sufficient to confer ethylene insensitivity. These results demonstrate that, despite the divergence in their primary structure, the function of maize ethylene receptors is highly conserved with ethylene receptors in Arabidopsis."
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P34034 Isolation and characterization of the first full-length gymnosperm auxin conjugate hydrolase from *Picea sitchensis* (Sitka Spruce)
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"Both vascular and non-vascular plants regulate the balance of the phytohormone auxin through interactions among de novo synthesis, degradation, efflux, influx, and conjugate synthesis/hydrolysis. An interspecific knowledge of the regulation and character of these pathways and their interactions is key to delineating auxin influences on plant growth and development. We have examined how the ILR1-like auxin conjugate hydrolase family of genes has functionally evolved in the gymnosperm model species sitka spruce (*Picea sitchensis*). We have isolated and cloned from spruce an ortholog (PsIAR31) for the *Arabidopsis* IAR3 auxin amidohydrolase. We are presently characterizing the enzymatic activity of PsIAR31. We will compare its enzymatic activity to hydrolase genes isolated from angiosperm species that have already been characterized, as well as moss species."
(a) Montclair State University (b) Technical University of Dresden

P34035 Is Soybean More SLEEPY Then *Arabidopsis*? : Sorting Out Multiple Putative Orthologs Of The *Arabidopsis* Gene SLEEPY-1
Janssens, Derek (a) Tsou, Pei-Lan-presenter tsoup@gvsu.edu(b)
"Bioactive gibberillic acid (GA) signaling is critical for multiple developmental processes throughout the life cycle of several plants. Elements involved in the GA response pathway in *Arabidopsis* have been identified and studied. The F-Box protein Sleepy 1 (SLY1) has been characterized in *Arabidopsis* as a positive regulator of GA signaling. The *SLY1* gene is up regulated in response to an increase in bioactive GA and functions to degrade inhibitors of GA signaling. Two putative soybean *SLY1* orthologs have identified by screening a cDNA library and bioinformatic methods. While both putative orthologs contain an F-Box motif as well as several other domains common to the *Arabidopsis* SLY1 protein, it has not been determined whether these orthologs fulfill the same function as SLY1. We treated soybean seedlings with either GA or paclobutrazol (an inhibitor of GA biosynthesis); studies in *Arabidopsis* suggest that the former treatment increases SLY1 activity while the latter treatment decreases SLY1 activity. Using RT-PCR, we are determining if these treatments have similar affects on the expression of either soybean putative ortholog. We are also transforming both genes into *Arabidopsis sly1* mutant to rescue the phenotype in order to understand the role of SLY1 in soybean. "
(a) Biomedical Science, Grand Valley State University (b) Cell and Molecualr Biology, Grand Valley State University

P34036 The effect of cytokinin signaling mutants on auxin signaling
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The plant hormones cytokinin and auxin are known to act coordinately in regulating plant growth and organ development. Cytokinin responses are mediated by a two-component signaling pathway. In Arabidopsis a family of transcription factors referred to as type-B response regulators (ARRs)

function as targets of the primary cytokinin signaling pathway. We have undertaken a mutational analysis to characterize the contribution of specific type-B ARRs to cytokinin responses. Arabidopsis lines with T-DNA insertions in single and multiple type-B ARRs have been assembled and characterized. In order to examine the impact of cytokinin signaling mutants on auxin signaling the auxin reporter constructs DR5:GUS and BA3:GUS have been incorporated into Arabidopsis lines with mutant type-B ARRs.
(a) University Of New Hampshire, Department of Molecular, Cellular and Biomedical Science (b) Dartmouth College, Department of Biological Sciences

P34037 The Arabidopsis ASA1 gene is important for jasmonate-mediated regulation of auxin biosynthesis and transport during lateral root formation
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"Plant roots show an impressive degree of plasticity in adapting their branching patterns to ever-changing growth conditions. An important mechanism underlying this tremendous ability of adaptation is the interaction between hormonal and developmental signals. Here we analyze the interaction of jasmonate with auxin to regulate lateral root (LR) formation through characterization of an Arabidopsis mutant, jasmonate-induced defective lateral root1 (jdl1/asa1-1). We demonstrate that, whereas exogenous jasmonate promotes LR formation in wild type, it represses LR formation in jdl1/asa1-1. JDL1 encodes the auxin biosynthetic gene ANTHRANILATE SYNTHASE1 (ASA1), which is required for jasmonate-induced auxin biosynthesis. Inspection of auxin distribution through monitoring expression patterns of the auxin responsive-proIAA2:GUS reporter reveals that jasmonate elevates local auxin accumulation in the basal meristem of wild-type roots, whereas reduces local auxin accumulation in the basal meristem of mutant roots, suggesting that in addition to activate ASA1-dependent auxin biosynthesis, jasmonate also affects auxin transport. Indeed, jasmonate modifies the expression of auxin transport genes in an ASA1-dependent manner. We further provide evidence showing that the action mechanism of jasmonate to regulate LR formation through ASA1 differs from that of ethylene. Our results highlight the importance of ASA1 in jasmonate-induced auxin biosynthesis and, reveal a role for jasmonate in the attenuation of auxin transport in the root. Jasmonate action therefore results in fine-tuning local auxin distribution in the root basal meristem that appears to be critical for LR formation. "
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P34038 Molecular basis of the hormonal control of citrus fruit pigmentation: an overview
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"Citrus fruit served for the last 40 years as a model system for the study of ripening and senescence-associated pigment changes. This concerns two major, not directly linked processes occurring during chloroplast-chromoplast transition: the breakdown of chlorophyll and the buildup of carotenoids. Most prominent is the effect of ethylene which enhances both processes (and is used commercially to improve fruit coloration), and that of gibberellins (and cytokinins) which oppose the effect of ethylene. Recent years elucidation of the biochemical pathways of chlorophyll catabolism and carotenoid biosynthesis opened the way for identification of events regulated hormonally at the molecular level. Ethylene upregulates transcriptional as well as post-transcriptional induction of chlorophyllase; subsequent steps of chlorophyll catabolism also appear to be enhanced. Key regulatory steps of the citrus carotenoid pathway have recently been identified; genes which are differentially regulated during natural citrus fruit ripening are further accentuated by ethylene. Citrus pigment mutants [i.e. stay-green *nan*, yellow *Pinalate*] serve to decipher the regulatory mechanisms. The interplay of environmental, nutritional and hormonal signals modulating fruit pigmentation is still fragmentary and requires clarification. A model/hypothesis of the molecular regulation of citrus fruit pigmentation, integrating new physiological, molecular and genetic evidence will be presented and discussed. "
(a) Hebrew Univ of Jerusalem (b) Instituto de Agroquimica y Tecnologia de Alimentos (IATA-CSIC) (c) ARO, Volcani Center

P34039 "SMAP1 is a positive regulator for 2,4-Dichlorophenoxyacetic acid mediated actin degradation and acts independent of known auxin signaling pathway"
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"2,4-dichlorophenoxyacetic acid (2,4-D), a chemical analogue of plant hormone auxin, Indole-3-acetic acid (IAA), is widely used as a growth regulator and exogenous source of auxin. Traditionally, it is believed that IAA and 2,4-D share a common signaling pathway. However, recent studies have challenged this idea both at physiological and genetic level. The isolation of a 2,4-D specific mutant *aar1* and identification of the protein SMAP1, which confers specific resistance to 2,4-D, provide evidence that IAA and 2,4-D use partially distinct molecular pathways. Similar to upstream events, 2,4-D and IAA also control the downstream physiological responses differentially. 2,4-D but not IAA, degrades the root actin cell cytoskeleton and inhibits the cell division. To provide a molecular explanation of 2,4-D specific action in Arabidopsis, we characterized the functionally unknown protein SMAP1. The molecular and cell biological analyses revealed that, 1) SMAP1 acts as a positive regulator for 2,4-D induced degradation of actin, cell division and cell elongation processes as the loss of SMAP1 nullify the effect of 2,4-D on all these processes, and 2) SMAP1 acts independent of known ubiquitin-proteasome mediated auxin signaling pathway, as the double mutant of SMAP1 and TIR1, a component of E3 ubuquitin ligaes (*aar1-tir1*), in root growth assay, shows complete insensitivity to 2,4-D at the concentration that inhibits eighty percent root growth in respective single mutants. Similarly, the loss of SMAP1 in *tir1* background makes the root actin resistant to 2,4-D mediated degradation. Our results, for the first time, identify a novel 2,4-D specific biologically significant pathway in plants, and also provide a molecular explanation of the difference between IAA and 2,4-D."
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P34040 Genetic study on the cytokinin-associated type-B ARR transcription factors in *Arabidopsis thaliana*
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"*Arabidopsis thaliana* has 11 members belonging to the typical type-B ARR (AUTHENTIC RESPONSE REGULATOR) family. Among them, 7 ARRs are implicated as DNA-binding transcription factors in the phosphorelay-mediated cytokinin signal transduction network. To gain insights into the functions of the cytokinin-associated type-B ARRs, here we established an *arr1 arr10 arr12* triple loss-of-function mutant, which showed remarkable and severe phenotypes. The observed cytokinin-associated phenotypes of *arr1 arr10 arr12* were highly analogous to those reported for certain *ahk2 ahk3 ahk4/cre1* triple mutants, which have virtually no cytokinin receptor to propagate the phosphorelay signal transduction. Taken together, it was demonstrated that ARR1, ARR10, and ARR12 together play essential (or general) roles in cytokinin signal transduction. These results will be presented together with the idea that the other type-B ARRs (ARR2, ARR11, ARR14, and ARR18) may play more specific roles spatially and temporarily in plants."
(a) Graduate School of Bioagricultural Sciences ,Nagoya University

P34041 Trp-aminotransferase (TA) is a major enzyme of the auxin indole-3-acetic-acid (IAA) biosynthesis in developing seeds of maize.

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"In maize, the highest levels of IAA are in developing seeds. Although IAA biosynthesis is entirely Trp-dependent (T-D) in seeds, very little is known on the genes and enzymes of the pathway. The loss-of-function mutation at the *Miniature-1* (*Mn1*) locus that encodes an endosperm-specific cell wall invertase is associated with several pleiotropic changes, including the reduced levels of IAA in developing embryo and endosperm (Phytochemistry 69: 692, 2008) and, as shown here, greatly reduced mass weights of the same tissues in the *mn1* mutant. Attempts to understand the basis of these changes have led us to two new maize genes, each of which represent the two major T-D branches of IAA pathway in plants. The *ZmTARelated-1* gene is a maize ortholog of the TA in Arabidopsis, a key enzyme in the indole-3-pyruvic acid (IPA) branch, and the *ZmYUC1* codes for a flavin mono-oxygenase-like enzyme in the tryptamine branch. Transcript levels of the two genes by q-PCR were the highest with an IAA peak at the cell division / elongation phase (8 - 12 DAP). Coincident with the second peak of IAA levels during the storage phase (> 20 DAP) was a steep decline in the levels of *ZmYUC1*; the *ZmTAR1*, however, showed only a moderate down-regulation. Importantly, the *TAR1* transcripts were > 10-fold higher than the *YUC1* throughout the seed development. Western blot analyses using a peptide antibody of a conserved 20 amino acid TA1 epitope showed a discrete band of the expected size in both the *E. coli*-expressed full-length cDNA clone of the *ZmTAR1* gene and in the endosperm and embryo, which showed copious amounts of the candidate TA1 protein. Overall, these gene expression data suggest that the *ZmTAR1* gene specifies a major pathway, the IPA branch, of IAA biosynthesis in developing seeds of maize."

(a) USDA-ARS and University of Florida

P34042 Arabidopsis Hormone Database: a comprehensive genetic and phenotypic information database for plant hormone research in Arabidopsis

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"Plant hormones are small organic molecules that influence almost every aspect of plant growth and development. Genetic and molecular studies have revealed a large number of genes that are involved in responses to numerous plant hormones, including auxin, gibberellin, cytokinin, abscisic acid, ethylene, jasmonic acid, salicylic acid, and brassinosteroid. We have developed an Arabidopsis hormone database, which aims to provide a systematic and comprehensive view of genes participating in plant hormonal regulation, as well as morphological phenotypes controlled by plant hormones (Peng et al., Nucleic Acids Research, 2009, Vol. 37:D975-982). Based on data from mutant studies, transgenic analysis and gene ontology (GO) annotation, we have identified a total of 1026 genes in the Arabidopsis genome that participate in plant hormone functions. Meanwhile, a phenotype ontology is developed to precisely describe myriad hormoneregulated morphological processes with standardized vocabularies. A web interface (http://ahd.cbi.pku.edu.cn) would allow users to quickly get access to information about these hormone-related genes, including sequences, functional category, mutant information, phenotypic description, microarray data and linked publications. Taking advantage of these collections, we have conducted several analyses on the interactions among multiple hormone pathways in regulating root elongation, seed germination, apical hook formation, and so on. These analyses demonstrate that our database is valuable in uncovering new mechanisms of plant hormonal regulation and cross-talks, and that our effort in developing hormone-related phenotypic ontology would be helpful to eventually create a comprehensive plant phenome database."

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P34043 Phenotypic characteristics of *LEACO1$_{0.821kb}$-ipt* plants vary between species

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"The phenotypic response of isopentenyl transferase (*ipt*) transgenic plants is greatly dependent on the promoter used to drive *ipt* gene expression. Earlier reports indicate that ipt under the control of a 821 bp fragment of the LEACO1 gene promoter (from *Lycopersicon esculentum*) produced a number of potentially useful modifications in growth form of the ornamental species *Dendranthema x grandiflorium* cv. Iridon (chrysanthemum). Chrysanthemum in the generative state exhibited increased flower bud counts that ranged from 3.8 to 6.7 times the number observed in wild-type plants. Increases in bud counts were sometimes associated with increased branching but sometimes not, and some transgenic lines displayed increased branching but no increase in the number of buds per branch. However, in *Nicotiana tabacum* (cv. Havana) the *LEACO1$_{0.821kb}$-ipt* construct produced a narrower range of phenotypes with plants showing either increased branching or increased bud counts but not both. In chrysanthemum, dramatic increases in flower number were associated with a delay of flower bud development and a decrease in flower bud diameter. RT-PCR analysis indicated differences in ipt gene expression between individual transgenic lines that exhibited a range of phenotypes. *LEACO1$_{0.821kb}$-ipt* chrysanthemum and tobacco both exhibited delayed senescence of excised leaves. Recently a number of transgenic poinsettia (*Euphorbia pulcherrima* Willd. cv. 'Red Success') lines have been recovered from culture and the effects of *LEACO1$_{0.821kb}$-ipt* expression on growth and development is under assessment. "

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P34044 Gibberellin 3β-Hydroxylase Gene Over-expression Alters Vegetative Development in Pea

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"Gibberellins (GAs) regulate many aspects of plant growth and development. To expand our understanding of the importance of GAs in plant growth and developmental processes, pea (*Pisum sativum* L. cv. Carneval) plants were transformed, utilizing a CaMV 35S promoter, to over-express *PsGA3ox1*, the gene that encodes for the activation step in GA biosynthesis (C-3β-hydroxylation which converts GA_{20} to bioactive GA_1). Homozygous transgenic lines had longer internodes and tendrils, and larger stipules than the control lines. Expression patterns of *PsGA3ox1* (transgene and the naturally occurring gene) were investigated in expanding pea internodes using qRT-PCR. Gene expression of the naturally occurring GA biosynthesis genes *PsGA20ox1* and *PsGA20ox2*, and the GA catabolic genes *PsGA2ox1* and *PsGA2ox2* (which encode for the 2β-hydroxylation inactivation step) were also monitored. The *PsGA3ox1* transgene was expressed differentially across the 3 transgenic lines. Transcript levels of one of the GA catabolic genes, *PsGA2ox1*, were substantially elevated, suggesting that substrate-induced feedback regulation is likely acting to maintain GA homeostasis in the rapidly elongating internodes. Changes in endogenous GAs, quantified by the stable isotope dilution method using GC-MS-SIM, were indicative of an increased flux in GA biosynthesis in the transgenic lines. "

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P34045 A Patatin-Like Protein Delays Flowering in Arabidopsis by Reducing Gibberellin Synthesis

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"*OSAG78* isolated from *Oncidium* Gower Ramsey encodes a patatin-like protein. Overexpression of *OSAG78* in *Arabidopsis* causes late flowering. The effects of OSAG78 on flowering were analyzed in this study. The localization of OSAG78 fused with green fluorescence protein in plants was

visualized using a confocal microscope, which revealed that OSAG78, unlike most cytosolic patatin-bearing proteins, presents in the plasma membrane. Crude proteins of transgenic *Arabidopsis* overexpressing *OSAG78* possess higher lipase activity than those from wild type. Phenotypes of transgenic plants exhibit small and round leaves, round flowers, and reduced cell surface. Also, the late-flowering phenotype in transgenic plants can be reversed by gibberellin acid (GA) treatment. The expression levels of several flowering-related genes were then analyzed, and a GA-stimulated gene was found to be depressed in plant overexpressing *OSAG78*. In addition, the levels of bioactive GAs, GA4 and GA7, were reduced in *Arabidopsis* overexpressing *OSAG78* from the assays of gas chromatography-mass spectrometry. Conclusively, OSAG78 in membrane may hydrolyze phospholipids to release free fatty acids, which reduce bioactive GA levels, and, consequently, late flowering occurs."
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P34046 The variable N-terminal domain of NtCDPK1 is required for the recognition of the target protein RSG that regulates transcription of GA biosynthetic genes
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"Gibberellins (GAs) are phytohormones that regulate many aspects of plant development, including germination, stem elongation, flower induction, and another development. Repression of Shoot Growth (RSG) is a transcriptional activator with a bZIP domain that is involved in the regulation of endogenous GA levels in tabacco. The 14-3-3 proteins negatively regulate RSG by sequestering it in the cytoplasm in response to GAs. The phosphorylation on Ser-114 of RSG is essential for 14-3-3 binding of RSG. Of particular importance is the identification of the protein kinase that catalyzes the phosphorylation on Ser-114 of RSG in response to GAs for understanding the molecular mechanisms by which GA regulates the function of RSG. We found that tobacco Ca^{2+}-dependent protein kinase (NtCDPK1) as an RSG kinase that promotes 14-3-3 binding to RSG by phosphorylation of Ser-114 of RSG. NtCDPK1 interacts with RSG in a Ca^{2+}-dependent manner in vivo and in vitro and specifically phosphorylates Ser-114 of RSG. Overexpression of CDPK1 inhibited the feedback regulation of a GA 20-oxidase gene and resulted in sensitization to the GA biosynthetic inhibitor, suggesting that NtCDPK1 plays a role in the GA homeostasis. Because CDPKs comprise a multi-gene family and the primary structures of CDPK isoforms are very similar, it was considered unlikely that they would have distinguishable substrate specificities. However, knock-down study of NtCDPK1 using RNAi uncovered a distinct physiological function of NtCDPK1 in the transgenic plants. To understand how an individual CDPK distinguishes its specific substrates, we investigated the RSG-interacting region of NtCDPK1. Our in vivo and in vitro analyses suggest that CDPK recognizes a specific substrate through the variable N-terminal domain of CDPK."
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P34047 The *Arabidopsis thaliana* STYLISH1 protein acts as a transcriptional activator regulating local auxin biosynthesis
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"The *SHI/STY* family consists of nine active members in *Arabidopsis* (*STY1, STY2, SHI, LRP1* and *SRS3-7*). We have previously reported that induction of *STY1* results in higher auxin levels, higher auxin biosynthesis rate and increased expression of the *YUC* family member *YUC4*. We have also shown that *SHI/STY* family members redundantly affect development of flowers and leaves and that the stylar defect in *sty1 sty2* mutants is restored by auxin application. Our new data show that STY1 is a nuclear protein, that STY1 interact with the *YUC4* promoter and that the STY1 dependent activation of *YUC4* does not require protein translation. Plants expressing a construct with STY1 fused to a repressor domain have phenotypes resembling *SHI/STY* family multiple mutants and have reduced *YUC4* transcript levels suggesting that STY1 normally functions as a transcriptional activator. A *C. roseus* SHI/STY orthologue have been described to interact directly with the *ORCA3* promoter. Analysis of the promoters of *YUC4* and *ORCA3* suggests a 7 bp fragment to be a potential SHI/STY binding site and when parts of this sequence are mutated STY1 can no longer interact with the *YUC4* promoter in yeast. The putative binding site is also found in another *YUC* gene, *YUC8*, and in *ORA59*, an *A. thaliana* gene closely related to *ORCA3* and potentially involved in auxin biosynthesis, and these genes are also activated by STY1. Taken together our results suggest that STY1, and probably other SHI/STY members, are DNA binding transcriptional activators that target genes involved in auxin biosynthesis, thereby being essential regulators of auxin mediated leaf and flower development. To identify other possible SHI/STY targets we have made an array study with an inducible STY1 line and are investigating genes induced by STY1."
(a) Swedish university of agricultural sciences, Department of plant biology and forest genetics (b) Royal institute of technology, Department of wood biotechnology

P34048 *Arabidopsis thaliana* bromodomain proteins BET9 and BET10 regulate BT2-mediated responses to hormones
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"The *Arabidopsis thaliana* BTB protein BT2 is involved in regulating various hormone, stress and metabolic responses in plants. In particular, loss of BT2 function results in plants that are resistant to auxin and are sensitive to ABA and sugar mediated inhibition of germination. Here, we report the interesting roles of potential BT2 interacting partners, the bromodomain proteins BET9 and BET10, in regulating BT2-mediated responses to auxin, ABA and sugars. The single mutants *bet9-1* and *bet10-1* phenocopy the *BT2*-null mutant responses, where inhibition of germination in both mutants is more sensitive to sugar and ABA stress. Furthermore, loss of either BET9 or BET10 in a BT2 over-expressing line blocked the resistance of the germination to sugars and ABA, suggesting that BET9 and BET10 are required for BT2 function. Unexpectedly, germination in a *bet9-1bet10-1* double mutant was not sensitive to inhibition by sugar or ABA. BET9 and BET10 also regulate BT2-mediated auxin responses. We propose a model where BET9 and BET10 individually repress BT2 and associated functions and work antagonistic to each other. Accumulation of *BT2* mRNA is unaltered in both *bet9-1* and *bet10-1*, suggesting that BET9 and BET10 regulate BT2 post-transcriptionally while mediating diverse responses. o"
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P34049 Abscisic Acid Regulation of Gibberellin Metabolism in Pea (*Pisum sativum* L.)
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"Gibberellins (GAs) have important roles in the regulation of fruit growth and seed development. The role of abscisic acid (ABA) as a GA antagonist has been well documented in several plant processes, but the interaction between ABA and the GA class of hormones during fruit and seed development has not been extensively studied. In pea (*Pisum sativum* L.) GAs are metabolized primarily through the early 13-hydroxylation pathway to bioactive GA_1 (GA_{12} converted sequentially to GA_{53},GA_{19}, GA_{20} to GA_1). To understand the effects of ABA on GA metabolism, seeds were treated at two different development stages with ABA using a split-pericarp technique. The expression of four GA biosynthesis genes (*PsGA3ox1*, *PsGA3ox2*, *PsGA20ox1*, and *PsGA20ox2*) and two GA catabolism genes (*PsGA2ox1* and *PsGA2ox2*) was monitored by qRT-PCR. Concentrations of extractable ABA [applied + endogenous], GA_{20}, GA_1 and their immediate catabolites (GA_{29} & GA_8, respectively) were quantified by the isotope dilution method using GC-MS-SIM. During early seed development, which is characterized by both rapid expansion of the seed coat and a pre-storage phase embryo (10 to 12 days after anthesis; DAA), applied ABA altered the expression levels of several GA biosynthesis genes as well as GA concentrations. This

suggests that ABA may be an important regulator of GA biosynthesis in both the seed coat and actively growing embryo. At a later developmental stage, one which is characterized by nutrient storage in the embryo (16-18 DAA), applied ABA also increased the expression of the GA catabolic gene *PsGA2ox2* in the embryo axis. These data suggest that ABA can regulate GA biosynthesis in a developmentally- and tissue-specific manner during pea seed development."
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P34050 Mitogen-activated protein kinases regulate *Botrytis cinerea*-induced ethylene production in Arabidopsis
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"Ethylene plays important roles in plant responses to pathogens. Plants challenged by pathogens, especially necrotrophic fungal pathogens including *Botrytis cinerea*, produce high levels of ethylene. At present, the signaling pathways underlying the induction of ethylene after pathogen infection are largely unknown. Arabidopsis stress-responsive mitogen-activated protein kinase 6 (MPK6) was previously shown to regulate the stability of ACS2 and ACS6. Phosphorylation of ACS2 and ACS6 by MPK6 prevents the rapid degradation of ACS2/ACS6 by the 26S proteasome pathway, resulting in an increase in cellular ACS activity and ethylene biosynthesis. Here, we show that MPK3, which shares high homology and common upstream MAPK kinases (MAPKKs) with MPK6, is also capable of phosphorylating ACS2 and ACS6. In the *mpk3* mutant background, ethylene production in the gain-of-function *GVG-NtMEK2DD* transgenic plants was compromised, suggesting that MPK6 and MPK3 function synergistically to stabilize ACS2 and ACS6. Using a liquid-cultured seedling system, we found that ethylene induction was greatly compromised in the conditionally rescued *mpk3/mpk6* double mutant seedlings after *B. cinerea* infection. In contrast, ethylene production decreased only slightly in the *mpk6* single mutant and not at all in the *mpk3* single mutant, demonstrating overlapping roles for these two highly homologous MAPKs in pathogen-induced ethylene induction. Consistent with the role of MPK3/MPK6 in regulating *Botrytis*-induced ethylene production, mutation of *ACS2* and *ACS6*, two genes encoding downstream substrates of MPK3/MPK6, also reduced *B. cinerea*-induced ethylene production in Arabidopsis. In addition, we found that other ACS isoforms were also involved and may be regulated by a MAPK-independent pathway."
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P34051 Transcriptional Control of The *Arabidopsis* Cytokinin Signaling Networks
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"Cytokinins regulate many different developmental and physiological processes in plants, such as cell division, root and shoot growth, chloroplast development, and leaf senescence. Cytokinin signal transduction is mediated by a multi-step phosphorelay that culminates in transcriptional regulation by the type-B response regulators (type-B ARR family). A type-B *ARR* triple mutant (*arr1,10,12*) shows a substantially reduced response to cytokinin based on physiological and molecular analysis. Based on genome-wide analysis, we identified 71 genes induced 3-fold or more in cytokinin-treated wild-type shoots. In the *arr1,10,12* mutant, expression for the vast majority of cytokinin-regulated genes is severely attenuated. Many of the genes whose cytokinin-induced expression is affected in the *arr1,10,12* mutant represent additional transcription factors, suggesting that the type-B ARRs act at the head of a transcriptional cascade to regulate the cytokinin response. Some of these **C**ytokinin-regulated **T**ranscription **F**actors (*CTFs*) are differentially expressed in the root and shoot, suggesting that transcriptional cascades are regulated spatially by cytokinin. Time-course analysis suggests additional temporal regulation of *CTF* expression also exists. "
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P34052 Physiological and Transcriptome Analysis of 1-MCP Inhibition of Adverse Ethylene Responses in Cotton
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"The phytohormone ethylene can cause adverse effects in plants, including inhibition of shoot elongation and abscission of leaves, flowers and fruits. 1-MCP is a competitive inhibitor of ethylene binding with the ethylene receptors and prevents ethylene responses. In this study, a flow-through controlled growth system was developed that integrated analyses of leaf declination, shoot elongation and ethylene-responsive gene expression. The optimal parameters of ethylene treatment in eliciting responses, and of 1-MCP treatment in preventing these responses, were determined. 1-MCP was proven to be very effective in preventing inhibition of shoot elongation and gene expression changes induced by ethylene. The effectiveness of gaseous 1-MCP delivery and solution-based foliar application was compared and it was found that the two methods were able to produce similar results. Global gene expression profiling of ethylene and 1-MCP treated tissues are providing insights into the associated molecular mechanisms."
(a) Department of Soil and Crop Sciences, Texas A&M University, Texas AgriLife Research

P34053 Characterization and functional analysis of strigolactone-related branching genes in maize
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"Strigolactones are a newly discovered class of branch inhibitory hormones. To understand their role in maize development, we have begun an investigation on the orthologous genes involved in strigolactone biosynthesis and perception in the maize genome. ZmCCD7 (MAX3) and ZmCCD8 (MAX4) are encoded by single copy genes, whereas ZmMAX1 and ZmMAX2 are encoded by at least two genes. Expression of ZmCCD7 in a β-carotene accumulating *E. coli* strain indicated that ZmCCD7 encodes a functional 9,10 CCD. Overexpression of ZmCCD7 and ZmCCD8 separately in *Arabidopsis max3* and *max4* mutants also can rescue their bushing phenotype. Yeast two-hybrid analysis shows that ZmCCD7 and ZmCCD8 do not physically interact with each other, though they presumably are both plastid-localized and potentially catalyze sequential carotenoid cleavage reactions. Expression profiles of branching pathway genes in B73 seedlings showed detection of mRNA's in every tissue tested. In mature tissues, the expression levels of *ZmCCD7* and *ZmCCD8* in nodes bearing lateral buds are higher than in internode tissues. Also, the expression level of *ZmCCD8* and *ZmMAX1a* in shank and ear are higher than in other tissues and coincident with the *ZmTB1* expression pattern, which correlates with suppression of branch growth. During grain development, strigolactone biosynthetic genes were detected by 29 day-after-pollination and specifically in embryo. Furthermore, we found that a short pulse of light treatment is enough for induction of *ZmCCD7* and *ZmCCD8* in etiolated B73 seedlings. In addition, they are also induced by increasing planting density. These results indicate that strigolactone-related branching genes are transcriptionally regulated by endogenous and environmental stimuli controlling bud outgrowth in maize. "
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P34054 Interplay of ABA and GA modulates transcriptome profiles and the developmental fate of maize embryos
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"Developmental processes are often controlled by the interplay of positive and antagonistic, or modulating signaling pathways. Our work concerns the interaction of abscisic acid (ABA) and gibberellic acid (GAs) signaling pathways that initiate and maintain embryo maturation in maize. Bioactive

GAs accumulate in young maize embryos, and then decline prior to the ABA peak that initiates maturation phase. The role of ABA in maize maturation is well-known -- ABA-deficient kernels are viviparous and desiccation-sensitive. However, maize embryos deficient in both ABA and GA exhibit the wild-type phenotypes of quiescence and desiccation tolerance, suggesting that these hormones are antagonistic signals that jointly govern the maturation of cereal seeds. To examine the interaction of these hormones at the level of gene expression, we made a transcription profile of developing maize embryos that compared four genotypes: wild-type (WT), GA-deficient (GA-) ABA-deficient (ABA-), and ABA-GA- double mutants. The level of expression of maturation-phase genes was hugely reduced in ABA- mutants but was restored to almost wild-type levels in the double hormone mutant. However, the temporal pattern of expression is very different from the early, rapid increase in transcript level that is seen in wild-type. Instead, double mutants show a surge in maturation transcript accumulation late in seed development (after both GA and ABA have fallen to basal levels in wild-types). This suggests that the direct interaction of signaling pathways does not account for the double mutant effect, and it implies that in addition to the ABA signal initiating maturation phase, there is a later cue that maintains high transcript levels in the absence of the ABA signal."
(a) Oregon State University (b) University of Florida

P34055 "A Novel Class of Gibberellin 2-Oxidases Control Semidwarfism, Tillering, and Root Development in Rice"
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"Gibberellin 2-oxidases (GA2oxs) regulate plant growth by inactivating endogenous bioactive gibberellins (GAs). Two classes of GA2oxs inactivate GAs through 2β-hydroxylation: a larger class of C_{19} GA2oxs and a smaller class of C_{20} GA2oxs. In this study, we show that members of the rice (*Oryza sativa*) GA2ox family are differentially regulated and act in concert or individually to control GA levels during flowering, tillering, and seed germination. Using mutant and transgenic analysis, C_{20} GA2oxs were shown to play pleiotropic roles regulating rice growth and architecture. In particular, rice overexpressing these GA2oxs exhibited early and increased tillering and adventitious root growth. GA negatively regulated expression of two transcription factors, *O. sativa* homeobox 1 and TEOSINTE BRANCHED1, which control meristem initiation and axillary bud outgrowth, respectively, and that in turn inhibited tillering. One of three conserved motifs unique to the C_{20} GA2oxs (motif III) was found to be important for activity of these GA2oxs. Moreover, C_{20} GA2oxs were found to cause less severe GA-defective phenotypes than C_{19} GA2oxs. Our studies demonstrate that improvements in plant architecture, such as semidwarfism, increased root systems and higher tiller numbers, could be induced by overexpression of wild-type or modified C_{20} GA2oxs."
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P34056 Analyzing protein phosphatase 2A regulation of ethylene production
Skottke, Kyle R-presenter Kyle_Skottke@Brown.edu(a) DeLong, Alison (a)
"Reversible protein phosphorylation is tightly regulated and controls myriad cellular functions. Protein phosphatase 2A (PP2A) is an abundant Ser/Thr phosphatase. PP2A complexes encompass an array of unique heterotrimeric holoenzymes comprised of regulatory A and B subunits and a catalytic C subunit. An Arabidopsis PP2A mutant, *rcn1*, exhibits a pleiotropic phenotype and a two-fold reduction in PP2A activity. Interestingly, dark-grown *rcn1* seedlings fail to down-regulate production of the gaseous hormone ethylene. Ethylene regulates many processes throughout plant development including inhibition of hypocotyl elongation in dark-grown seedlings. As a result of ethylene overproduction, dark-grown *rcn1* seedlings have short hypocotyls. In addition to tight transcriptional control of ethylene biosynthetic enzymes, ethylene production is largely regulated through phosphorylation-dependent stabilization of the rate-limiting enzyme ACC Synthase (ACS). Multiple kinases have been implicated in ACS phosphorylation, including members of Mitogen Activated Protein Kinase (MAPK) signaling networks and an unidentified Calcium Dependent Protein Kinase (CDPK). In contrast, little is known about the molecular details of phosphatase regulation in ethylene production. Previous work clearly demonstrates that PP2A regulates ethylene biosynthesis but the molecular mechanism has yet to be elucidated. We have used biochemical and inhibitor analysis to characterize the effect of PP2A on ethylene biosynthesis. In addition, multiple mutant and inhibitor analyses indicate that PP2A regulates ethylene production through a pathway independent of the previously described regulator *ETO1*. This work is supported by NSF award IOS-0846282"
(a) Brown University

SESSION P35 – INTRACELLULAR SIGNALING

P35001 "Like heat, L-N^G-monomethyl arginine (L-NMMA), an inhibitor of arginine-dependent nitric oxide (NO) production, blocks auxin signaling for gene expression and interferes with hormone-dependent cell expansion and division in cultured *Nicotiana glauca* guard cell protoplasts (GCP)."
Tallman, John G.-presenter gtallman@willamette.edu(a) Bufford, Jennifer L. (b) Anderson, David J. (a) Beard, Robert (a)
"At 32°C, an auxin, 1-naphthaleneacetic acid (NAA) and a cytokinin, 6-benzylaminopurine (BAP) cause cultured GCP to expand 20-30 fold, deposit cell walls, re-enter the cell cycle and divide. Both NAA and BAP are required for GCP to survive in high percentages (50-80%) at 32°C. After 9-12 h at 38°C GCP survive in the same high percentages as at 32°C but neither NAA nor BAP is required. GCP expand only 5-6 fold and neither deposit cell walls nor make the G1-to-S cell cycle transition. Transient gene expression analyses with thermostable *mGFP*-based reporters indicate that heat suppresses activation of the auxin-responsive BA promoter in cultured GCP, but not *mGFP* expression driven by the CaMV 35S constitutive promoter. Here we report that at the normally permissive temperature of 32°C L-NMMA mimics the effects of sustained heat on GCP. Like heat, L-NMMA limits cell expansion; prevents cell wall deposition; eliminates the survival requirement for NAA and BAP; prevents the G1-to-S transition; and suppresses BA promoter activation (but not 35S CaMV promoter activity). These data suggest that at 32°C arginine-dependent NO production is required for auxin signaling for gene expression and hormone-dependent cell expansion and cell division in cultured GCP."
(a) Department of Biology, Willamette University (b) Department of Botany, University of Hawaii

P35002 THE GmNAC6 TRANSCRIPTION FACTOR INDUCES CELL DEATH AND MAY ACT DOWNSTREAM TO NRPS IN THE ER STRESS-INDUCED PCD SIGNALING
Faria, Jerusa AQA-presenter jerusa.faria@ufv.br(a) Reis, Marco Tulio B (a) Fietto, Luciano G (a) Fontes, Elizabeth PB (a)
"The GmNAC6 gene is a member of the NAC transcription factor gene family in soybean. Transient localization studies with YFP-GmNAC6 fusion protein revealed its nuclear localization. Expression of GmNAC6 is induced by cycloheximide, a potent inducer of cell death in soybean suspension cells and repressed by senescence inhibitors, BAP and zeatin. GmNAC6 gene expression is also induced by wounding and cell wall degrading enzymes in systemic leaves. We have used a transient expression assay to investigate the role of GmNAC6 in cell death. Transient expression of GmNAC6 in planta caused leaf yellowing, chlorophyll loss, induction of the senescence marker gene CP1 and hypersensitive response marker genes. Collectively,

these results indicate that GmNAC6 is functionally involved in programmed cell death processes. Transcript levels of the GmNAC6 gene are rapidly induced by tunicamycin treatment, an ER stressor that promotes accumulation of unfolded proteins in the organelle. We have recently demonstrated that prolonged ER stress promotes cell death and apoptotic-like responses through activation of N-rich proteins (NRP-A and NRP-B). To investigate whether GmNAC6 was connected to the ER signaling pathway that transduces a PCD signal via NRPs, we examined the effect of transient expression of NRPs on GmNAC6 transcript accumulation in soybean protoplasts and in planta. We found that transient expression of NRPs promoted a 15-fold induction in GmNAC6 expression. Furthermore, NRPs and GmNAC6 share similar expression profile in response to different stimuli, indicating that GmNAC6 may act downstream to NRPs in the ER stress-induced PCD signaling. We are currently isolating the GmNAC6 promoter to evaluate directly promoter activation mediated by NRPs. "
(a) Universidade Federal de Vicosa

P35003 Nuclear β-amylases regulate gene expression in arabidopsis
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"Plant growth is tightly linked to carbon assimilation, its storage and distribution between tissues. However, the underlying regulatory mechanisms that control carbon allocation and growth remain unclear. In addition to their roles as metabolites and building blocks for growth, sugars such as glucose and sucrose have been shown to act as signalling molecules, and cross-talk between sugar- and hormone-signalling has been proposed. During the day plants store a fraction of their assimilated carbon transiently as starch in chloroplasts. This starch serves as a source of carbohydrate during the subsequent night to sustain respiration, growth and development. Maltose, the major product from transitory starch degradation, is released from starch by chloroplastic β-amylases and exported to the cytosol. Here we would like to introduce a novel concept of β-amylase-like proteins as regulators of gene expression. Our work focuses on two Arabidopsis β-amylases which carry an N-terminal DNA-binding domain also found in a family of transcriptional regulators involved in brassinosteroid signalling. These proteins are nuclear and deregulation results in altered plant growth and development. We provide evidence that these two proteins bind to a specific DNA motif in the promoters of genes and act as activators of transcription. The target motif is also found in promoters of brassinosteroid responsive genes. We suggest that these β-amylase-like proteins serve as maltose sensors, providing a regulatory link between night-time carbon availability and growth control. The possibility of cross-talk between hormone signalling and transitory starch metabolism will be discussed."
(a) Institute of Plant Sciences, ETH Zurich, 8092 Zurich, Switzerland (b) Department of Biology, James Madison University, Harrisonburg, VA 22801, USA

P35004 Signaling function of the Arabidopsis heterotrimeric G protein β and γ subunits during the oxidative and ER stress responses
Iwata, Yuji-presenter yui2@psu.edu(a) Narendra, Savitha (a) Fedoroff, Nina V. (a)
"The *Arabidopsis thaliana* genome encodes a single canonical heterotrimeric G protein α and β subunit and two γ subunits. There is growing recognition that the Gβ and Gγ subunits, often referred to as the Gβγ dimer, function as a single entity independent of its function in the Gαβγ heterotrimer. Using *Arabidopsis thaliana*, we reported that the Gα and Gβ subunits serve both separable and synergistic functions in signaling by reactive oxygen species in the oxidative stress response. We showed that plants homozygous for a null mutation of the Gβ subunit are substantially more resistant to endoplasmic reticulum (ER) stress-induced cell damage and death than either wild-type plants or plants homozygous for a null mutation of the Gα subunit. These observations support our previous conclusion that the G protein subunits serve different functions during some stress responses in plants. To elucidate the signaling function of the *Arabidopsis* Gβγ dimer during the oxidative and ER stress responses, we are investigating the effect of these stresses on the subcellular localization of the Gβγ dimer. Since the Gβγ dimer exerts the signaling function through its downstream target proteins, we are also in attempting to identify Gβγ-interacting proteins by using immunopurification and mass spectrometry."
(a) Huck Institutes of the Life Sciences, Pennsylvania State University

P35005 A J domain protein that physically interacted with ARC1 is involved in pollination response in *Brassica* stigma
Lan, Xing-guo-presenter lanxingguo1979@nefu.edu.cn(a) Li, Yu-hua (a) Kawabata, Saneyuki (b)
"Self-incompatibility (SI) is a physiological strategy of many flowering plants to prevent inbreeding. In *Brassica*, the *S*-haplotype specific interaction between the pollen-borne SP11/SCR and the stigmatic SRK receptor activates an intracellular signaling pathway in the stigmatic papilla cell. Arm repeat containing 1 (ARC1), a stigmatic U-box protein with E3 ubiquitin ligase activity, functions as a positive mediator of SI signaling. Upon self-pollination, ARC1 is activated by SRK and then promotes the ubiquitination and degradation of unknown compatibility factors in the pistil, which in turn results in pollen rejection. To determine potential targets of ARC1, we used yeast two-hybrid library screening to identify stigmatic proteins that interact with ARC1. JDP1, a J domain-containing protein, was indentified and interacted specifically with ARC1 but failed to interact with two different *Arabidopsis* U-box E3 ligases, AtPUB14 and AtPUB17. The interaction between ARC1 and JDP1 was confirmed through *in vitro* pull down assays and subcellular colocalization analysis. Domain-mapping studies revealed this interaction was mediated by the C-terminal region of JDP1. In pollination, the ubiquitination level of stigma proteins was increased substantially after incompatible pollination compared with compatible pollination. However, the JDP1 protein level of stigmas is noticeable reduced after incompatible pollination compared with compatible pollination, and had a high peak within 45-60 min after compatible pollination, suggesting an important role of JDP1 in compatible pollination. Taken together, these data suggest that JDP1 interacted with ARC1 is involved in pollination response in *Brassica*. "
(a) College of Life Sciences, Northeast Forestry University (b) Graduate School of Agricultural and Life Sciences, University of Tokyo

P35006 Identification of new ABI1-mediated ABA signaling components in Arabidopsis.
Nishimura, Noriyuki-presenter nonishi@ucsd.edu(a) Sarkeshik, Ali (b) Nito, Kazumasa (c) Park, Sang-Youl (d) Wang, Angela (a) Lee, Stephen (a) Cutler, Sean (d) Chory, Joanne (c) Yates, John R (b) Schroeder, Julian I (a)
"Abscisic acid (ABA) regulates physiologically important stress and developmental responses. ABI1 encodes a protein phosphatase 2C that functions in early in ABA signaling. To address the mechanism of ABI1-mediated ABA signaling, we generated tagged ABI1 Arabidopsis expression lines in an abi1 knockout mutant and performed affinity column purification of ABI1-associated proteins. Transgenic tagged ABI1 plants show a strong ABA insensitive phenotype at the seed germination root elongation and stomatal response similar to abi1-1. After silver staining, visible bands overlapped with controls, and specific bands associated with purified tagged ABI1 samples were consistently observed. Mass-spectrometrical analyses allowed identification of proteins associated with ABI1. These included some known ABA signaling components. These results suggested that our strategy has the potential of identifying ABA signaling components. We found that a sub-group of gene family members interacted with ABI1 in an ABA dependent manner. The functional relationship between ABI1 and this protein family is being characterized."
(a) University of California, San Diego (b) The Scripps Research Institute (c) SALK Institute (d) University of California, Riverside

P35007 Identification of nucleotidyl cyclase activity of a unique membrane receptor and its role upstream from Ca channel activation in pathogen response intracellular signaling

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"Influx of Ca into cells initiates numerous plant intracellular signaling cascades. Few if any proteins and molecular mechanisms acting upstream of Ca elevation in these signaling cascades are known. We provide a new model for such a signaling system. A family of Arabidopsis peptides (AtPeps) are endogenous amplifiers of innate immune responses. Their plasma membrane receptor, the leucine-rich receptor-like kinase AtPepR1 is involved in pathogen response signaling in an unknown manner. Cytosolic Ca elevation is an early and paramount signal in innate immune responses. How nonself perception is linked to this Ca signaling is unknown. The Arabidopsis dnd1 mutant lacks a cyclic nucleotide activated Ca channel. Pathogen activation of Ca currents and signaling downstream from cytosolic Ca elevation involved in immune responses is impaired in this mutant. Here we show that recombinant affinity-purified AtPepR1 protein has nucleotidyl cyclase activity. Patch clamp studies show AtPeps activate an inward Ca current in mesophyll cells from wild type (WT) but not atpepr1 null mutant plants. AtPep application to WT leaves initiates a cytosolic Ca rise that does not occur in either the atpepr1 or dnd1 mutant. Pathogen-dependent cytosolic Ca elevation is also impaired in atpepr1 mutants. These results link this receptor protein to intracellular Ca signaling during pathogen responses through cyclic nucleotide generation, which activates a cyclic nucleotide gated Ca conducting channel. The resulting cytosolic Ca elevation is a critical secondary message initiating a cascade of intracellular pathogen defense responses. This is the first report linking cyclic nucleotides, Ca, and specific receptor and channel proteins in a plant signaling cascade. Supported by NSF award 0844715."

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P35008 Identification of CO_2-binding Proteins that Function as Upstream Mediators of CO_2-Induced Stomatal Movements

Hu, Honghong-presenter hohu@biomail.ucsd.edu(a) Boisson-Dernier, Aurelien (a) Israelsson-Nordstrom, Maria (a) Bohmer, Maik (a) Rie, Amber (a) Schroeder, Julian (a)

"Guard cells form stomatal pores in the plant epidermis that allow CO_2 influx for photosynthesis and transpirational water loss from plants. The continuing rise in atmospheric CO_2 causes closing of stomatal pores in leaves and thus globally regulates CO_2 influx into plants and plant water use efficiency, which increasing leaf heat stress. However, the CO_2 sensing mechanisms that control this CO_2 response remain unknown. Moreover, the cell type that responds to CO_2, mesophyll cells or guard cells, and whether photosynthesis mediates this response remain matters of debate. Here, we show that *Arabidopsis* mutant plants in leaf-expressed CO_2 binding proteins (CO_2 Responsive Proteins: CORP) display strongly impaired CO_2-regulation of gas exchange and CO_2-regulated stomatal movements, but retain functional abscisic acid and blue light responses. Data will be presented demonstrating whether CORP-mediated stomatal CO_2 signaling is linked to photosynthesis or not and which leaf cell type mediates this response. Furthermore, cell type targeted over-expression of *CORP* in wild-type plants greatly enhances water use efficiency. These findings, together with epistasis and biochemical CO_2 response analyses demonstrate that CORP functions early in the stomatal CO_2 signaling pathway that controls gas exchange between plants and the atmosphere. "

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P35009 Calcium/Calmodulin: The Grand Conductor of Signal Orchestration in Plants

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http://molecularplants.wsu.edu/calcium

"The central role of calcium/calmodulin in plant growth and response to external stimuli is well recognized. However, only recently the underlying mechanisms are beginning to be understood. Recently, our laboratory has documented the unique roles of three intriguing calcium/calmodulin-regulated proteins DWF1, CCaMK and AtSRs. DWF1 catalyses an early step in brassinosteroid biosynthetic pathway. Our results have revealed that calcium/calmodulin regulates the function of DWF1 (1). CCaMK, first introduced to the scientific community by our team in 1995 (2), has unique structural features and is subject to two steps of calcium regulation. It is now established as a key signal component in both bacterial and fungal symbioses. We have shown that the release of autoinhibition/CaM-binding from CCaMK is a central switch that is sufficient to activate nodule morphogenesis (3). AtSRs are a family of transcription factors involved in plant response to multiple stress signals (4, 5). We recently reported that calcium/calmodulin-regulated AtSR1 is a negative regulator of salicylic acid-mediated plant immunity revealing a novel mechanism connecting calcium/calmodulin and salicylic acid signaling (6). These studies provide new strategies to optimize plant growth and development under varying conditions. Supported by NSF and USDA. ***1***. *Du, L and Poovaiah, B.W., Nature 437:741-745, 2005.* ***2***. *Patil, S., Takezawa, D. and Poovaiah, B.W., PNAS 92: 4897-4901, 1995.* ***3***. *Gleason, C., Chaudhuri, S., Yang, T., Munoz-Gutierrez, A., Poovaiah, B.W. and Oldroyd, G.E.D., Nature 441:1149-1152, 2006.* ***4,5***. *Yang, T and Poovaiah, B.W., JBC 275:38467-38473, 2000; JBC 277:45049-45058, 2002.* ***6***. *Du, L., Ali, G. S., Simons, K. A., Hou, J., Yang, T., Reddy, A.S.N. and Poovaiah, B. W., Nature 457:1154-1158, 2009.*"

(a) Washington State University

P35010 "Activation of MKK9 induces ethylene and camalexin biosynthesis, and enhances sensitivity to salt stress in *Arabidopsis*"

Xu, Juan (a) Li, Yuan (a) Wang, Ying (a) Liu, Hongxia (a) Lei, Lei (a) Yang, Hailian (a) Liu, Guoqin (a) Ren, Dongtao-presenter ren@cau.edu.cn(a)

"Mitogen-activated protein kinase (MAPK) cascades play important roles in regulating plant growth, development and responses to various environmental stimuli. We demonstrate that MKK9, a MKK, is an upstream activator of the MPKs MPK3 and MPK6, both in vitro and in planta. Expression of active MKK9 protein in transgenic plants induces the synthesis of ethylene and camalexin through the activation of the endogenous MPK3 and MPK6 kinases. As a consequence, transcription of multiple genes responsible for ethylene biosynthesis, ethylene responses, and camalexin biosynthesis are coordinately up-regulated. The activation of MKK9 inhibits hypocotyl elongation in the etiolated seedlings. MKK9-mediated effects on hypocotyl elongation were blocked by the ethylene biosynthesis inhibitor, AVG, and ethylene receptor agonist, Ag+. Expression of active MKK9 protein enhances the sensitivity of transgenic seedlings to salt stress, while loss of MKK9 activity reduces salt sensitivity indicating a role for MKK9 in the salt stress response. The results reported here reveal that the MKK9-MPK3/MPK6 cascade participates in the regulation of the biosynthesis of ethylene and camalexin, and may be an important axis in the stress responses of Arabidopsis. This work was supported by NSF China (30721062, 30870220) to D. Ren, NSF China (30771124) to H. Yang. "

(a) China Agricultural University

P35011 Structural Basis of *Arabidopsis* Receptor for Activated C Kinase 1 (RACK1) scaffold protein mediated protein-protein interactions: Functional insights into the role of post-translational modifications.

Ullah, Hemayet-presenter hullah@howard.edu(a) Dozier, Uvetta (a) Kundu, Nabanita (a) Fennell, Herman (a) Haidara, Moulaye (a)

http://www.biology.howard.edu/Faculty/FacultyBios/Ullah.htm
"Scaffold proteins are uniquely poised to integrate signals from multiple pathways by bringing interacting signaling components to proximity. Scaffold protein RACK1 in metazoan mediates diverse signaling pathways and is reported to interact with more than ninety diverse proteins. Loss-of-function alleles of three *rack1* genes in *Arabidopsis* implicate the proteins in diverse environmental stress signaling pathways. Despite the functional conservation of RACK1-mediated protein protein interaction regulated signaling modes; the structural basis of such interactions is largely unknown. In this effort, the first crystal structure of a RACK1 protein, RACK1 isoform A from *Arabidobsis*, at 2.4 A^{o} resolution is deduced. The structure shows the highly conserved surface residues that could play critical roles in protein protein interactions and reveals the surface location of proposed post-transcriptionally modified residues. Site-directed mutagenesis studies in the potential tyrosine phosphorylation sites and sumoylation sites reveal functional role of these sites in water stress responses. Earlier, through Split-ubiquitin based cDNA library screen we have identified dozens of putative *Arabidopsis* RACK1 interacting proteins from diverse stress pathways. The availability of this structure provides a structural basis for dissecting RACK1-mediated cellular signaling mechanisms in diverse environmental stress signaling pathways."
(a) Howard University, Biology Department

P35012 Calcium-dependent regulation of abiotic stress responses and ion transport processes by CBL calcium sensor protein / CIPK-type protein kinase complexes
Held, Katrin (a) Eckert, Christian (a) Waadt, Rainer (a) Batistic, Oliver (a) Kudla, Joerg-presenter jkudla@uni-muenster.de(a)
"Intracellular releases of calcium ions belong to the earliest events in signal perception by plant cells. Calcineurin B-like proteins (CBLs) represent a group of calcium sensor proteins likely to function in deciphering calcium signals. CBLs interact with a group of serine/threonine protein kinases designated as CBL-interacting protein kinases (CIPKs). In Arabidopsis, 10 CBL-type calcium sensor proteins form an interaction network with 26 CIPKs. Preferential complex formation of individual CBLs with defined subsets of CIPKs appears to be one of the mechanisms generating the temporal and spatial specificity of calcium signals in plant cells. Reverse genetics approaches have begun to unravel the function of several members of both protein families. We will present results of our characterization of cbl and cipk loss-of-function mutants and of our investigation of the sub-cellular localization of all CBLs from Arabidopsis. These studies suggest that CBL/CIPK complexes function predominantly at cellular membranes and can decode Ca^{2+} signals in different compartments. In this context, dual lipid modification by myristoylation and palmitoylation appears to play an important role in determining the membrane targeting of CBL/CIPK complexes. Our reverse genetics analyses indicate that alternative complex formation of CIPK-type kinases with different CBLs enables the simultaneous regulation of ion transport processes at different compartments of the plant cell. We will provide examples for CBL/CIPK complexes contributing to regulating the extrusion of Na^{+} ions in root tissues and the sequestration of Na^{+} into the vacuole in green tissues as well as for CBL/CIPK complexes regulating K^{+} channels by novel mechanisms. "
(a) Universitaet Muenster, Institut fuer Botanik

P35013 The BAK1-FLS2 receptor complex: Dynamics of heteromerization and phosphorylation in response to flagellin perception
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"PAMP-triggered innate immunity, the first line of plant defense, is activated by recognition of pathogen associated molecular patterns (PAMPs). These are perceived by highly specific receptors at the plasma membrane, such as the flagellin receptor FLS2 (Flagellin sensing 2) [1]. Previously, we demonstrated that BAK1 interacts with FLS2 after binding of the ligand flg22 and is required for activation of physiological responses [2]. In the present work, we refine our kinetic analysis of receptor heteromerization and show that FLS2 can interact with BAK1 within less than 1 s after stimulation. While FLS2 is responsible for ligand binding, the kinase domains of both FLS2 and BAK1 are believed to be activated by phosphorylation leading to cellular signal transduction. Using in vivo labeling with [33P]phosphate, we characterized de novo phosphorylation events on FLS2 and BAK1 and followed the stability of the phosphorylated proteins over time. In Arabidopsis cell cultures both, FLS2 and BAK1, are phosphorylated within 15 s of treatment with flg22. Thus, de novo phosphorylation within this receptor complex clearly precedes activation of other signaling steps involved in the induction of innate immune responses. Funding by the SNF (31003A-120655 and 31003A-105852) and a post-doctoral grant from the Deutsche Akademie der Naturforscher Leopoldina (BMBF-LPD 9901/8-152) to BS are gratefully acknowledged. [1] Boller T., Felix G. 2009. Annu. Rev. Plant Biol. 60:379-406 [2] Chinchilla D., Zipfel C., Robatzek S., Kemmerling B., Nurnberger T., Jones J. D., Felix G., Boller T. 2007. Nature. 448:497-500. "
(a) Botanical Institute, University of Basel, Switzerland (b) Institute of Plant Biochemistry, ZMBP, University of Tuebingen, Germany

P35014 Calcium-dependent protein kinases: substrate specificity and pollen tube growth and tropism
Harper, Jeffrey F-presenter jfharper@unr.edu(a) Curran, Amy (a) Barron, Yoshimi (a,d) Myers, Candace (a,d) Romanowsky, Shawn (a) Zinn, Kelly (a) Azuse, Corinn (a) Chang, Ing-Feng (a,c) Harmon, Alice (b)
"While all eukaryotes use calcium (Ca^{2+}) as a signaling molecule, there is considerable diversity in how Ca^{2+}-signals are created and decoded. In a model plant, *Arabidopsis thaliana*, the 67 kinases implicated in Ca^{2+}-signaling are not present in animal systems. Of these, 34 belong to a family of Ca^{2+}-dependent protein kinases (CPKs), which are defined by a unique structure in which a calmodulin-like domain is fused to the kinase domain. In a systematic analysis of the 12 different isoforms expressed in mature pollen, we discovered that a double mutation of *CPK17* and *CPK34* results in pollen tubes with reduced growth rates and a defect in tropism. Pollen transmission is reduced by approximately 350-fold. These defects can be rescued through expression of a transgene encoding CPK34-GFP. To begin identifying potential substrates, we used an *in vitro* kinase assay to survey more than 500 potential peptide substrates using 5 different CPK isoforms. Each of the 5 CPKs tested showed distinct preferences for different substrates. While CPK34 showed strong phosphorylation of 99 different peptides, CPK16 only recognized 3. We found that the restricted substrate specificity of CPK16 could be modified to be more like CPK34 by engineering four CPK34-like substitutions into the kinase domain. The engineered substrate specificity change was found to enable CPK16-(modified) to partially rescue the *cpk17/34* pollen transmission defect. These studies provide *in vitro* and *in planta* evidence that differences in substrate specificity among CPKs is a critical feature contributing to their diversity of biological functions."
(a) U of Nevada, Reno (b) U of Florida, Gainesville (c) National Taiwan University, Taipei, Taiwan (d) U of Arizona, Tucson

P35015 ABA Receptors? Not Sure. Signaling Molecules? Yes.
Lin, Wan-Chi-presenter linwc@mail2000.com.tw(a) Huang, Kuan-ying (a) Lu, Yung Yu (a) Ho, Tuan-hua David (a,b)
"Several putative ABA receptors have been reported in recent years, however the validity of some of these reports has been seriously questioned (e.g. McCourt and Creelman [2008], Curr Opin Plant Biol, 11:474; Risk et al [2008] Nature, 456:E5&−6). We have investigated the role of these proteins in the cereal aleurone tissue where well-defined ABA responses can be analyzed with precision. Three established signaling pathways exist in this tissue: ABA induction of LEA genes, and GA induction & ABA suppression of α-amylase. ABA induction of LEA genes is enhanced by over-expression of either GCR2 or ABAP1, but significantly suppressed by GCR2 RNAi or ABAP1 RNAi. However, the other two signaling pathways, i.e. GA induction & ABA suppression of α-amylase, are not affected at all by either GCR2 and ABAP1 over-expression or their RNAi. Furthermore, the

suppression effect of GCR2 RNAi can be overcome by over-expression of ABAP1, suggesting that ABAP1 works downstream from GCR2. Following a similar approach, we have determined that both GCR2 and ABAP1 work downstream from a plasma membrane-localized receptor kinase, RPK1, which has been shown to be an important signaling molecule for ABA action. The GCR2-GFP fusion protein is localized in the cytoplasm with a punctate pattern near plasma membrane. The ABAP1-GFP protein is initially localized in the cytoplasm with a punctate pattern more pronounced than that of GCR2-GFP. Upon prolonged incubation, the ABAP1-GFP is preferentially localized in the nucleus. Based on these observations, we propose that these regulatory molecules work in the sequence of RPK1 (plasma membrane) → GCR2 (cytoplasm) → ABAP1 (cytoplasm to nucleus) → ABI5 (nucleus) → LEA expression. "

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P35016 A Receptor-like kinase interacts with RopGEF1 and regulates defense-related responses in *Arabidopsis thaliana*

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"Rac/Rop GTPases act as molecular switches in many important signaling pathways. Functionally, Rac/Rop signaling has been implicated in many aspects of plant growth and development, including polar cell expansion, hormonal signaling and responses to biotic and abiotic stress. Rac/Rop activation is mediated by guanine nucleotide exchange factors, referred to as GEFs, which stimulate their exchange of GDP for GTP. GEF1 is a highly phosphorylated protein whose promoter GUS is broadly expressed throughout plant development, including the primary root, root hairs, lateral root tips, and leaf pavement cells. In order to investigate the role of GEF1 in Rac/Rop signaling pathways, both T-DNA insertion mutants and transgenic plants over-expressing GEF1 are being analyzed. To identify regulators and/or effectors of GEF1, the yeast two-hybrid system was utilized. GEF1 was used as the bait protein to screen for potential protein interacting partners. A novel interaction was discovered between GEF1 and a receptor-like kinase (referred to as RLK1). GEF1 and RLK1 physically interact when expressed in leaf protoplasts. They also appear to act synergistically to regulate defense response-related gene expression. Finally, studies to examine how loss of function *rlk1* mutants and RLK1 over-expression transgenic plants are affected in their pathogen response are underway. Results that implicate a role for RLK1 in Rac/Rop mediated stress-related signaling will be presented. "

(a) University of Massachusetts, Amherst/Molecular and Cellular Biology Department

P35017 "Characterization of *sac9*, a phosphoinositide phosphatase mutant of *Arabidopsis thaliana* involved in phospholipid signaling; early development and ultrastructure of primary roots"

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"Primary root development and ultrastructure of *sac9*, a phosphoinositide phosphatase mutant of *Arabidopsis thaliana* involved in phospholipid signaling, were investigated. Observations of the development of *sac9* for two weeks indicate that the mutant grew shorter primary roots than wild type and ceased to grow midway during the experiment, whereas wild type showed an almost continuously linear growth. When transferred to media containing NaCl the growth kinetics changed differentially. Mild and moderate salt stress promoted primary root elongation in the mutant but did not affect wild type. Severe salt stress inhibited root elongation in both the mutant and wild type. Morphological studies revealed differences in cell wall structure and shape between the mutant and wild type and a higher presence of agranular membrane inclusions in the mutant. These membrane inclusions or whorls are similar to those observed in invertebrates that have been treated with drugs, such as promazine. They are also found in cells that produce surfactants, such as lung cells of vertebrates. Our studies of the developmental and morphological differences are important since they will serve as a base for future investigations designed to determine the spatio-temporal localization and function of Sac9 and its substrate phosphatidylinositol 4,5-bisphosphate under normal and saline growth conditions in *A. thaliana*."

(a) Utah State University

P35018 Root cell shape is altered in T-DNA knockout Eukaryotic Elongation Factor One Alpha *Arabidopsis thaliana* seedlings

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"Eukaryotic Elongation Factor One Alpha (eEF1A) is a GTP binding protein involved in protein synthesis and degradation, binding actin and microtubules, and several different signal transduction pathways in the cell. The regulation of eEF1As many functions is not understood. *Arabidopsis thaliana* has four eEF1A genes forming a small family. We have taken a loss of function approach to gain a better understanding of the importance and functions of each eEF1A family member. To accomplish this task we identified forty T-DNA knockout lines containing a T-DNA insertion in the promoter, coding, or 3 UTR region of each eEF1A gene. Seeds were germinated on vertical MS plates under normal growth conditions and the phenotypes were noted. The most dramatic phenotype observed was a change in root morphology. Several T-DNA lines displayed a short root length compared to wild type at six days post germination. Observation of the eEF1A T-DNA seedlings primary root at twelve days post germination showed a change in cell shape. Wild type root cells were rectangular in shape whereas the eEF1A T-DNA cells were square in the region of maturation. The root phenotype was observed in T-DNA lines spanning the four genes and two different insertion locations. T-DNA knockout lines obtained for actin genes ACT1 and ACT2, and ACT7 showed a similar phenotype. The interaction between actin and eEF1A in altering root cell morphology will be discussed."

(a) Western Michigan University

P35019 "Arabidopsis Calcium Dependent Protein Kinase, CPK6 Functions in Methyl Jasmonate Signaling in Guard Cells"

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"Previous studies implied that a volatile phytohormone, methyl jasmonate (MeJA) induces stomatal closure dependent on change of cytosolic Ca^{2+} concentration in guard cells. However, details of this molecular mechanism remain far from clear. Calcium-dependent protein kinases (CDPKs) function as Ca^{2+} signal transducers in various plant physiological processes. It was suggested that Arabidopsis four CDPKs, *CPK3*, *CPK6*, *CPK4*, and *CPK11* function as positive regulators in abscisic acid (ABA) signaling in guard cells with functional redundancies between *CPK3* and *CPK6* (1) and between *CPK4* and *CPK11* (2). In guard cells, MeJA signaling is partly overlapped with ABA signaling and some signaling factors (e.g. protein phosphatase 2A, reactive oxygen species, and nitric oxide) function in both phytohormone signalings (3, 4). Here we attempted to identify CDPKs, which function in MeJA signaling in guard cells using these four CDPK gene disruption mutants. In *CPK6* gene disruption mutants, MeJA-induced stomatal closure was impaired whereas in *CPK3*, *CPK4*, and *CPK11* gene disruption mutants, MeJA-induced stomatal closure were not altered. We evaluated roles of CPK6 in MeJA regulation of second messenger production and ion channel activity in guard cells. Our results provide genetic evidence that CPK6 has a different role from CPK3 and functions as a positive regulator of MeJA signaling in Arabidopsis guard cells. (1) Mori et al (2006) *PLoS Biol* 4: e327 (2) Zhu et al (2007) *Plant Cell* 19: 3019-036 (3) Munemasa et al (2007) *Plant Physiol* 143: 1398-1407 (4) Saito et al (2008) *Plant Cell Physiol* 49: 1396-1401"

(a) Okayama University

P35020 "A Hydrophilic Ca^{2+}-binding Protein with PEVK-rich Domain, PCaP2, is Associated with Plasma Membrane via *N*-myristoylation and Interacts with Calmodulin and Phosphatidylinositol Phosphates in *Arabidopsis thaliana* "

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http://celld.agr.nagoya-u.ac.jp/Cell_Dynamics_HP_E.html

"Here we report a PCaP1-related protein named PCaP2 of *Arabidopsis*. PCaP1 (plasma membrane associated cation-binding protein-1) found a novel protein in *Arabidopsis thaliana* is localized in the plasma membrane via *N*-myristoylation and binds Ca^{2+} and Cu^{2+} (Nagasaki *et al*. 2008). PCaP2 is composed of 168 amino acids and rich in proline, glutamate, valine and lysine residues (PEVK-rich domain). PCaP2 has no transmembrane domain and a putative *N*-myristoylation motif in its N-termini. In this study, we investigated the expression profile and molecular properties of PCaP2. The promoter-GUS reporter expression analysis revealed that PCaP2 is predominantly expressed in germinating pollens and roots including root hairs. When PCaP2-GFP proteins were expressed under the control of its own promoter, green fluorescence was clearly detected on the plasma membrane in root maturation zone and root hairs. Then we examined a possibility of *N*-myristoylation by transient expression of fusion proteins with GFP in cultured cells. A normal PCaP2 linked with GFP at the C-termini was localized in plasma membrane. However, a mutated PCaP2, whose Gly2 was replaced with Ala, was detected in the cytosol. From the results, we estimate that PCaP2 binds to the plasma membrane via *N*-myristoylation at Gly2. Further characterization was done using recombinant PCaP2. PCaP2 bound Ca^{2+}, Ca^{2+}/calmodulin complex, and specific phosphatidylinositol phosphates. Interaction of PCaP2 with PtdInsPs was weakened by Ca^{2+}/calmodulin complex. We estimate that PCaP2 is involved in signal transduction in epidermal cells of roots. Wang *et al*. (2007) reported that PCaP2 is a microtubule-associated protein. We will discuss physiological meanings of PCaP2 in relation to microtubules in plant cells. "

(a) Laboratory of Cell Dynamics, Graduate School of Bioagricultural Sciences, Nagoya University

P35021 "Analysis of guard cell signaling events of *fia*, the ABA-insensitive mutant of *Vicia faba*"

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"Stoma surrounded by a pair of guard cells is an important organ that facilitates transpiration and gas exchange. Abscisic acid (ABA) that is synthesized upon drought stress induces stomatal closure hence reduces water loss through transpiration. *fia* mutant of *Vicia faba* was isolated as an easy-wilting mutant under strong light or high temperature from a field in Kagoshima, Japan, in 1997. It has been reported by Iwai et al. (*Plant Cell Physiol.* 44: 909-913, 2003) that stomata of fia mutant demonstrate ABA insensitivity. To position FIA in the ABA signal network, we examined signal events in ABA signaling in guard cells of the *fia* mutant. External Ca^{2+}, H_2O_2 and a NO donor, SNAP induced stomatal closure of *fia* mutant, while ABA-induced stomatal closure was not observed. ROS production and activation of AAPK, which are early ABA signal events in guard cell, were impaired in guard cells of fia mutant. Furthermore, inactivation of inward-rectifying K^+ channel in the plasma membrane by ABA was not observed in the mutant. Unlike ABA, methyljasmonate induced stomatal closure of *fia* mutant. These results indicate that FIA functions in early ABA signaling in guard cells."

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P35022 Role of glutathione in ABA-induced stomatal closure in *Arabidopsis thaliana*

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"ABA-induced signal cascades, which control mechanisms of stomatal closing, involve redox regulation of proteins and low-molecular-weight compounds. Glutathione (GSH), one of the most abundant low molecular-weight thiol compounds maintains redox homeostasis in plants under normal and stressful conditions. We found that guard cells accumulate more GSH that other epidermal cells do and that ABA induces stomatal closure along with decreasing GSH level but dark condition induces stomatal closure without changing GSH content. Next, in order to clarify whether GSH is involved in the redox regulation of stomatal closure in *Arabidopsis thaliana*, ABA-induced stomatal closure was studied using GSH deficient mutants, *cad2-1* and *ch1-1* which are deficient in the key GSH biosynthesis enzyme, γ-glutamylcystein synthetase (GCS), and a GSH-decreasing chemical, 1-chloro-2,4-dinitrobenzene (CDNB). The *CAD2* or *CH1* mutation, or depletion of GSH by CDNB enhanced stomatal sensitivity to ABA. Meanwhile, in *cad2-1* and *ch1-1* plants, GSH monoethyl ester (GSHmee) increased GSH level and lowered stomatal sensitivity to ABA. Neither *CAD2* nor *CH1* mutation significantly increased ABA-induced production of reactive oxygen species (ROS) in guard cells. Moreover, GSH did not affect activation of I_{Ca} currents by ABA. Taken together, these results suggest that GSH could modulate some signaling factors downstream of ROS production in ABA signaling to control ABA sensitivity in guard cells."

(a) Okayama University (b) Darul Iman University (c) Research Institute for Biological Sciences OKAYAMA

P35023 TGG1 and TGG2 redundantly function in ABA and MeJA signaling in Arabidopsis guard cells

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"Thioglucoside glucohydrolase (myrosinase, TGG) is the enzyme in glucosinolate-myrosinase system, and hydrolyze glucosinolates to toxic compounds as thiocyanate, isothiocyanate and nitrile which relate to plant defense against insect, fungi and bacteria. Six *TGG* genes are identified in Arabidopsis genome. Recently, it is shown that TGG1 and TGG2 redundantly function in defense to insect herbivory and TGG1 is abundant in guard cell (1). To clarify whether myrosinase function during stomata response, we employed *tgg1-3* and *tgg2-1* single mutants and *tgg1-3 tgg2-1* double mutants in this study. Compare to wild-type, myrosinase activity slightly decreased in *tgg1-3* single mutants and significantly decreased in *tgg1-3 tgg2-1* double mutants. ABA, MeJA and H_2O_2-induced stomatal closure were observed in wild-type, *tgg1-3* and *tgg2-1*, but failed to induce stomatal closure in *tgg1-3 tgg2-1*. Unlike ABA, MeJA and H_2O_2, Ca^{2+} induced stomatal closure in all mutants and wild-type. In addition, ABA induced ROS productions in all mutants and wild-type. Interestingly, cytosolic alkalization in guard cell were induced by ABA in all mutants and wild-type, however pH elevation in cytosol of *tgg1-3 tgg2-1* was significantly lower than wild-type. These results suggest that TGG1 and TGG2 redundantly function in response to ABA and MeJA in guard cell and work at the downstream of ROS production and upstream of cytosolic Ca^{2+} elevation in ABA and MeJA signaling in guard cells. (1) Barth C. and Jander G. (2006) Plant J. 46: 549-562 "

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P35024 Studies on Pti4 and SEBF Interaction: β-Lactamase-Based Protein Fragment Complementation Assay in Tobacco Protoplasts

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"For doing β-lactamase-based PCA, the small and monomeric enzyme was split into two fragments. The fragment 1 was fused to the amino terminal of SEBF by a 10 amino acid flexible linker $(Gly_4Ser)_2$. The fragment 2 was fused to the amino terminal of Pti4 by a similar approach. These recombinant gene fusions were placed downstream of the 35S promoter and followed by NOS terminator. Electroporation-mediated transformation

was employed to deliver these DNA constructs into tobacco protoplasts for transient expression assays. For each sample, the transformation mixture contained the following plasmid DNAs: 15µ;g of Lac[1]-SEBF, 15µ;g of Lac[2]-Pti4, 10µ;g of pBI221. The negative control had 15µ;g of Lac[1] instead of Lac[1]-SEBF. After electroporation, the protoplasts were incubated in the dark for 18 hr at room temperature. The protoplasts were then lysed and centrifuged in a table centrifuge for 5 min at full speed. The supernatant was used to determine β-lactamase and β-glucuronidase activities. β-Glucuronidase activity was used to correct for variations in electroporation efficiency. The results show that the sample containing SEBF and Pti4 presented a higher β-lactamase activity. The slope of the sample Lac[1]-SEBF and Lac[2]-Pti4 was 2.6 times higher than that of the negative control. The higher β-lactamase activity suggested that SEBF could interact with Pti4 in tobacco protoplasts by reconstructing β-lactamase activity."
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P35025 Intracellular localization of two cytokinin responsive factors
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"Cytokinins are a small group of plant hormones which are involved in different processes, most prominently in the induction of cell division. They mediate their effects through a signal transduction cascade which finally involves transcription factors, for example the CYTOKININ RESPONSIVE FACTORS (CRFs) which are a subgroup of the AP2 proteins. The six CRFs from *Arabidopsis thaliana* are highly up-regulated during cytokinin treatment. We analyzed these CRFs with computer-based prediction algorithms for their subcellular localizations. CRF5 and CRF6 were predicted to be located both in mitochondria as well as in the nucleus, thus dually targeted. To analyze the localization of these transcription factors the genes encoding the full length proteins or the putative presequences were cloned into an expression vector between a 35S CaMV promoter and the gene encoding the green fluorescent protein (GFP). These constructs were introduced into protoplasts derived from an Arabidopsis cell culture by PEG mediated transformation and into onion epidermal cells as well as into *Funaria hygrometrica* leaf cells by particle-bombardment. It turned out that both transcription factors did not show the predicted localization in mitochondria but instead could be localized in the nucleus and the cytoplasm only. Even the putative presequences did not enable the GFP being transported into mitochondria. This is in accordance with recent studies by Rashotte and colleagues of CRF-GFP-fusion proteins in *Arabidopsis thaliana* protoplasts. An interesting finding, however, was that the GFP fluorescence of the CRF5-fusion protein is not spread evenly in the nucleus in contrast to the homogeneous spreading in the cytoplasm but instead appears as yet unidentified nuclear bodies in variable numbers."
(a) University of Tromsoe

P35026 Two rice hexokinases OsHXK5 and OsHXK6 play a key role in glucose signaling
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"The Arabidopsis hexokinase 1 (*AtHXK1*) is recognized as an important glucose sensor. However, the function of hexokinases as glucose sensors has not been clearly demonstrated in other plant species including rice. To investigate the functions of rice hexokinase isoforms, we characterized *OsHXK5* and *OsHXK6*, which are evolutionarily related to *AtHXK1*. Transient expression analyses using GFP fusion constructs revealed that OsHXK5 and OsHXK6 are associated with mitochondria. Interestingly, the OsHXK5ΔmTP-GFP and OsHXK6ΔmTP-GFP fusion proteins, which lack N-terminal mitochondrial targeting peptides (mTP), were present mainly in the nucleus with a small amount of the proteins seen in the cytosol. In addition, the OsHXK5NLS-GFP and OsHXK6NLS-GFP fusion proteins harboring nuclear localization signals (NLSs) were targeted predominantly in the nucleus, suggesting that these OsHXKs retain a dual-targeting ability to mitochondria and nuclei. In transient expression assays using promoter::LUC fusion constructs, these two *OsHXKs* and their catalytically inactive alleles dramatically enhanced the glucose-dependent repression of the maize *RbcS* and rice α-amylase (*RAmy3D*) genes in mesophyll protoplasts of maize and rice. Notably, the expression of *OsHXK5, OsHXK6*, or their mutant alleles, complemented the Arabidopsis *glucose insensitive2-1* (*gin2-1*) mutant, thereby resulting in wild type characteristics in seedling development, glucose-dependent gene expression, and plant growth. Furthermore, transgenic rice plants overexpressing *OsHXK5* or *OsHXK6* exhibited hypersensitive plant growth retardation and enhanced repression of the photosynthetic gene *RbcS* in response to glucose treatment. These results provide evidence that rice *OsHXK5* and *OsHXK6* can function as glucose sensors."
(a) Graduate School of Biotechnology & Plant Metabolism Research Center

P35027 "Roles of RCN1, Regulatory A Subunit of Protein Phosphatase 2A, in Methyl Jasmonate Signaling and Signal Crosstalk between Methyl Jasmonate and Abscisic Acid"
Okamoto, Hiroyuki-presenter gag20007@cc.okayama-u.ac.jp(a) Saito, Naoki (a) Munemasa, Shintaro (a) Nakamura, Yoshimasa (a) Mori, Izumi C (b) Murata, Yoshiyuki (a)
"Abscisic acid (ABA) induces stomatal closure via reactive oxygen species (ROS) and/or nitric oxide (NO) generation in guard cells and methyl jasmonate (MeJA), which mediates various plant defense responses, has also been reported to induce stomatal closure. Munemasa et al. (2007) reported that ROS and NO are involved in MeJA signaling, and suggested that there are a crosstalk between ABA and MeJA signaling in guard cells. However, the crosstalk in both signaling and how they branch remain to be clarified. *Arabidopsis rcn1* protein phosphatase 2A (PP2A) A subunit knockout mutant is impaired in ABA-induced stomatal closure, whereas hydrogen peroxide (H_2O_2) successfully induces stomatal closure (Kwak et al. 2002). So, we focused on *rcn1* mutant to investigate the function of RCN1 in MeJA signaling, and to shed light on the signaling crosstalk between MeJA and ABA in guard cells. Both MeJA and ABA failed to induce stomatal closure in *Arabidopsis rcn1* knockout mutants unlike in wild-type plants. Neither MeJA nor ABA induced ROS production and suppressed activations of calcium (I_{Ca}) channel and inward-rectifying K^+ (I_{Kin}) channel in *rcn1* mutants but not in wild-type plants. These results suggest that RCN1 functions upstream of ROS production and downstream of the branch point of MeJA and ABA signaling in *Arabidopsis* guard cells. Munemasa et al (2007) *Plant Physiol.* 143: 1398-1407, Kwak et al (2002) *Plant cell* 14: 2849-2861"
(a) Graduate School of Natural Science and Technology, Okayama University (b) Research Institute for Bioresources, Okayama University

P35028 "OsBWMK1, rice MAP kinase, mediates the SA-dependent defense responses by activation of WRKY transcription factor"
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"Mitogen-activated protein kinases (MAPKs) play important roles in various developmental processes and in environmental stress responses. A number of plant MAPK-related genes appear to be involved in the defense response against pathogens. We reported earlier that BWMK1, a rice mitogen-activated protein kinase, locates in the nucleus and mediates pathogenesis-related gene expression by activation of a transcription factor, ethylene-responsive element-binding protein 1 (plant physiol. 132, 1961-1972). Here, we report that BWMK1 also phosphorylates OsWRKY33 that binds to the W-box element (TTGACCA) of the several PR- gene promoters, which in turn enhances DNA-binding activity of the factor to the cis element in vitro. Transient co-expression of the BWMK1 and OsWRKY33 in Arabidopsis protoplasts elevates the expression of the GUS-reporter gene driven by the W-box element. Furthermore, 35S-BWMK1 tobacco plants, which showed the enhancement of PR-genes expression and pathogen resistance, was identified to elevate the SA and H_2O_2 contents. These results suggest that OsBWMK1 may mediate the SA-dependent defense

responses by activation of WRKY transcription factor in plant. [This work was supported by a grant from BioGreen 21 (20080401034018) and KRF-2006-331-C00261]"
(a) Department of Bio-Environmental Science, Sunchon National University, Korea (b) Division of Applied Life Science, Gyeongsang National University, Korea (c) Center of Herbal Resources Research, Korea Institute of Oriental Medicine, Korea

P35029 "14-3-3 proteins bind to the Brassinosteroid receptor kinase, BRI1 and are positive regulators of brassinosteroid signaling"
Oh, Man-Ho-presenter manhooh@life.uiuc.edu(a) Wang, Xiaofeng (b) Clouse, Steven D. (b) Huber, Steven C. (a)
" Multiple members of the 14-3-3 protein family have been found in all eukaryotes, the biological functions of which are to interact physically with specific client proteins and thereby effect a change in the client. Thus, 14-3-3s are involved in many processes. The plant brassinosteroid (BR) receptor, BRASSINOSTEROID INSENSITIVE 1 (BRI1), is a member of the large family of leucine-rich repeat receptor-like kinases (LRR-RLKs) that contain cytoplasmic protein kinase domains. At least two LRR-RLKs are involved in BR perception and signal transduction: BRI1 and BRI1-associated receptor kinase 1 (BAK1). Pharmacological and molecular genetic results suggested that 14-3-3s are positive regulators of BR signaling. 14-3-3 proteins CO-IP with BRI1-Flag and both BRI1 and BAK1 bind to 14-3-3 proteins in vitro. The binding of recombinant 14-3-3σ to the recombinant cytoplasmic domain of BRI1 and BAK1 in vitro is dependent on autophosphorylation of the receptor kinase and is strongly stimulated by Mg2+. Generally, 14-3-3 proteins bind to pSer/pThr sites of client proteins. We determined that 14-3-3ω binds to the juxtamembrane domain of BRI1-CD and mutagenesis analysis suggest that Ser-858 and Thr-872 are involved in 14-3-3 binding and are critical for plant growth and development in BR signaling. The potential for serine and threonine phosphorylation to be directly involved in 14-3-3 binding is established, and adds a new dimension to the functionality of these signaling proteins. "
(a) US. Department of Agriculture, Agricultural Research Service, and Department of Plant Biology, University of Illinois (b) Department of Horticultural Science, NC State University

P35030 Non-Selective Ca^{2+} Channels Control Basal Cytosolic Ca^{2+} Levels in *Arabidopsis*
Pei, Zhen-Ming-presenter zpei@duke.edu(a) Nam, Edward (a) Han, Shengcheng (a) Choi, Christopher S (a) Lee, Michelle (a) Wang, Wei A (a) Zhu, Xueyi (a,b) Kong, Dongdong (a)
"Cytosolic Ca^{2+} plays a vital role as a second messenger in perceiving a large array of abiotic and biotic signals in plants. In addition, the homeostasis of cytosolic Ca^{2+} is important for plant growth and development[1, 2]. Elevation in cytosolic Ca^{2+} arises from Ca^{2+} entry across the plasma membrane and/or release from internal stores, and is mediated by Ca^{2+}-permeable channels. However, the molecular nature of these channels is poorly understood. We have used a Ca^{2+} imaging-based functional assay in human embryonic kidney (HEK293) cells[2], and cloned a Ca^{2+}-permeable channel (CPC) in *Arabidopsis*. CPC localizes to the plasma membrane in HEK293 cells, forms non-selective Ca^{2+}-permeable channels, and mediates Ca^{2+} influx. The *Arabidopsis cpc* knockout mutant displays reduced cytosolic Ca^{2+} levels, which affect Ca^{2+}-regulated growth and development. The isolation of CPC may shed light on the molecular identifying of Ca^{2+} permeable channels in plants. This study is supported by grants from NSF and USDA to Z-MP 1. Tang R-H, Han S, Zheng H, Cook CW, Choi CS, Woerner TE, Jackson RB, Pei Z-M (2007) Coupling diurnal cytosolic Ca^{2+} oscillations to the CAS-IP_3 pathway in *Arabidopsis*. ***Science*** 315, 1423-1426. 2. Han S, Tang R, Anderson LK, Woerner TE, Pei Z-M (2003) A cell surface receptor mediates extracellular Ca^{2+} sensing in guard cells. ***Nature*** 425, 196-200. "
(a) Duke University (b) Xiamen University

SESSION 36 – LIPIDS

P36001 Role of fatty acid-based signaling in coordinating plant stress responses
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"Fatty acids and fatty acid-metabolites are not only major structural and metabolic constituents of the cell, but they also function as modulators of signal transduction pathways. To determine the mechanisms of action of fatty acids and their metabolites in stress signaling we have focused on the oxylipin pathway. The relative levels of oxylipins provide a species-specific signature and help determine the ability of plants to adapt to various developmental and environmental stimuli. Among the several branches of this pathway, we have concentrated on the *AOS* branch of the oxylipin pathway to explore its critical role in plant stress responses via production of jasmonates (JAs). To elucidate the role of fatty acid-mediated signaling in the oxylipin pathway network we have employed transgenic plants that produce minor but easily detectable levels of eicosapolyenoic acids, specifically eicosadienoic acid (EDA, C20:2 $\Delta^{11,14}$) and arachidonic acid (AA, C20:4 $\Delta^{5,811,14}$). We have established that this perturbation of the *in vivo* fatty acid composition of membrane lipids profoundly alters the expression levels of JA-biosynthetic and JA-responsive genes, and modifies the levels of JAs. The physiological consequences of these alterations are modified plant resistance to a number of pathogens examined. These findings suggest that *in vivo* perturbation of fatty acid or fatty acid-derived metabolites, directly or indirectly, regulates *AOS* pathway gene expression. "
(a) University of California, Davis Plant Biology Department (b) University of California, Davis Plant Pathology Department (c) University of California, Entomology Department (d) University of California, Davis Plant Sciences Department

P36002 Lipoxygenase-mediated oxidation of polyunsaturated *N*-acylethanolamines in *Arabidopsis* seedlings
Kilaru, Aruna-presenter kilaru@unt.edu(a) Keereetaweep, Jantana (a) Goebel, Cornelia (b) Hornung, Ellen (b) Feussner, Ivo (b) Chapman, Kent D (a)
http://www.biol.unt.edu/~chapman/research%20projects/nae/naegroup.htm
"*N*-Acylethanolamines (NAEs) are fatty acid ethanolamides (endocannabinoids) that are widely distributed in eukaryotes. Their activity is modulated by hydrolysis *via* a fatty acid amide hydrolase (FAAH) to generate free fatty acid and ethanolamine. In mammals, NAEs are involved in the regulation of neurotransmission, cognition, embryo development and feeding behavior. In plants, lauryl ethanolamide (NAE 12:0) was shown to affect cytoskeletal organization, endomembrane trafficking, cell wall formation and cell shape. With the availability of *Arabidopsis* T-DNA knockout (KO) mutants and overexpressors (OE) of FAAH, we demonstrated that NAEs can act as negative regulators of seedling growth and development and modulators of stress responses. Moreover, we noted depletion of NAE content in KO seedlings despite the lack of FAAH activity, which suggested the occurrence of an alternate NAE catabolic pathway. Previously, *in vitro* studies demonstrated that polyunsaturated (PU) NAEs such as NAE 18:2 and NAE 18:3 can serve as substrates for several plant-derived LOXs to yield NAE-oxylipins. To ask whether NAE oxidation is a prominent pathway *in vivo*, we first generated NAE-oxylipins *in vitro* utilizing commercially available 13- and 9-LOX enzymes and developed a comprehensive chromatography and mass spectrometry methods to characterize and quantify NAE-oxylipins, including a novel compound, NAE -oPDA. Here, we report the profiling capability, evidence for endogenous NAE-oxylipins in Arabidopsis seedlings with altered NAE metabolism and the ability of *Arabidopsis* 13- and 9-LOX to oxidize PU-NAEs. Understanding LOX-mediated NAE catabolism in plants is pertinent because their products, NAE-oxylipins, may have functional bioactive roles in plant growth and signaling. "

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P36003 Sphingolipids and programmed cell death in *Arabidopsis thaliana*
Lin, Jiusheng (a,b) Stone, Julie M-presenter jstone2@unl.edu(a,b)
"Classically viewed as membrane structural components, sphingolipids also modulate cell proliferation and death. The fungal toxin fumonisin B1 (FB1) perturbs sphingolipid metabolism and induces plant programmed cell death (PCD). The FB1-resistant mutant *fbr6* is disrupted in a gene encoding an SBP domain DNA-binding transcription factor. Double mutants between *fbr6* and selected *accelerated cell death acd* mutants, the single mutants, and wild-type controls were tested for FB1 sensitivity. *fbr6* effectively suppressed the enhanced sensitivity of *acd5* to FB1-induced cell death and ROS accumulation, but failed to suppress these in *acd1* and *acd2*. Because *ACD1* and *ACD2* encode proteins involved in the metabolism of chlorophyll breakdown products and *ACD5* encodes a ceramide kinase, FBR6 target geneslikely regulate sphingolipid metabolism/signaling. Sphingolipid profiles for wild-type, *fbr6*, *acd5*, and *acd5 fbr6* mutant plants were determined. FB1 treatment clearly caused a shift in sphingolipids pools, as predicted given its function as a competitive inhibitor of ceramide synthase (sphinganine N-acyl transferase). There was a shift from very long chain fatty acids (VLCFAs; C20 to C26) to shorter chain C16 FAs, but this shift was circumvented by the fbr6 mutation. FB1 also caused significant accumulation of saturated LCBs and LCB-Ps. The *fbr6* mutant, however, accumulated very different levels and types of LCBs and LCB-Ps relative to wild-type plants, and these were also suppressed in the *acd5 fbr6* double mutant. Sphingolipidomics profiling link FBR6-mediated gene expression modulation to sphingolipid-dependent signal transduction pathways providing new information on the mechanisms by which plants control cell death."
(a) University of Nebraska, Department of Biochemistry (b) University of Nebraska, Center for Plant Science Innovation

P36004 Long chain base hydroxylation and unsaturation play important but distinct roles in metabolism and function of *Arabidopsis* sphingolipids
Chen, Ming-presenter mchen4@unlnotes.unl.edu(a) Cahoon, Edgar B (a)
"Sphingolipids are major structural components of endomembranes and function in a number of cellular processes including endocytosis, protein trafficking and programmed cell death. More than 200 different sphingolipid molecular species occur in *Arabidopsis*, but the functional basis for this immense structural diversity is largely unknown. To address this question, we have conducted studies with *Arabidopsis* mutants that have altered levels of hydroxylation and unsaturation of their long-chain bases (LCBs). Typically, ~~ 80 to 90% of the LCBs in sphingolipids of *Arabidopsis* leaves contain a C-4 hydroxyl group and a Δ8 double bond. Double mutants of the two C-4 hydroxylase genes *SBH1* and *SBH2* were generated that completely lack LCB C-4 hydroxylation. These mutants are greatly reduced in size due to defects in cell expansion and division and do not progress to reproductive stages of growth. The total sphingolipid content of the *sbh1 sbh2* mutants is increased two- to three-fold relative to wild type plants, due primarily to the aberrant accumulation of sphingolipid species with C16 fatty acids. These findings highlight the importance of LCB C-4 hydroxylation for growth and for mediating the content and composition of sphingolipids in *Arabidopsis*. By contrast, double mutants of the two Δ8 desaturase genes *SLD1* and *SLD2* are deficient in sphingolipid LCB ~{&$~}8 unsaturation, but do not display growth phenotypes under normal conditions. Growth of these mutants, however, is impaired in response to certain stresses, suggesting a more subtle, but important role of LCB Δ8 unsaturation in the physiology of *Arabidopsis*. "
(a) University of Nebraska-Lincoln

P36005 "Nutritional Oils, A Tale of two Crops"
Hildebrand, David-presenter dhild@uky.edu(a) Thoguru, JR (a) Jamboonsri, Wachareewan (a) Ratliff, Gary (a) Sterling, Paul (b) Geneve, Bob (a) Phillips, Tim (a)
"There is growing interest in oils with enhanced nutritional value with oils high in σ3 fatty acids and shorter chain mono-unsaturated fatty acids being of particular interest. Palmitoleate (δ9-*Z*-hexadecenoic acid, 16:1) is a particularly healthful monounsaturated fatty acid. *Macadamia integrifolia* seed oil is highest in 16:1. Accumulation of 16:1 in membrane lipids does not impair membrane function. The occurrence and accumulation of 16:1 in different macadamia tissues was monitored. Some 16:1 is seen in leaves with higher levels in inflorescences with highest levels in mature seeds. During seed development no 16:1 is detected until the phase in early-mid maturation wherein 16:1 and triacylglycerol accumulate rapidly. Chia, *Salvia hispanica*, averages ~ 10% more than the main plant source of σ3 fatty acids, flax (*Linum usitatissimum*) or ~ 60% or more of the σ3 fatty acid, α-linolenic acid, in its seed oil. In areas such as Kentucky chia is a much more robust plant with much higher yield potential than flax. However, chia is a short-day plant and unable to set seed before frost in temperate areas. To develop lines able to flower and set seeds in higher latitudes ethyl methanesulfonate (EMS) and gamma radiation were used to induce altered flowering mutants. Mutagenized M1 plants were grown and induced to flower under short days. M2 seeds were planted in the field at Lexington, KY. Early flowering plants were found 55 days after planting while non-mutagenized plants had not produced any flower buds. 0.012% of the EMS-treated M2 population and 0.021% of the gamma ray treated population flowered considerably earlier than the controls under natural field conditions. These lines can be used to extend the range of cultivation of this useful crop."
(a) University of Kentucky (b) Dr. Paulos Farms

P36006 Redox regulation of recombinant VDE
Emek, Sinan cem-presenter sinan_cem.emek@biochemistry.lu.se(a) Akerlund, Hans-Erik (a)
"VDE (violaxanthin de-epoxidase) is a 43 kDa enzyme, soluble in the lumen of thylakoids at high pH and membrane bound at low pH. Under light stress in higher plants, VDE converts violaxanthin to zeaxanthin as part of the xanthophyll cycle. VDE possesses three specific regions; a cycteine-rich N-terminus followed by a lipocalin region and highly a charged C-terminus. The cycteine rich N-terminus contains 11 of the total 13 cycteine residues of the mature protein. The function of this highly conserved cycteine-rich N-terminus region is unknown. In order to examine the function of the cycteine-rich N-terminus, we have expressed the VDE in E.Coli. The use of signal peptide sequences, recombinant inactive VDE is secreted out of E.Coli cytoplasm to the periplasmic space, in which disulfide bonds formed to active VDE due to its more oxidative environment. Results showed that thiol groups in the N-terminus take important part of the stability of the VDE. "
(a) Biochemistry

P36007 Oleosins have an important role in protecting oilseeds against freezing stress
Shimada, Takashi L-presenter takashi@gr.bot.kyoto-u.ac.jp(a) Shimada, Tomoo (a) Takahashi, Hideyuki (a) Fukao, Yoichiro (b) Hara-Nishimura, Ikuko (a)
"Oleosins are major oil-body membrane proteins in seeds. Here we demonstrate a novel role for oleosins in protecting oilseeds against freeze/thaw-induced damage of their cells (1). We detected four oleosins in oil bodies isolated from seeds of *Arabidopsis thaliana*, and designated them *OLE1*, *OLE2*, *OLE3* and *OLE4* in decreasing order of abundance in the seeds. We isolated oleosin-deficient mutants (*ole1*, *ole2*, *ole3* and *ole4*), and

generated three double mutants (*ole1 ole2*, *ole1 ole3* and *ole2 ole3*). Electron microscopy showed an inverse relationship between oil body sizes and total oleosin levels. The double mutant *ole1 ole2*, which had the lowest levels of oleosins, had irregular enlarged oil-containing structures throughout the seed cells. The seeds of *ole1 ole2* and *ole1 ole3* poorly germinated, while the other mutants normally germinated. Interestingly, we found that freezing followed by imbibition at 4 ^{o}C abolished seed germination of single mutants (*ole1*, *ole2* and *ole3*). Germination rates were positively associated with oleosin levels, suggesting that defects in germination are related to the expansion of oil bodies due to oleosin deficiency. The treatment accelerated the fusion of oil bodies and the abnormal-positioning and deformation of nuclei in *ole1* seeds, which caused seed mortality. In contrast, *ole1* seeds that had undergone freezing treatment germinated normally when incubated at 22 ^{o}C instead of 4 ^{o}C, because degradation of oils abolished the acceleration of fusion of oil bodies during imbibition. Taken together, our findings suggest that oleosins increase the viability of over-wintering oilseeds by preventing abnormal fusion of oil bodies during imbibition in the spring. (1) Takashi L. Shimada et al., Plant J, 2008, vol. 55, 798-809"

(a) Department of Botany, Graduate School of Science, Kyoto University (b) Nara Institute of Science and Technology

P36008 Functional analysis of sphingolipid fatty acid hydroxylase in Arabidopsis

Nagano, Minoru-presenter ss077173@mail.ecc.u-tokyo.ac.jp(a) Uchimiya, Hirofumi (a,b) Kawai-Yamada, Maki (a,c)

"Sphingolipids are a large class of lipids ubiquitously present in eukaryotic cell membranes and essential for various cellular reactions such as signal transduction, protein transport and programmed cell death. The structural diversity of sphingolipids derives from numerous distinct head groups as well as various modifications on hydrocarbon chains of the hydrophobic ceramide moiety. One of the main modifications of ceramide structure is 2-hydroxylation of the fatty acids, which are catalyzed by fatty acid hydroxylase (FAH). In Arabidopsis, saturated and monenoic 2-hydroxy fatty acids account for over 90% of the total fatty acids of glucosylceramide, ceramide and glycosylinositolphosphoceramide. Nevertheless, it remains unclear what hydroxylates fatty acids in Arabidopsis. We identified two Arabidopsis *FAH*s (*AtFAH1* and *AtFAH2*) homologous to *S. cerevisiae FAH1*. These include H$X_{(2\text{-}3)}$(XH)H motif, which are characteristics of membrane-bound fatty acid hydroxylases. However, AtFAHs had no cytochrome b_5 (Cb5)-like domains, which characteristically existed in S. cerevisiae and mammals FAHs. Instead, we showed that AtFAHs interacted with Cb5 in the ER with a bimolecular complementation (BiFC) assay. In addition, a complementation test with yeast FAH1-deletion variant demonstrated that AtFAHs had fatty acid 2-hydroxylase activity with Cb5. Moreover, we revealed that AtFAH2 hydroxylated palmitic acid, whereas AtFAH1 hydroxylated very-long-chain fatty acids in Arabidopsis. Here we show the functions and roles of AtFAHs in Arabidopsis. "

(a) University of Tokyo (b) Iwate Biotechnology Research center (c) Saitama University

P36009 The fatty acid σ-hydroxylase CYP86A22 is required for the formation of stigmatic exudate essential for pollination in *Petunia hybrida*

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"Plant flower stigmas can be classified into two types: wet stigma that is covered by a copious exudate on the surface; and dry stigma that, in contrast, has little or no exudate on the surface. The stigmatic exudate is rich in σ-hydroxy fatty acids that form estolide, a polyester constituting a major component of the exudate. The primary roles of the exudate include tracking pollen and keeping a moisture environment for pollen hydration. Although a series of studies on the stigmatic exudate have been carried out, the molecular mechanism of exudate biosynthesis remains unknown. We have cloned and characterized the cytochrome P450 gene *CYP86A22* that encodes a fatty acid σ-hydroxylase involved in estolide biosynthesis in the stigma of *Petunia hybrida*. In transgenic petunia in which the expression of *CYP86A22* has been decreased by RNAi, σ-hydroxy fatty acids were dramatically decreased or not detectable. Surprisingly, no exudate was found on the surface of the knockdown stigma, creating a dry stigma phenotype. This fundamental change dramatically altered overall carbon flux in the stigma. In the dry stigma the high carbon flux normally secreted as exudate instead accumulated in cytosolic lipid bodies and, further upstream in carbon flow, there was a large accumulation of starch in chloroplasts of the secretory cells. In the total lipid extract of the dry stigma, in place of the estolide, triacylglycerols as well as diacylglycerols accumulated. Furthermore, the dry stigma phenotype seriously reduced the number of pollen tubes and seed setting. The possible mechanism for exudate secretion in stigmas will be discussed."

(a) Donald Danforth Plant Science Center (b) Metabolix

P36010 Synthesis of hydroxylated sterols in transgenic Arabidopsis plants alters growth and steroid metabolism

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"Sterols are isoprenoid-derived lipids that play multiple roles in eukaryotes, e.g. as essential components of the plama membrane, or as metabolic precursors to steroidal hormones and defence substances. Sterol composition in plants is characterized by a complex mixture of several sterol species. The most dominant plant sterols are sitosterol, campesterol and stigmasterol whereas cholesterol is a minor sterol in most species. To explore mechanisms in plant sterol homeostasis, we have here increased the output of sterols in Arabidopsis plants through overexpression of four mouse cDNA encoding cholesterol hydroxylases (CH); thus hydroxylating cholesterol at the C-7, C-24, C-25, or C-27 position. In mice, these enzymes are involved in the normal turnover of sterols into bile acids by hydroxylating cholesterol at specific positions. The four types of transformant showed different phenotypes, the strongest one being in CH25 lines, which were dark-green dwarfs resembling brassinosteroid-related mutants. The expected hydroxylated cholesterol products in CH7, CH24 and CH25 transformants were identified by GC-MS. However, additional forms of hydroxylated sterols were also identified, particularly in CH25 plants. In CH24 and CH25 lines, but not in CH7, the presence of hydroxysterols was correlated with a considerable alteration of the sterol composition. Associated with this was a three-fold higher enzymatic activity of the sterol-biosynthetic enzymes sterol methyltransferase type 1 and type 2, as revealed in CH25 lines. In addition, CH25 lines contained clearly reduced levels of brassinosteroids. The results suggest that an increased output of cholesterol and/or other sterols in Arabidopsis triggers compensatory metabolic processes acting to maintain sterols at adequate levels. "

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P36011 "Identification of *ERG3*, a gene involved in the biosynthesis of ergosterol in *Chlamydomonas reinhardtii*"

Brumfield, Kristy M. (a) Donze, David (a) Moore, Thomas S. (a) Simms, Tiffany A. (a) Moroney, James V.-presenter btmoro@lsu.edu(a)

"The major sterol found in the membranes of the green alga *Chlamydomonas reinhardtii* is ergosterol. While studies in the past have identified some *C. reinhardtii* ergosterol mutants, very little is known about sterol biosynthesis in this species. With the elucidation of the *Chlamydomonas* genome, we now have the ability to identify many of the genes likely to be involved in sterol pathways in *C. reinhardtii*. One such gene has significant similarity to *S. cerevisiae ERG3*, a sterol C-5 desaturase in yeast. Sequence analysis identifies putative histidine rich motifs that are present in *ERG3* of higher plants as well as yeast. To verify the role of *ERG3* of *C. reinhardtii* in sterol biosynthesis, yeast *erg3* knockout strains were created and

tested for complementation. Yeast cells defective in *ERG3* are unable to survive on the non-fermentable carbon source, acetate, and show hypersensitivity to cycloheximide. They also exhibit an overaccumulation of the ergosterol precursor, episterol. These *erg3*Δ mutants were then transformed with a yeast vector designed to express *ERG3* cDNA from *C. reinhardtii*. Transformants were screened on cycloheximide plates for resistance to the drug as well as an ability to grow on acetate. Qualitative GC/MS analysis also confirms that ergosterol biosynthesis is restored in the *erg3*Δ mutants transformed with *ERG3* from *C. reinhardtii*. These data strongly support *ERG3* as a participant in ergosterol biosynthesis in *C. reinhardtii*. This study is supported by the National Science Foundation."
(a) Department of Biological Sciences, Louisiana State University

P36012 Higher plant Cb5 genes display differential fatty acid desaturation
Kumar, Rajesh-presenter kumarr@missouri.edu(a) Cahoon, Edgar (b) Tran, Lam-Son (a) Neelakandan, Anjanasree (a) Nguyen, Henry (a)
"Cytochrome b5 (Cb5) is a small haem-binding tail anchored class of proteins in higher eukaryotes located in endoplasmic reticulum (ER) and outer mitochondrial membranes. In higher plants, animals, and fungi, the ER resident Cb5 provides electrons in desaturation of Acyl CoA fatty acids. Also it provides reducing equivalents in hydroxylation of Acyl-CoA fatty acids, desaturation and hydroxylation of complex sphingolipids and sterols desaturation in higher plants. Desaturation of fatty acid is an important enzymatic reaction resulting into unsaturated fatty acids that are essential membranes glycerolipid components, constituent of storage TAG in oil storing seeds. Higher plants are uniquely endowed with multiple forms of Cb5s as opposed to single in mammals and yeast respectively. To understand the role of higher plants Cb5 genes in fatty acid desaturation, we co-expressed various ER resident Cb5 genes from soybean and Arabidopsis with FAD2/FAD3 gene in a mutant yeast disrupted in its lone endogenous Cb5 gene. We observed that certain Arabidopsis Cb5 genes exhibit distinct and differential ~1.5-2 fold increase in the 18:2 level, a product of σ6 desaturation, however in case of soybean the differences were subtle. On the other hand, we consistently found that some Cb5 genes from both Arabidopsis and soybean demonstrate differential σ3 desaturation, as judged from significant differences in their product outcome. We reconfirmed above result by further analyses in which Arabidopsis Cb5 genes were replaced by soybean Cb5 genes or vice versa. The present study provides the first report of the differential nature of higher plant Cb5 genes in carrying out fatty acid desaturation reaction and offers a potential genetic tool to modulate poly-unsaturated fatty acid (PUFA) level. "
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SESSION P37 – MEDICINAL PLANT BIOLOGY

P37001 Bio Substances Extracted From Certain Medicinal And Ornamental Plant Against Root Knot Nematode M.incognita
Haroon, Sanaa Abu Serie-presenter sanaaharoon@hotmail.com(a)
"Eight plant species used as medicinal and ornamental plants in Egypt, were tested in microplots and in small field plots to determine their effects on the root knot nematode *Mlidogyne incognita*. Five of these plants, *Linum Usitatissimum, Calendula afficinalis, Hyoscyamus Muticus,Ambrosia maritime and Origanum Vulgare*, significantly reduced the number of nematodes when planted into microplots inoculated with Root Knot Nematode *M .incognita* or in naturally infested field plots. No Juveniles were recovered fromthe roots or soils four months after planting with all five plant species individualy. Further, when the micrplots were replanted with susceptible tomato cultivars, a significant reduction in Root Knot Nematode occurred. Studies were then conducted under laboratory conditions to observe the effect of root extract of these plants on hatch rate and / or mortality of Root Knot Nematode. Hatch rate was increased in the presence of *A.Moluccama H. niger, and C. afficinalls* when compared to the controls, whereas a lower hatch rate occurred with extracted of *O.vulgare* and *A .trijida*. Survival of hatched juveniles was significantly reduced after 24 hours in extracted of *A. trijida, C. afficinalis* and *O. vulgare* when compared to the controls. Juveniles, inoculated onto tomato root explant cultures with extracted added, failed to penetrate and / or develop. These results indicated that some medicinal and ornamental plant work as natural nematicides to reduce the population level of Root Knot Nematode. We can recommend that these plants can be used in crop rotation system in IPM program to control Root Knot nematode Melidogyne incognita Keywords: allelopathy, crop rotation, *Melidogyne incognita*, explant culture "
(a) Fayoum University

P37002 Molecular mechanisms for the health benefits of cinnamon and green tea polyphenol extracts
Cao, Heping-presenter Heping.Cao@ars.usda.gov(a,b) Anderson, Richard A (a)
http://www.ars.usda.gov/Aboutus/docs.htm?docid=18166
"Medicinal plants have long been used for the prevention and treatment of various diseases including diabetes and cardiovascular disease. Plant polyphenols found in seeds, fruits, leaves, and bark are generally present in the diet and often considered important for human health. For example, cinnamon and green tea extracts (CE and GTE) exhibit insulin-like functions with anti-inflammatory, anti-diabetic, antioxidant, and anti-obesity activities. However, the molecular mechanisms responsible for these activities have not been fully elucidated. We hypothesized that CE and GTE induced molecular changes in the insulin signal transduction and related pathways. Real-time PCR was used to compare the effects of CE, GTE, and insulin on the expression of >40 genes encoding insulin signaling components, the glucose transporter (GLUT) family, the anti-inflammatory tristetraprolin (TTP) family, and pro-inflammatory cytokines in mouse cell-line adipocytes and macrophages, and in the muscle and liver of rats fed a high-fructose diet known to induce insulin resistance. Immunoblotting confirmed protein expression of some of the PCR findings. Our analyses indicated that CE and GTE exhibited insulin-like effects including rapid TTP mRNA induction, and reduced mRNA for vascular endothelial growth factor and components of the insulin-signaling pathway. CE and GTE also possessed insulin-independent effects such as sustained increases in GLUT1 and TTP expression. This study demonstrated that CE and GTE regulated the expression of multiple genes in cell-line adipocytes and macrophages in vitro and in rats, and suggested that the health benefits of cinnamon and green tea are due to both their insulin-like and insulin-independent activities. "
(a) USDA-ARS-Human Nutrition Research Center (b) USDA-ARS-Southern Regional Research Center

P37003 Distribution and evolution of cyclotides in flowering plants
Craik, David J-presenter d.craik@imb.uq.edu.au(a) Daly, Norelle L (a) Elliott, Alysha G (a) Gruber, Christian W (a) Oku, Basar (a)
"Cyclotides are disulfide-rich mini-proteins that have the unique structural features of a circular backbone and knotted arrangement of three disulfide bonds. These features make them exceptionally stable and they have applications as host defense (insecticidal) agents [1] and stable drug frameworks [2]. So far they have been found mainly in two plant families, including in every species of the violet family (Violaceae) so far examined, and in a few species of the coffee family (Rubiaceae). Rubiaceae is the fourth largest flowering plant family, comprising approximately 13,000 species, and is one of the largest and most important living biomasses due to their geographical distribution and economic importance. In this study we analyzed over 200 Rubiaceae species, distributed in many tribes, for the occurrence of cyclotides. Their presence was confirmed in more than 20

species and mass spectrometry was used to obtain sequences of a selection of the novel cyclotides. On the basis of the phylogeny of cyclotide-bearing plants, we hypothesize that the evolution of circular mini-proteins occurred as independent events after the divergence of Asterids and Rosids. This is further supported by recent findings on the biosynthesis of cyclotides [3], which involves ubiquitously present enzymes for folding and processing. We predict that the number of cyclotides within the Rubiaceae could be as great as 50,000, potentially making cyclotides one of the largest protein families in the plant kingdom [4]. [1] Barbeta B L et al: PNAS (2008) 105, 1221 [2] Craik D J et al: Curr. Opin. Drug Discovery and Development (2006) 9, 251 [3] Saska et al, J. Biol. Chem., (2007), 282, 29721; Gillon et al, The Plant Journal (2008), 53, 505 [4] Gruber C W, et al: Plant Cell, (2008) 20, 2471 "
(a) The University of Queensland

P37004 Survey of the presence of lectin activity in south texas plants
Miranda, Paul E-presenter eddie_miranda@students.tamiu.edu(a) Perez, Eric (a) Portillo, Melinda (a) Ynalvez, Ruby A (a)
"Lectins are sugar-binding proteins. They serve many different biological functions but the most recognized one is their role in plant defenses. Plant lectins have many interesting properties which include ability to inhibit HIV-1 reverse transcriptase in addition to anti-bacterial and anti-fungal activities. In biotechnology, lectin genes can be expressed in transgenic plants to confer resistance against insects. There is an increasing interest in lectins for medical therapeutic effects and potential in crop protection and thus crop production. Plant lectins have been isolated and characterized from many plant sources but very few studies on lectins from plants from South Texas have been conducted. The objective of this study is to identify new sources of plant lectins specifically from plants found in the South Texas region. This work will then be extended to the isolation and characterization of the specific plant lectins. In this study we will present evidence that some South Texas plants have lectin activity. "
(a) Texas A&M International University

P37005 "Production, Purification, and Functional Characterization of Novel Chlorogenic acid Glycoside Using the Glucansucrase "
Yang, Kwang-Yeol (b) Nam, Seung-Hee-presenter namsh@jeonnam.go.kr(a) Park, Jamg-Hyun (a) Kim, Young-Ok (a) Kim, Joung-Keun (a)
"Chlorogenic acid is major polyphenol compounds in the human diet such as coffee bean, mullberry leaves, or apple skins and is highlighted as its cancer chemopreventive properties, free radical scavenging activity, and oxidized DNA repairing function. Chlorogenic acid glucoside was synthesized via the acceptor reaction of a glucansucrase from ''*Leuconostoc mesenteroides* '' B-512FMCM with chlorogenic acid and sucrose due to improve their functionality. Chlorogenic acid glucoside was synthesized with a 28% yield and purified by silica gel column chromatography and C18 reverse-phase HPLC after removing unreacted chlorogenic acid and sucrose using ethylacetate (1:1, v/v). Chlorogenic acid glucoside was further confirmed by LC/MS/MS. Chlorogenic acid glucoside exhibited slightly higher antioxidant activity (RC50=1.02mM), compared to chlorogenic acid (RC50=1.64mM) but no difference on NO scavenging. Chlorogenic acid glucoside showed 20% higher effects than chlorogenic acid on anti-lipid peroxidation using chemiluminescent assay. As the result of MTT assay using colon cancer cell (Hep2), chlorogenic acid glucoside prevented more cancer cell growth (IC50=4.44mM), compared to chlorogenic acid (IC50=7.44mM). This work was supported by the Korea Research Foundation Grant funded by the Korean Government (KRF- 2008-531-F00019). "
(a) Food Research Institute, Jeonnam Agricultural Research and Extension Services (b) Department of Plant Biotechnology, Chonnam National University

P37006 Tissue culture and active compounds analysis of wild and transgenic *Salvia miltiorrhiza* Bunge
Tsay, Hsin-Sheng-presenter hstsay@cyut.edu.tw(a) Chen, Emily Chin-Fun (a) Ho, Hsin-Chveh (a)
"*Salvia miltiorrhiza* Bunge is a well known oriental medicinal herb. Roots of *S. miltiorrhiza*, generally known as Dan-shen, contain bio-active compounds such as tanshinone-I, tanshinone-IIA , cryptotanshinone and salvianolic acid B. Dan-shen have been used for multiple therapeutic remedies (cardiovascular disorders and other diseases) based on their biological activities such as antibacterial, antioxidant, anti-inflammatory, cytotoxic and anti-platelet aggregation. On the basis of Dan-shen are mainly imported from mainland China, we have developed the plant regeneration system from *S. miltiorrhiza* callus as well as the transgenic system. In this study, wild type and one of the transgenic *S. miltiorrhiza* were used as plant material for tissue culture, active compounds and antioxidant detection. Results showed that transgenic plant with low budding ability in 1/2 MS (1mg//L BA) medium could grow faster in height as well as in biomass. No difference in tanshinone-I, tanshinone-IIA and cryptotanshinone content were detected both in 4-month-old wild type and transgenic plant. Salvianolic acids B in transgenic plant is 3.4 times more than wild type. And transgenic plants also have higher total polyphenol content and antioxidant ability."
(a) Chaoyang University of Technology

P37007 "Effects of gibberellins on the growth, development, and artemisinin content of *Artemisia annua* plants grown in controlled environments."
Sponsel, Valerie M.-presenter valerie.sponsel@utsa.edu(a) Brice, Joshua R. (a) Cantu, Donaciano (b) Groshel, Carla (a) Mullens, Conor (b) Bach, Stephan (b)
"Artemisinin is a sesquiterpenoid lactone endoperoxide which is produced in shoots of *Artemisinin annua* (sweet wormwood) plants. Artemisinin derivatives are essential components of Artemisinin-based Combination Therapy (ACT), which is recommended by the World Health Organization (WHO) for the treatment of malaria that is resistant to conventional monotherapies. Zhang et al (2005) reported that treatment of vegetative *Artemisia* plants with gibberellic acid increases the yield of artemisinin by approximately four-fold. We are examining the effect of GAs, and of daylength, on the yield of artemisinin in *Artemisia*, which is an obligate short day plant. Plants are grown in controlled environments at 24 degrees in continuous light or long days, with or without eventual transfer to short days. Vegetative plants transferred to short days have visible flower buds within 7d of transfer. Plants are sprayed with an aqueous solution of gibberellic acid. Artemisinin, which accumulates in glandular hairs on leaves, stems and sepals, is extracted with chloroform, and extracts are analyzed by reverse phase liquid chromatography-mass spectrometry. Artesunate (obtained from Walter Reed Army Institute of Research) is used as an internal standard. Quantitative analyses of artemisinin will be presented, along with information on glandular hair distribution in treated plants. Since 2001, 56 countries have adopted a WHO-recommended ACT, underscoring the urgent need for artemisinin. (This work is funded by an award from the UTSA Collaborative Research Seed Grant Program). "
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SESSION 38 – MEMBRANE BIOLOGY & TRANSPORT

P38001 Cloning and characterization of plant oxalate transporters
Wyman, Aaron J-presenter awyman@aurora.edu(a,c) Anderson, Carrie M (b) Webb, Mary Alice (c)
http://www.aurora.edu/biology/index.htm
"The *Slc26a* gene family encodes for ion transporters responsible for exchanging monovalent and divalent anions. In mammals, the Slc26a6 transporter exchanges chloride for formate, sulfate, and the toxic antinutrient oxalate. Elimination of Slc26a6 transporters in rats leads to oxalate accumulation and kidney stone formation in renal tissues. While many anion transporters are known in plants, none responsible for oxalate transport have been identified. A better understanding of how plants mobilize oxalate *in vivo* may aid in developing more nutritious food crops. Using a bioinformatics approach, we identified plant proteins homologous to mammalian Slc26a6 transporters, including several polypeptides in the model plant *Arabidopsis thaliana* and grape (Vitis) species. Interestingly, some of these Slc26a6-like transporters in *Arabidopsis thaliana* are responsible for sulfate transport *in vivo*. Mutant Arabidopsis lines carrying T-DNA insertions in the genes encoding for these sulfate transporters have been obtained and are being characterized for differences in growth and development and oxalate transport. Vitis species contain high concentrations of calcium oxalate crystals throughout their tissues. From a Vitis leaf cDNA library, we have cloned a putative Vitis *Slc26a6* predicted to encode a polypeptide having strong homology to mammalian Slc26a6 transporters. We are currently working toward expressing this Vitis sequence in yeast to generate recombinant protein which will be used to raise antibodies for Slc26a6 immunochemical studies."
(a) Aurora University Department of Biology (b) Purdue University Department of Biology (c) Purdue University Department of Botany & Plant Pathology

P38002 Manipulating sucrose/proton symporter genes to understand their role in biomass partitioning and plant productivity
Dasgupta, Kasturi-presenter kd0064@unt.edu(a) Srivastava, Avinash C (b) Ayre, Brian G (a)
"Phloem vascular tissues transport sugars from photoautotrophic source leaves to sink tissues along hydrostatic pressure gradients that are generated in part by Suc/H^+ symporter. *AtSUC2* encodes the principal Suc/H^+ symporter involved in phloem transport in Arabidopsis, and is regulated by environmental and physiological conditions. In this study, we demonstrate that *AtSUC2* cDNA fused to different phloem-specific promoters can substitute for endogenous *AtSUC2* gene activity, and that phloem transport can be uncoupled from endogenous *AtSUC2* regulation. *AtSUC2* cDNA expression from *AtSUC2p*, Cucumis melo Galactinol synthase promoter (*CmGAS1p*), promoter element from Commelina yellow mottle virus (*CoYMVp*) were all sufficient to restore transport to an *Atsuc2* knockout mutant to varying extents. Expression of *AtSUC2* cDNA from *CmGAS1p* was relatively weak, and source leaves had elevated levels of starch, indicating compromised sucrose transport. *AtSUC2* cDNA expression from *CoYMVp* was high relative to *AtSUC2* expression in wild type plants, and source leaves had reduced levels of starch, suggesting that elevated *AtSUC2* expression may improve sucrose transport. Further we tested the activity of each promoter in the presence of exogenous sucrose. Expression from *AtSUC2p* was repressed as expected, but expression from *CoYMVp* was elevated, implying that driving *AtSUC2* cDNA from this promoter may promote phloem transport under conditions where it would otherwise be repressed. Further characterization of expression under various stress conditions will tell us whether plants expressing Suc/H^+ symporter from exotic promoters are more productive in adverse conditions. An applied goal of this research is to manipulate phloem transport to alter biomass partitioning and improve plant productivity."
(a) University of North Texas, Department of Biological Sciences (b) Samuel Roberts Noble Foundation, Plant Biology Division

P38003 Mighty but shorthanded: the microdomain hypothesis of the vacuolar Ca^{2+} release via SV channels
Pottosin, Igor I-presenter pottosin@ucol.mx(a) Dobrovinskaya, Oxana R (a) Perez, Vadim (a) Wherrett, Tim (b) Shabala, Sergey (b)
"Vacuole is the largest Ca^{2+} store in a plant cell, but the principle routes of the vacuolar Ca^{2+} release remain elusive. To date, the only established by electrophysiological, molecular, and proteomics studies tonoplast Ca^{2+} permeable channel is the SV (TPC) one. The SV channel is activated by the cytosolic Ca^{2+} and is down-regulated by the luminal Ca^{2+}. In this study on sugar beet vacuoles we found that at physiological vacuolar pH and cation concentrations the SV activity was 'buffered' and not affected by vacuolar Ca^{2+} variations within the physiological range. Ca^{2+} release by intact vacuoles was measured by the MIFE technique. It was stimulated by cytosolic Ca^{2+} and Mg^{2+} and abolished by 0.1 mM Zn^{2+}. Among a wide range of second messengers tested such as IP_3, cADPR, ABA, ATP, cAMP, cGMP, H_2O_2 and CaM, both the Ca^{2+} fluxes and the SV channel activity were affected by adenine nucleotides and H_2O_2, whereas other substances were inefficient. Our patch-clamp and MIFE data suggest that at all conditions the vacuolar Ca^{2+} release was dominated by the SV channels, and that it was strongly limited. Even at high (20 µ;M) cytosolic Ca^{2+} only few tens of SV channels per vacuole (0.5%) were open, mediating a net Ca^{2+} release about 5 pA. Such a strict control prevents irreversible feed-forward vacuolar Ca^{2+} release via SV channels. Instead of a global (i.e. across the entire tonoplast surface) Ca^{2+} release, the SV channels might mediate local Ca^{2+} signaling, especially within the contact zones between the tonoplast and plasma membrane or organelles containing other Ca^{2+} channels, thus, forming high Ca^{2+} microdomains. Supported by CONACyT grants to IIP and ARC Discovery grant to SS."
(a) Universidad de Colima (b) University of Tasmania

P38004 Enlightening the mechanism of polarized cell wall biosynthesis in the root-hair tip
Park, Sungjin-presenter sungjinp@umich.edu(a)
"The functional identification of cellulose synthase-like D3 (CSLD3) has been made an additional step forward by combination of subcellular localization and following biochemical and genetic analyses. Adding to the previous studies revealing the Golgi localization of CSLD3 in tobacco leaves, its targeting towards plasma membrane in root hairs was verified using N-terminal YFP fusion CSLD3 in Arabidopsis. The tip-focused aggregation of CSLD3 in the tips of growing root hairs and its structural similarity to cellulose synthase connote its pivotal role in root-hair elongation. In addition, the treatment of isoxaben, a cellulose synthase inhibitor, failed to impede CSLD3 functionality and its endogenous localization in the growing root-hair tips, which insinuates CLSD3 is involved in synthesizing cell walls and plasma membranes in root-hair tips"
(a) University of Michigan

P38005 "Cloning and characterization of a vacuolar iron transporter, TgVit1, from *Tulipa gesneriana*"
Kazumi, Momonoi-presenter momonoi@agri.pref.toyama.jp(a,b) Shoji, Mano (c,d) Chihiro, Nakamori (c) Mikio, Nishimura (c,d) Kazuaki, Shoji (e) Kumi, Yoshida (a)
"Petals of *Tulipa gesneriana* cv. Murasakizuisho exhibits blue color at the inner perianth-bottom, although the upper petals are purple. We found that the difference in iron content was the only factor of the different coloration; in blue cells iron content was 25 times more than that in purple cells (Shoji *et al.*, 2007). This strongly suggested an existence of blue cell-specific vacuolar iron transport and/or storage system. Here, we have isolated a vacuolar iron transporter gene in *T. gesneriana* (*TgVit1*) from the blue petals of the tulip. The amino acid sequence of TgVit1 was similar to that of the Arabidopsis thaliana vacuolar iron transporter AtVIT1 (Kim *et al.*, 2006), and Ca2+-sensitive cross-complementer 1 (CCC1) in yeast (Li *et al.*, 2001). The tissue- and stage- specific expression of *TgVit1* gene indicated a strong correlation between the expression of *TgVit1* mRNA and blue color development. Furthermore, accumulation of TgVit1 protein also confirmed these results. We will discuss function of TgVit1 protein with involvement in a vacuole iron transport and blue flower color development."
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Fisheries Research Center (c) Department of Cell Biology, National Institute for Basic Biology (d) Department of Basic Biology, School of Life Science, The Graduate University for Advanced Studies (e) Agricultural Research Institute, Toyama Prefectural Agricultural, Forestry and Fisheries Research Center

P38006 Plant lessons: understanding ABCB functionality through structural modelling

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"Attempts to fight multidrug resistance by inhibiting the unspecific drug export activity of ABCB1/MDR1 in cancer cells have been hampered by a lack of unequivocal identification of substrate and inhibitor binding sites in HsABCB1. To gain a clearer understanding of how eukaryotic ABCB proteins interact with their transport substrates, we computed structures of plant ABCB transporters, which exhibit a high degree of substrate specificity, employing the crystal structure of the bacterial ABC drug exporter, Sav1866. Sequence and structural comparisons showed that plant and mammalian ABCB transporters share a common architecture. Using different approaches, our analysis identified candidate substrate binding sites in the transmembrane domains of the proteins near the inner leaflet of the plasma membrane but surprisingly also in the intracytoplasmic loop domain of ABCB1. Conserved *gate* sequences in the animal ABCB1 transporters explain their substrate promiscuity, while a divergent *gate* region in plant ABCBs accounts for their substrate specificity. Comparative models with putative plant ABCB importers recognized potential coiled coil domains that might regulate the directionality of the plant ABCB transporters by recruiting interacting proteins. Finally, molecular *in silico* docking between Arabidopsis auxin exporter ABCB1 and its regulator of transport activity and specificity, FKBP42/TWISTED DWARF1 (TWD1), predicted an ABCB1 docking surface. In summary, our bioinformatics approaches provide a framework for the molecular investigation of ABCB transport activity, specificity and directionality that might have also a direct clinical impact."

(a) University of Zurich (b) Purdue University

P38007 Saport: A semi-automatic transporter annotation system

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"Membrane transport proteins play crucial roles in living cells. Experimental characterization of transporters is costly and time-consuming. Current computational methods of automatic transporter annotation still require extensive curation efforts due to low predictive accuracy, especially for plant genomes. Hereby, we develop a semi-automatic system, called Saport, to aid the systematic transporter annotation on a genome scale. Saport takes simple DNA or protein sequences as input and outputs all predicted candidate transporters with various supportive evidences derived from the input sequences, including highly accurate automatic predictions, membrane topology and domains or Gene Ontology distribution, phylogenetic analysis related to known transporters. Saport also includes a series of tools to facilitate the manual curation such as visualizing, sorting, ranking, filtering and cross-linking of the supportive evidences. The embedded automatic transporter prediction program integrates sequence homology-based and machine learning-based methods in a two-phase approach with classification rules learned from well-curated proteomes. Through cross-validation using the yeast proteome as training and ten other proteomes as testing, we validated 80.0% of predictions on the tested proteomes and our predictions exactly matched 83.0% of the corresponding putative transporters in TransportDB. In an independent test using the Arabidopsis proteome as training and seven plant proteomes as testing, we validated 71.1% of the predictions via manual curation and our predictions exactly matched 75.8% of the manual curation. Saport is freely available at *http://bioinfo3.noble.org/saport*."

(a) The Samuel Roberts Noble Foundation

P38008 Structure-Function Relations Underlying the Functionality of the ALMT and MATE-type Transporters Involved in the Organic Acid Release Al Tolerance Response

Pineros, Miguel A.-presenter map25@cornell.edu(a) Ligaba, Ayalew (a) Kochan, Leon V (a)

"Many plants species overcome aluminum (Al) toxicity by releasing Al-chelating organic acids from the roots, consequently detoxifying Al^{3+} at the root surface, and preventing it from entering the root. Two distinct families of transporters [Al-activated malate transporter (ALMT) and multidrug and toxin efflux (MATE)] underlie this organic acid efflux response in different plant species. We are conducting a comparative analysis of the structural and functional (electrophysiological) features of diverse members from these two major families of transporters. Functional (TEVC recordings in *Xenopus* oocytes) analysis of ALMTs from different plant species indicate that a high degree of amino acid identity and conserved predicted structure does not necessarily correlate with common transport function as revealed by electrophysiological analysis. That is, transporters with similar predicted structures could exhibit different functional (e.g. the type and affinity of the anion transported) and regulatory (e.g. the degree or lack of transport enhancement by Al^{3+}) properties. Structure/function relationships for ALMT1 proteins were examined by generating specific ALMT1 mutants and expressing them in *Xenopus* oocytes. Using this approach, we are identifying protein domains and specific amino acids residues (i.e. single point mutations) involved in TaALMT1 transport regulation (e.g. enhancement of transport activity by extracellular Al^{3+}). The predicted structures of the MATE transporters, as well as their transport characteristics, are significantly different than the predicted structure of ALMT-type transporters. These differences in transport and regulation between MATE and ALMT anion transporters will be discussed in terms of their structure-function relations."

(a) Robert W. Holley Center for Agriculture and Health, USDA-ARS, Cornell University

P38009 Characterization of Arabidopsis mutants lacking multiple vacuoler/endosomal Na+/H+ antiporter functions

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"Na^+/H^+ antiporters play a major role in pH and Na^+ homeostasis of cells throughout the biological kingdom, from bacteria, algae, fungi and worms to plants and humans. Na+/H+ antiporters are integral membrane proteins residing in the plasma membranes and endomembranes of different cells. According to their amino acid sequence similarity, the Arabidopsis thaliana vacuolar/endosomal Na^+/H^+ (NHX) antiporters can be grouped in two different sub-groups, AtNHX1-4 and AtNHX5-6. While the first group is >75% similar, the second group is <30% similar to the first. AtNHX1 have been shown to play important roles in cellular homeostasis, including the sequestration of Na^+ and K^+ ions into the vacuole, the regulation of vacuolar pH and leaf development. On the other hand, the contribution of the additional members of this gene family to plant development and ion homeostasis has not been studied and their roles remain unknown. In order to establish the roles of all endosomal AtNHX members, we characterized single knockout lines of AtNHX1-6 and generated multiple knockout lines of the different NHXs. Single to quadruple knockouts lines of *nhx1/nhx2/nhx3/nhx4* were generated separately from single and double knockouts of *nhx5* and *nhx6*. NHX-GFP- and NHX-YFP chimeras and different intracellular-specific probes were used to assess the localization and function of each family member. In addition, phenotypical differences among the single and multiple knockout mutants were characterized. Our results revealed unique and diverse functions for each member and the individual role(s) in the regulation of cell volume, ion homeostasis, cell growth and development will be presented and discussed."

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P38010 "Genes controlling Ca accumulation in specific leaf cell types are also necessary for regulating apoplastic Ca, stomatal conductance and growth"

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http://www.agwine.adelaide.edu.au/plant/plant_phys/pcp/

"The way calcium (Ca) is stored and can be subsequently manipulated in leafy vegetables impacts upon plant, human and animal nutrition. A large-scale study on the leaves of 40 plant species using X-ray microanalysis has highlighted accumulation patterns for Ca within specific leaf cell types to the exclusion of other cell-types that are conserved within different plant families. Calcium accumulation occurs within the vacuole of epidermal cells of grass monocots, which contrasts to Ca accumulation in the mesophyll cell vacuoles of eudicots. To correlate gene expression with Ca accumulation profiles we micropipetted RNA from epidermal and mesophyll cells of the eudicot Arabidopsis thaliana. Cell specific RNA libraries were then analysed by microarray, followed by qPCR validation of a number of candidate membrane transporters with greater relative expression in each of the respective cell-types. Knockout mutagenesis of one candidate, a Ca^{2+}-transporter which is expressed predominantly in the mesophyll, resulted in no mutant phenotype. When the expression of a complementing family member was also abolished, this resulted in a reduction in total leaf [Ca] and perturbance of the Ca distribution pattern. The double knockout plant had a 3-fold higher apoplastic [Ca] which correlated with reduced stomatal conductance, photosynthetic rate and consequently growth. Apoplastic [Ca], stomatal conductance and growth rate, but not leaf Ca accumulation patterns, could be recovered to wild-type levels by Ca deprivation. We will discuss the role and identity of the candidate gene and its homologues as regulators of calcium distribution in leaves, and as necessary regulators of apoplastic calcium."

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P38011 Interactions among nitrate sensor CHL1 and CBL-interacting protein kinases (CIPKs) in nitrate signaling

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"Nitrate is a critical nutrient. In addition to being a nutrient source, nitrate also serves as a signaling molecule for plant development and gene expression. A central question in past decade is how nitrate levels in soil are sensed by a plant and which signaling components and molecule mechanisms are used in plants. However, no ion sensor has been identified in higher plants. Recently, we have found several members of the CBL-interacting protein kinase family (CIPK) participate in nitrate signaling and can interact with putative ion sensor CHL1. Our previous study indicated that CHL1 is a dual affinity nitrate transporter, and two action modes are switched by phosphorylation/dephosphorylation at residue theronine 101. Kinetic analysis of nitrate-induced transcriptional response (primary nitrate response) indicated that similar to the kinetic properties of nitrate uptake, primary nitrate response also displayed two saturable phases with K_m~20µ;M for high-affinity phase and K_m~0.7mM for low-affinity phase. Moreover, CHL1 and CIPKs are involved in the primary nitrate responses. Primary nitrate responses of *cipk* mutants indicated that high-affinity and low-affinity response are differentially regulated by different members of CIPKs. Further studied on the effluences of CIPKs on CHL1 and nitrate sensing indicated that using phosphorylation switch, CHl1 can sense wide-range of nitrate concentration changes and led to different levels of responses."

(a) Institute of Molecular Biology, Academia Sinica. (b) Taiwan International Graduate Program, Academia Sinica

P38012 Protoplasts and intact vacuoles as experimental models to study the mechanisms of solute transport and compartmentation and tonoplast energization in grape cells

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"Sugars, acids, phenolics and water are mostly accumulated in the vacuole of the grape berry cells, with a great impact into plant productivity and wine quality, but the knowledge about tonoplast transport in higher plants is still rather limited. As in many species, the major bottleneck to study these aspects in grapevine is the obtention of highly purified vacuoles with a good yield. This work describes the preparation of intact and viable protoplasts and vacuoles from grape cells, and their feasibility as a model system to study the mechanisms underlying tonoplast energization and solute transport. Protoplasts were prepared by enzymatic digestion of grape cells, and vacuoles were released and purified by a Ficoll step gradient centrifugation. The tonoplast stained strongly with the fluorescent dye FM1-43 and most vacuoles maintained an internal acidic pH, as assessed by Neutral Red. Flow cytometry analysis of vacuole samples incubated with the calcium-sensitive fluorescent probe Fluo4-AM revealed a well-defined sub-population of intact vacuoles. As assessed by the pH-sensitive probe ACMA, intact vacuoles generated and maintained a pH gradient through the activity of V-ATPase and V-PPase and were able to transport Ca^{2+} via a proton-dependent transport system. HPLC analysis showed that intact vacuoles preferentially accumulate glucose and fructose to concentrations up to 1 M, but no evidence was obtained for sugar-induced proton movement. This work was supported by the Fundacao para a Ciencia e a Tecnologia (grant no. SFRH/BD/23169/2005 to N.F) and Accoes Integradas Luso-Francesas N F-25/08 (CRUP/CPU). "

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P38013 Evolution of Plant Major Intrinsic Proteins

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"Major Intrinsic Proteins (MIPs, also called Aquaporins, AQPs) are channel forming membrane proteins. Although initially functionally characterized and named after their water channeling property in human red blood cells, it has become increasingly evident that MIPs are present in almost all organisms and transport a variety of small, uncharged molecules besides water. MIPs have a highly conserved structure and it is believed that the pore structure, with a constriction region and an electrostatic repulsion filter, is responsible for the high transport rate and selectivity of MIPs. In plants, MIPs form a large and varied protein family, with roughly two to three times the number of MIP isoforms found in animals. Although this abundance implies that MIPs have important functions in plants, the roles of individual MIPs have so far only been described for very few isoforms. Differences in the filter regions and experimental data suggest differences in substrate specificities as well as localization for different MIP subfamilies. However actual functions of different isoforms remain largely unknown as knock out/knock down experiments have failed to reveal any clear phenotypes. Using another approach to decipher the different functions of MIP isoforms, we try to link the emergence of specific subfamilies with different traits. The single celled algae *Chlamydomonas reinhardtii* contain only 2 MIPs, suggesting that the plant MIP family expansion is linked to multicellularity and different aspects of land colonization. Previously we identified 23 MIPs in the moss *Physcomitrella patens*, showing that already early non vascular land plants had a diverse MIP family and we are now trying to identify MIPs in charales, in order to see how this expansion is related to land colonization. "

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P38014 Sodium and potassium transport selectivities of the OsHKT2;1 and OsHKT2;2 transporters in plant cells

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"Na^+ and K^+ homeostasis is crucial for plant growth. Two HKT transporter/channel classes have been characterized that mediate either Na^+ transport

or Na^+ and K^+ transport when expressed in heterologous systems. But their Na^+/K^+ selectivities have not yet been studied in plant cells. Here we analyze two highly homologous HKT transporters from indica rice, cv Pokkali, OsHKT2;1 and OsHKT2;2, that show differential Na^+/K^+ selectivities in heterologous systems. When these transport proteins were stably expressed in plant BY2 cells, OsHKT2;1 mediated only Na^+ uptake, but not K^+ or Rb^+ uptake, consistent with findings in heterologous systems and in rice *oshkt2;1* knockout mutants (T. Horie et al., 2007 *EMBO J.*). In contrast, OsHKT2;2 mediated Na^+-K^+ co-transport in plant cells. Furthermore, extracellular K^+ stimulates OsHKT2;2-mediated Na^+ influx. OsHKT2;2 also mediates Na^+-stimulated $K^+(Rb^+)$ influx, showing that OsHKT2;2 is a Na^+ and K^+ transporter/channel."
(a) University of California, San Diego (b) Wuhan University

P38015 Functional analysis of a rice spermidine transporter gene by heterologous expression in yeast mutants
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"Polyamines are ubiquitous nitrogenous compounds found in all eukaryotic and prokaryotic cells. In plants, they regulate several growth and developmental processes and the levels of polyamines are also correlated with the plant response to stresses such as drought, salinity and temperature. Polyamines are known to be actively translocated through the vascular tissues. However, the transport of polyamines in and out of the plant cells has not been yet linked to any plant genes. In the present study, we used a comparative genomics approach to identify and characterize polyamine transporters in rice and *Arabidopsis thaliana* genomes. We identified three and four candidate transporter genes in *A. thaliana* and rice, respectively. The full-length cDNA clone of one of the rice candidate genes was transferred to the Gateway expression vector, pDEST 52, by recombination cloning. The heterologous expression of this gene in *AGP2*-deficient yeast mutant was mediated under the control of *Gal1* promoter. Radiological time-dependent, uptake studies of the above rice gene showed an enhanced uptake of spermidine by transformants relative to the mutant strain. The kinetic characterization of this gene for spermidine showed a Km of 18.48 µ;M indicating that the gene is a high affinity spermidine transporter in rice. When tested with putrescine as an alternate substrate, the rice gene was found to be a spermidine specific importer. This is the first report of a characterization of a plant polyamine transporter. The future work will focus on the phenotypic characterization of mutants of these predicted transporter genes under different environmental stress conditions. The functional analysis will enable a systems approach to polyamine homeostasis in plants."
(a) Bowling Green State University

P38016 A systems model of vesicle trafficking in Arabidopsis pollen tube
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"Pollens show persistent polarized growth during pollination. This polarized growth, in a part, depends on vesicle trafficking that regulates the flow of macromolecules to the tip of these cells. Although the biological importance of individual genes that are involved in vesicle trafficking in pollens could be revealed through knock-out or -down analyses, their roles in the physiological system that leads to specific vesicle trafficking and the polarized growth are difficulty to elucidate. To study their roles at a systems level, we established a hypothetical model that describes the behavior of vesicle trafficking in polarized pollens (pollen tubes). Our model is a combination of a mathematical model, which is originally proposed by Heinrich and Rapoport (JCB, 2005, 271-), and bioinfomatic data. The model requires selected SNAREs (N-ethylmaleimide sensitive factor attachment protein receptors) and GTPases, as a minimum machinery of vesicle trafficking in a pollen tube, and predicts the rate of the polarized growth. By analyzing publicly available data of gene ontology and expression, we identified Arabidopsis vesicle-trafficking genes whose expressions are regulated by the signaling pathway of the pollen development. We further analyzed subcellular localizations and protein-protein interactions of these gene products in Arabidopsis cells. In this meeting, we will explain how these genes could control vesicle trafficking and lead the pollen tube growth in the physiological system, based on the mathematical model and our cell-biological data."
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P38017 The ABC transporter AtABCB14 is a malate importer and modulates stomatal response to CO_2
Lee, Miyoung-presenter anny98@postech.ac.kr(a) Choi, Yongwook (a) Burla, Bo (b) Kim, Yu-Young (a) Jeon, Byeongwook (a) Maeshima, Masayoshi (c) Yoo, Joo-Yeon (a) Martinoia, Enrico (b,a) Lee, Youngsook (a)
"Carbon dioxide uptake and water vapour release of plants occur through stomata that are formed by the guard cells, which respond to light intensity, CO_2, H_2O availability and plant hormones. The predicted increase of atmospheric [CO_2] is expected to have a profound impact on our ecosystem, however, many aspects of CO_2-dependent stomatal movements are still not understood. Here we show that the ABC transporter AtABCB14 modulates the stomatal closure upon transition to elevated [CO_2]. High [CO_2] induced stomatal closure was accelerated in plants lacking AtABCB14. Apoplastic malate has been suggested to be one of the factors mediating stomatal response to [CO_2] and indeed exogenously applied malate induced a similar AtABCB14-dependent response as high [CO_2]. In isolated epidermal strips which contained only guard cells, malate-dependent stomatal closure was faster from plants lacking the AtABCB14, and slower in AtABCB14 over-expressing plants, than in wild type, indicating that AtABCB14 catalyzes the transport of malate from the apoplast into guard cells. Indeed, when AtABCB14 was heterologously expressed in *E. coli* and HeLa cells, increases in malate transport activity were observed. We therefore suggest that AtABCB14 modulates stomatal movement by transporting malate from the apoplast into guard cells, thereby increasing their osmotic pressure. "
(a) Department of Life Science, Pohang University of Science and Technology (b) Institute of Plant Biology, University of Zurich (c) Graduate School of Bio agricultural Sciences, Nagoya University

P38018 Characterization of the novel aquaporin subfamily XIP in *Nicotiana benthamiana*
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"Aquaporins or Major Intrinsic Proteins (MIPs) are pore forming membrane proteins that facilitate the passive transport of water and/or other small, polar molecules. The aquaporin superfamily is ancient and can be found in all kingdoms of life. Especially in plants they form a highly divergent group of proteins, able to transport a large variety of solutes. Bioinformatic analysis of the recently sequenced genome of the bryophyte *Physcomitrella patens* revealed two aquaporins belonging to a hitherto unrecognized subfamily. Further analysis suggested that this subfamily is still present in many dicots but seem to have been lost in monocots. It was named X Intrinsic Proteins (XIPs) to emphasize the lack of information about these proteins. Curiously, it is not present in *Arabidopsis thaliana* which explains why it was not recognized earlier. Currently the expression pattern of a member of the XIP subfamily in *Nicotiana benthamiana* is being elucidated along with its specificity and subcellular localization to gain insight into the function of the members of this novel subfamily of aquaporins. "
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P38019 Vacuolar localization and tonoplast transport of salicylic acid glucose conjugates in *Arabidopsis thaliana*
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"In most plants salicylic acid (SA) is metabolized to glucose conjugates. These glucose conjugates are then typically transported into the vacuole through either an ATP-binding cassette (ABC) transporter-type mechanism or an H^+-antiporter. In Arabidopsis, exogenously supplied SA is converted into SA 2-O-β-D-glucose (SAG), SA glucose ester (SGE) and 2,5-dihydroxybenzoic acid 2-O-β-D-glucose (DHB2G). In order to determine the intracellular localization of SA and its metabolites, we isolated protoplasts and vacuoles from Arabidopsis leaves following a 12 h incubation with ^{14}C-SA. From these studies we were able to determine that SA, SAG and DHB2G were all localized primarily in the vacuole. However, SGE was localized outside the vacuole, presumably in the cytoplasm. The vacuolar transport of SAG was measured using tonoplast vesicles isolated from Arabidopsis cell cultures. The uptake of SAG was stimulated 3-fold by the addition of ATP. The ATP stimulated uptake of SAG was inhibited 23% by vanadate (ABC transporter inhibitor), but not by bafilomycin A_1 (vacuolar H^+-ATPase inhibitor). Therefore, vacuolar uptake of SAG in Arabidopsis appears to be due, in part, to an ABC transporter-type mechanism. We conducted a preliminary study using T-DNA insertion mutants to determine if a multidrug resistant associated protein- (MRP-) type ABC transporter might be involved in the vacuolar uptake of SAG in Arabidopsis. SAG uptake into tonoplast vesicles isolated from *atmrp2*, *atmrp4* and *atmrp10* mutants was similar to that of the wild-type (Col-0). However, SAG uptake into vesicles isolated from the *atmrp1* knockout mutant was only 58% of the uptake observed in the wild-type. Therefore, it appears as if AtMRP1 may play a significant role in the vacuolar transport of SAG in Arabidopsis."
(a) DePaul University

P38020 Potential Role of Multiple Cation/H^+ Exchangers (CHX) in Endomembrane Trafficking
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"Membrane trafficking is critical for growth, development and adaptation, though the participation of ion transporters in membrane trafficking in plants is poorly understood. Several Arabidopsis AtCHX genes predicted as cation/H^+ exchanger were functionally tested using yeast mutants lacking a CHX homolog, ScKHA1. Expression of group I genes (CHX17-19) conferred growth at basic pH and low K^+, and resistance to hygromycin B (HygB). However, group II genes (CHX16 & CHX20) conferred growth at acidic and basic pH with low K^+, and sensitivity to HygB. Furthermore, CHX20 acidified cytoplasmic pH in yeast as monitored by pHluorin whereas CHX17 had no effect. GFP-tagged CHX16 or CHX20 displayed a reticulate pattern in leaf protoplast. In contrast, group I protein, such as CHX17-GFP, labeled punctate structures and the cell periphery in protoplasts and in transgenic plants, consistent with localization to prevacuolar compartment (PVC) and to plasma membrane (PM). CHX17 stimulated the secretion of carboxypeptidase Y (CPY) which is usually sorted to the yeast vacuole, though CHX20 decreased CPY secretion. The membrane localization of CHX17 and the enhanced CPY secretion mediated by ScKHA1 or AtCHX17 in yeast, would point to a potential role of CHX17 in exocytosis. We propose that two subgroups of CHX affect trafficking of distinct endomembranes possibly through their effect on localized K^+ and pH homeostasis. (Supported by NSF 2010 & by Dept Energy BES to HS) "
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P38021 The Cyclic Nucleotide-Gated Channels AtCNGC19 and AtCNGC20 Localize to the Vacuole Membrane of Arabidopsis
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"The twenty members of the cyclic nucleotide-gated channel family in Arabidopsis can be divided into five phylogenetic groups, designated I-III, IV-A and IV-B. All plant CNGCs possess overlapping cyclic nucleotide-binding and calmodulnin-binding domains at their C-terminal ends. However, the two group IV-A members, AtCNGC19 and AtCNGC20, are unusual in that their N-terminal tail regions are approximately 50 - 100 residues longer than that of other CNGC paralogs, and are predicted by ChloroP to contain chloroplast transit peptides. To investigate the subcellular localization of these channels, we have generated translational fusion constructs of AtCNGC19 and AtCNGC20 to the fluorescent marker GFP, using both their full-length and N-terminal tail sequences. Co-transfection of Arabidopsis leaf protoplasts with these and a series of RFP-tagged subcellular marker constructs revealed that the full-length AtCNGC19::GFP and AtCNGC20::GFP fusion proteins did not target to chloroplasts, mitochondria, or peroxisomes. Instead, their principle site of localization appears to be the tonoplast. Slight overlap with RFP markers for the golgi and endoplasmic reticulum was also occasionally observed. In addition, the subcellular localization patterns of AtCNGC19::RFP and AtCNGC20::GFP were strongly overlapping, raising the possibility that the gene products of *AtCNGC19* and *AtCNGC20* may interact to form heteromeric channels in plants. Interestingly, the full-length and truncated versions of AtCNGC19 do not co-localize, indicating that the N-terminal tail region alone is insufficient for proper targeting to the vacuole. Their positioning within the tonoplast suggests AtCNGC19 and AtCNGC20 as possible candidates for mediation of the fast-activating vacuolar (FV) conductance in plants."
(a) University of Hawaii - Manoa

P38022 "Functions of Vacuolar Two Pore Channel 1 (TPC1), in *Arabidopsis* Stomatal Closure"
Islam, Mohammad Mahbub (a) Shintaro, Munemasa (a) Hossain, Mohammad Anowar (a) Nakamura, Yoshimasa (a) Mori, Izumi C (a) Murata, Yoshiyuki-presenter muta@cc.okayama-u.ac.jp(a)
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"Stomatal closure, essential for plant survival in dry conditions, requires activation of Ca^{2+} permeable channels on both plasma membrane tonoplast, and activation of S-type anion channels in the plasma membrane of the guard cell during stomatal closure. *Arabidopsis* Two Pore Channel 1 (AtTPC1) is a slow vacuolar (SV) Ca^{2+}-dependent Ca^{2+} release channel (1). TPC1 functions as a vacuolar cation channel without a major impact on cytosolic Ca^{2+} homeostasis (2). Thus, it is under debate whether TPC1 functions in Ca^{2+} signaling in guard cells. To investigate whether TPC1 is involved in stomatal closure, we examined effect of Abscisic acid (ABA), methyl jasmonate (MeJA) and Ca^{2+} on stomatal movements, second messenger production and S-type anion channel activation involving stomatal closure in the *tpc1-2* mutant. In *tpc1-2* mutant as well as wild type plants, both ABA and MeJA induced stomatal closure, cytosolic alkalization and production of reactive oxygen species (ROS) and nitric oxide (NO) in guard cells. ABA and MeJA elicited cytosolic calcium $[Ca^{2+}]_{cyt}$ oscillations in *tpc1-2* guard cells as wild-type. However, in *tpc1-2* mutant, external Ca^{2+} failed to induce stomatal closure and activate plasma membrane S-type anion currents, but Ca^{2+} elicited $[Ca^{2+}]_{cyt}$ oscillation. Our results indicate that in guard cells TPC1 functions in response to external Ca^{2+}, but not to ABA and MeJA and that TPC1 is involved in priming of plasma membrane S-type anion channels by external Ca^{2+} in Arabidopsis guard cells. (1) Peiter *et al* (2005) *Nature* **434**: 404-408 (2) Ranf *et al* (2008) *Plant Journal* **53**: 287-299 "
(a) Okayama Univ

P38023 Bimolecular fluorescence complementation for *in vivo* detection of SNARE interacting partners
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"Bimolecular fluorescence complementation (BMFC) is an emerging technique used to study the interaction of two cytoplasmic proteins. Here, we show that BMFC can also be used to confirm interactions between two membrane bound proteins. In BMFC, the N-terminus of the Enhanced Yellow Fluorescent Protein (nEYFP) is fused with a protein-of-interest, and the C-terminus of EYFP (cEYFP) is fused with its putative interacting partner. Interaction of the proteins will reconstitute a functional EYFP molecule and result in fluorescence. This study used two Soluble N-ethylmaleimide-sensitive factor attachment protein receptor proteins (SNARE) that are associated with gravitropism. The vesicle-SNARE (v-SNARE), VTI FAMILY 11 (VTI11), was fused with nEYFP, and the cognate target-SNARE (t-SNARE), SHOOT GRAVITROPISM 3 (SGR3), was fused with cEYFP. Both constructs

were simultaneously coated onto gold particles and co-bombarded into onion peels for transient expression. Although constructs bombarded individually did not produce fluorescence, YFP fluorescence was observed in four out of 1200 (approx.) cells, where both the constructs were expressed simultaneously. Fluorescence in co-bombarded cells indicated an interaction between SGR3 and VTI11. Optical sections using confocal microscopy revealed that interaction was localized to the tonoplast. Results showing YFP fluorescence on tonoplast confirmed the predicted function of VTI11 in association with SGR3. This study confirms that BMFC can be used for *in vivo* detection of proteins whose function is membrane-association dependent."
(a) Ohio University (b) Southern Illinois University Edwardsville

P38024 Monolignol Transporters and Cell Wall Oxidases Screens
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"Currently, biofuels, such as ethanol are produced largely from starch contained in grains, but it represents only a little proportion of sugar polymer availability on Earth. Large quantities of sugar from polysaccharides that are not utilized thus far are cellulose and hemicellulose, which are the main constituent of plant cell walls (95-70%). The rest of the plant cell walls are mainly composed of lignin, which inhibits efficient extraction of sugars from the cell wall thus prevents cost-effective lignocellulosic ethanol production, and is a very strong phenolic polymer recalcitrant to degradation. Unfortunately, lignin cannot simply be genetically removed without incurring deleterious consequences on plant productivity, because it gives a strong structural support to the plant but also protects the plant against biotic and abiotic stresses. Therefore, it is important to better control of lignin deposition and cross-linking within the cell wall, which may increase sugar recovery from the cell wall polysaccharides. In order to be able to modify lignin deposition, polymerization and cell wall cross-linking, a better knowledge of the enzymes (oxidases) that participates in the polymerization of lignin and cross-linking to other cell wall components is required. Similarly, to be able to control monolignol export into the apoplast, monolignol transporters need to be identified and characterized. Therefore, we are currently developing a strategy using yeast complementation to try to indentify protein involved in lignin deposition. Plant cDNA libraries, a complete Arabidopsis MDR transporters library and selected genes will be heterologous expressed in yeast and tested for their ability to detoxify phenolic compounds by export or polymerization. "
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P38025 "Characterisation of barley P_{1B}-ATPases, HvHMA1 and HvHMA2 "
Mills , Rebecca F (a) Baekgaard, Lone (b) Dalgaard Roed, Maria (b) Palmgren, Michael G (b) Williams, Lorraine E-presenter lew@soton.ac.uk(a)
"Manipulation of crops to improve their nutritional value (biofortification) and optimization of plants for removal of toxic metals from contaminated soils (phytoremediation) are major goals. Further understanding of membrane transporters with roles in zinc and cadmium transport would be useful for both aspects. The eight P_{1B}-ATPases identified in Arabidopsis play important roles in heavy metal allocation and detoxification. Relatively little is known about these pumps in monocots and therefore we have studied members of this family in barley. Nine P_{1B}-ATPases were identified in barley from EST analysis. This work focuses on the functional characterization of HvHMA1 and HvHMA2 and provides evidence for their role in heavy metal transport. cDNAs were cloned for both and structure/function analysis was performed by heterologously expressing these pumps in yeast and carrying out metal sensitivity/resistance tests. Results indicate that: both pumps have motifs and key residues characteristic of P_{1B}-ATPases; HvHMA1 can transport Cu, Zn and Cd in yeast; HvHMA2 transports Cd in yeast while HvHMA2 truncated at the C-terminus functions in Zn transport. Transport rather than binding is suggested by the fact that mutating a key residue critical for transport function in P-type ATPases abolished the particular metal-dependent sensitivity/resistance. We also investigated the *in planta* functioning of these pumps by expressing them in wild-type Arabidopsis and mutants. The Arabidopsis *hma2/hma4* mutant is severely stunted due to the lack of Zn translocation from roots to shoots. HvHMA2 restored the wild-type phenotype in this mutant indicating that HvHMA2 may have functional equivalence to AtHMA2 and AtHMA4 in zinc transport in the plant. "
(a) University of Southampton, UK (b) University of Copenhagen, Denmark

P38026 Biochemical evidences for identification of a plastid Na+ dependent pyruvate transporter in plants
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" Pyruvate is an essential metabolite in plastids as an initial substrate of lipid-, isoprenoid-, branched-chain amino acid synthesis and a major metabolite of C4 cycle. The cross-talk between cytosol and plastid has been speculated, however, a molecule of specific transporter is not identified. By transcriptome analysis between C3 *Flaveria pringlei* and C4 *F. trinervia*, we isolated a novel type plastid transporter gene as a candidate of the pyruvate transporter functioning in C4 cycle. This gene product was localized at chloroplast envelop in mesophyll cells and was commonly abundant in several Na+-dependent pyruvate transporter type-C4 species. By using the whole cell of heterologous expressed *E. coli*, sodium-dependent pyruvate uptakes were detected. These results indicate that these genes encode plastid Na+-dependent pyruvate transporters, functioning in C4 carbon cycle."
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P38027 Mechanism regulating *HvAACT1* expression in barley
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"Barley is the most Al-sensitive species among small grain cereals, but there are large genotypic variations in the Al tolerance (Ma et al., 1997). Physiological study has shown that Al-tolerant cultivars of barley detoxify Al by secreting citrate from the roots (Zhao et al., 2005). Recently, a gene responsible for Al-activated citrate secretion (*HvAACT1*) has been identified (Furukawa et al., 2007). The expression of this gene is not induced by Al and Al-tolerant cultivars constitutively have higher expression than Al-sensitive cultivars. HvAACT1 protein is located at the root epidermal cell. In the present study, to examine the mechanism regulating the expression of *HvAACT1*, we compared the sequence of UTR regions and genome of *HvAACT1* between Al-sensitive and tolerant cultivars. There was no correlation between Al tolerance and the sequence difference in the 3'-UTR region and Intron region. In the 5'-region, we found that a specific sequence (about 1 kb) was inserted in the genome of ORF upstream only in the Al-tolerant cultivars. 5'-RACE analysis showed that in Al-tolerant cultivar, there were multiple transcription start sites (TSS) in the specific region except for common TSS with Al-sensitive cultivar. We then designed specific primers and compared the expression of different 5'-UTR regions. There was no difference in the expression of common TSS region between Al-sensitive and tolerant cultivars, however, the expression of other 5'-UTR region originating from the specific region was very high. Moreover, results from promoter assay experiment using GFP fused with the specific region showed that this region has promoter activity. These results suggest that this specific region in the 5'-UTR may be involved in the higher expression of *HvAACT1*. "
(a) Research Institute for Bioresources Okayama University

P38028 Vacuolar transport of nicotine is mediated by a MATE-type transporter in *Nicotiana tabacum*
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"Alkaloids are nitrogen-containing low-molecular-weight organic substances that have a wide variety of chemical structures and often show diverse biological activities. Some alkaloids are transported from the source organ after their biosynthesis and moved to sink organs via long distance transport. One representative of such translocation of alkaloids is nicotine, a pyridine alkaloid, which is biosynthesized in root tissues, then translocated to the leaves, and finally accumulated in the leaf vacuoles in *Nicotiana* species. Although it is more than 10 years ago since nicotine translocation was identified, no transport protein has been characterized concerning the inter-organ movement of this alkaloid. Recently, we characterized a novel multidrug and toxic compound extrusion (MATE)-type transporter, Nt-JAT1 (*Nicotiana tabacum* jasmonate-inducible alkaloid transporter 1). Nt-JAT1 was identified as a gene that was co-regulated with nicotine biosynthetic genes following methyl jasmonate treatment of tobacco BY-2 cells. This MATE gene was expressed in the leaves, stems, and roots in tobacco plants. Biochemical analyses using a yeast cellular transport system and proteoliposome system suggested that Nt-JAT1 transported nicotine using the H+ gradient across the membrane as its driving force. The location of Nt-JAT1 was shown to be the tonoplast. These data suggested that Nt-JAT1 plays an important role in the nicotine translocation by acting as a transporter responsible for the unloading of nicotine in the aerial parts of the plant and its deposition in the vacuoles. To our knowledge, this is the first identification of a vacuolar transporter for alkaloids in plant. A possible application of this alkaloid transporter for the production of valuable alkaloids is also discussed. "
(a) Research Institute for Sustainable Humanosphere, Kyoto University (b) Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University (c) Department of Plant Biotechnology and Genetics, Ghent University (d) VTT Technical Research Centre of Finland (e) Department of Plant Systems Biology, Flanders Institute for Biotechnology

P38029 The *in planta* function and regulation of AHA1 and AHA2 analyzed using reverse genetics
Nelson, Rachel B.-presenter rachelnelson06@alum.wellesley.edu(a) Haruta, Miyoshi (a) Burch, Heather (a) Sussman, Michael R. (a)
"The plant/fungal plasma membrane proton pump belongs to the family of P-type ATPases. This enzyme uses up to one third of the cellular ATP to dynamically control the proton electrochemical gradient across the PM and drive diverse downstream functions including secondary transport of ions, sugars, and amino acids and turgor regulation. In Arabidopsis there are 11 PM proton pumps (abbreviated AHA for Arabidopsis H^+-ATPase); however, *AHA1* and *AHA2* are the most highly expressed and account for 75% of the overall mRNA in vegetative tissue. The function and regulation of these proteins is of great interest in plant biology since changes in AHA activity have been correlated with a large number of environmental perturbations, including pathogen attack and hormonal control of cell elongation. Studying AHA1/2 via yeast heterologous expression systems and the 3D crystal structure have been informative concerning possible mechanisms of regulation, yet little is known about their regulation and structure/function roles in planta. We are using loss of function *AHA1/2* mutants to replace endogenous wildtype *AHA1/2* with site-directed point mutants chosen based on PMA1 mutagenic studies. Our current study is focused on W71C, F96A/W, A649R, R871Δ, R880A, T881D, S904, and T948A/D. Additionally, replacing endogenous AHA1/2 with TAP- and GFP-labeled fusion proteins will allow us to investigate interacting protein partners and post-translational modification of AHA1/2, specifically phosphorylation, via highly sensitive mass spectrometry techniques. In this poster, we will present the results of our genetic complementation studies with point mutants and our initial work to understand the regulation of AHA1/2 by phosphorylation using state of the art mass spectrometry instrumentation."
(a) University of Wisconsin Madison

P38030 Analysis of amino acid transporter function in legumes
Tan, Qiumin-presenter qiumin_tan@wsu.edu(a) Tegeder, Mechthild (a)
"Nitrogen is essential for plant growth, and amino acids represent the predominant organic nitrogen (N) transport form in most plants. In legume species, following atmospheric N_2 fixation through a plant-bacteria interaction in root nodules, amino acids, especially glutamine and asparagine, are synthesized and then transported via the xylem to leaves, where they are metabolized, transiently stored or redistributed in the phloem to developing sinks (e.g. flowers and seeds) for metabolism, development and storage reserve accumulation. To understand the underlying mechanism of amino acid partitioning in legumes, French bean (*Phaseolus vulgaris L.*) and pea (*Pisum sativum L.*) amino acid transporters (*AAP*s and *CAT6*) were isolated by functional cloning using yeast transport mutants, and their function in amino acid transport was demonstrated by heterologous yeast complementation. Further, expression patterns of *AAP* and *CAT* transporters within the plant were determined as well as their cellular localization examined. *In situ* RNA hybridization experiments and protein-GFP localization studies suggest that the transporters are involved in cellular import processes and that their function is highly cell-specific. The results also indicate that the transporters are important to amino acid partitioning within the nodules, long distance transport of N in plants as well as amino acid import into seed cotyledons. We are currently analyzing transgenic Arabidopsis and pea plants with altered amino acid transporter expression to understand how N partitioning processes affect plant metabolism and productivity. This work is funded by the National Science Foundation (grant IOS 0448506). "
(a) School of Biological Sciences, Center for Reproductive Biology, Washington State University

P38031 Phloem loading of amino acids by Arabidopsis AAP2 influences carbon and nitrogen metabolism in source and sink
zhang, lizhi-presenter lizhi@wsu.edu(a) Lee, Yong-Hwa (a) Tegeder, Mechthild (a)
"Nitrogen (N) is one of the most important nutrients for plant productivity. Plants acquire N mainly in form of ammonium and nitrate from the soil, and following root uptake, the N is reduced to amino acids that are the dominant N transport compounds within plants. Synthesis of amino acids occurs in source organs such as mature roots and leaves that export the N to supply sinks like flowers and seeds with the essential nutrient for growth. Translocation of amino acids to sinks happens in the phloem and to achieve this, the N compounds have to be loaded into the companion cell-sieve element complex of the phloem. While plasma membrane transporters have been suggested to be involved in this process, physiological evidence was missing. Here, we describe the function of the Arabidopsis amino acid transporter AAP2 in phloem loading. Localization of AAP2-GFP was performed and the proteins were detected in the phloem throughout the plant. Further, the phenotype of AAP2 T-DNA insertion lines was examined and effects on seed carbon, N, and amino acid levels as well as storage compounds were examined. Phenotypic analyses of aap2 compared to wild-type plants revealed changes in seed yield, silique number and in total biomass. In addition, levels of total N and seed storage compounds were altered. When analyzing the consequences of AAP2 repression on source leaves, we found that leaf morphology was changed and leaf metabolism affected. Overall, our results demonstrate that AAP2 is involved in phloem loading and amino acid distribution to sink organs, which influences source physiology, sink storage compound accumulation, sink number and overall plant biomass production. This work is funded by the National Science Foundation (grant IOS 0448506). "
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P38032 Modification of the root/shoot Zn/Cd distribution in tobacco by expression of HMA4 from A.thaliana and from Cd/Zn hyperaccumulator A.halleri

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"Engineering plants for enhanced accumulation and alteration of root/shoot distribution of heavy-metal micronutrient and non-essential ones remains an important biotechnological goal (e.g. biofortification or phytoremediation. In the current research, tobacco was transformed with the P1B-ATPase AtHMA4, recently found to be involved in the regulation of the root-to-shoot transport of zinc and cadmium. The following constructs were introduced into the model plant tobacco: 35S::AtHMA4 full-length; 35S::AtHMA4 C-terminus (from A.thaliana), and pAhHMA4::AhHMA4 (from Zn/Cd hyperaccumulator A.halleri). The AtHMA4-GFP protein was localized to the plasma membrane of tobacco. The regulation of the expression of pAhHMA4::AhHMA4 in tobacco was studied under the exposure to Cd and under Zn deficient and Zn sufficient conditions to compare whether the regulation is similar to that found in A.hallerii. Tolerance and accumulation was studied under the exposure to the whole range of metal concentrations (low to high). Almost all transformants showed and increase in sensitivity to Zn and Cd. For plants expressing either full-length AtHMA4 or AhHMA4, this was accompanied by moderate changes in the concentrations of both metals in roots and shoots. A dramatic alteration in Zn concentration and distribution at the tissue level was detected in AtHMA4-Cterminus plants. Acknowledgements: This work was supported by FP6 EU PHIME project (FOOD-CT-2006-016253) "
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SESSION 39 – METABOLIC ENGINEERING

P39001 Structural and kinetic basis of *glycine max* (soybean) ATP sulfurylase regulation by sulfur assimilation pathway intermediates
Ravilious, Geoffrey E-presenter geravili@artsci.wustl.edu(a) Francois, Julie A (b) Jez, Joseph M (a,b)
"Soybeans are a component of livestock feed, yet lack adequate amounts of the essential amino acids cysteine and methionine. One strategy used to produce soybeans with sufficient amounts of these amino acids involves the introduction of exongenous proteins high in sulfur amino acid content, but this results in decreased levels of endogenous sulfur-rich proteins. This suggests either that the net flux of sulfate (SO_4^{2-}) through the sulfur assimilation pathway (SAP) provides an insufficient supply of sulfide (S^{2-}) or that increased concentrations of pathway intermediates inhibit enzymes in the pathway. Entry of sulfate into the pathway is controlled by ATP sulfuryase (ATPS), which catalyzes the formation of adenosine 5'-phosphosulfate (APS) and PP_i from ATP and SO_4^{2-}. GmATPS favors the reverse reaction ($K_{eq} = 4.5*10^{-3}$). Further decreases in the rate of APS synthesis by feedback inhibition would drastically reduce sulfate production. The specific activity of GmATPS is 0.095 µ;mol/min/mg while the specific activities of proteins downstream of ATPS, APS kinase and APS reductase, are 1.6 and 0.2 µ;mol/min/mg, respectively. Hence, it appears these proteins are adequate to kinetically drive sulfate reductions and that feedback inhibition is a likely cause of reduced flux. We have initiated crystallographic and kinetic studies to identify sulfur assimilation pathway intermediates that inhibit APS synthesis and to analyze structural motifs responsible for their binding. Crystals of ATPS and APSK in complex with SAP intermediates have been obtained, and an initial 3.2 A resolution structure of APSK determined. Findings from this work will suggest strategies for engineering SAP enzymes with reduced sensitivity to feedback inhibition for improved sulfur assimilation."
(a) Department of Biology, Washington University, St Louis, MO (b) Donald Danforth Plant Science Center, St Louis, MO

P39002 A photorespiratory bypass must shift the release of CO_2 from the mitochondrion to the chloroplast in order to maximize the increase of photosynthesis
Devloo, Vincent-presenter vdevloo@msn.com(a) Zhu, Xinguang (a,b)
"An increased rate of photosynthesis is an important target for metabolic engineering of crop plants towards a higher productivity. Most of current research focuses on the following targets: the photosynthetic electron transport chain, Rubisco activity and specificity, Rubisco activation state, photorespiration and the photosynthetic carbon reduction cycle. In particular, photorespiration is a promising target because it releases CO_2 and NH_3 that needs to be reassimilated at the cost of ATP consumption. However, it has been shown that shutting down the photorespiratory pathway is deleterious to photosynthesis in normal air. A photorespiratory bypass can instead increase photosynthesis without the negative impact of completely shutting down the photorespiratory pathway. Using a kinetic model of C_3 photosynthesis with added pathways, we analyzed these reengineering approaches in detail. Results show that shifting the release of CO_2 from the mitochondrion to the chloroplast is required to maximize the increase of photosynthesis. In particular, we confirmed that the *E. coli* glycolate catabolic pathway, recently introduced in the chloroplasts of *A. thaliana*, results in a higher CO_2 concentration inside the chloroplast, which is responsible for the increased photosynthetic rate. Moreover, we show that reducing NH_3 release and thus decreased ATP consumption has only a small effect on photosynthesis, especially under high light conditions. Together, this theoretical analysis strongly suggests that a photorespiratory bypass is an attractive solution for a higher productivity only when the release of CO_2 is shifted from the mitochondrion to the chloroplast."
(a) CAS-MPG Partner Institute for Computational Biology (b) Shanghai Institute of Plant Physiology & Ecology

P39003 RNAi-mediated elimination of toxic gossypol from cottonseed: a powerful model demonstrating the effectiveness of molecular tools to enhance global food security
Rathore, Keerti S-presenter rathore@tamu.edu(a) Sundaram, Sabarinath (a) Sunilkumar, G. (c) Campbell, LeAnne M (a) Puckhaber, Lorraine (b) Stipanovic, Robert D (b)
"Cottonseed, a byproduct of lint production, remains an abundant but greatly underutilized source of protein because of the presence of toxic gossypol. Annual, worldwide production of 44 million metric tons (MMT) of cottonseed contains ~10 MMT of protein, enough to meet the basic protein requirements of 500 million people. We utilized RNA interference to inhibit the expression of the δ-cadinene synthase gene in a seed-specific manner, thereby disrupting a key step in the biosynthesis of gossypol in cotton [Gossypium hirsutum L.; Sunilkumar et al. (2006) Proc. Natl. Acad. Sci., 103: 18054-18059]. Compared to an average gossypol value of 10 µ;g/mg in wild-type seeds, seeds from RNAi lines showed values as low as 0.2 µ;g/mg. Importantly, the levels of gossypol and related terpenoids that are derived from the same pathway were not diminished in the foliage and floral parts of mature plants and thus remain available for plant defense against insects and diseases. Further, we found that the germinating, RNAi seedlings are capable of launching terpenoid-based defense pathway when challenged with a pathogen. Thus, the silenced state of the δ-cadinene synthase gene that existed in the seed, does not leave a residual effect that can interfere with the normal functioning of the cotton seedling during germination. Evidence will be presented showing that transgene-encoded RNAi can be restricted in a spatial and temporal manner in cotton plants. "
(a) Texas A&M University (b) USDA-ARS, Southern Plains Agricultural Research Center (c) Syngenta US

P39004 Genetic modification of nitrogen use efficiency in potato and rice plants by introducing a fungal glutamate dehydrogenase
Aoki, Naohiro (a) Egami, Tomohito (a) Kawakami, Akira (a) Abiko, Tomomi (a) Wakayama, Masataka (a) Sasaki, Haruto (a) Ohsugi, Ryu-presenter aohsugi@mail.ecc.u-tokyo.ac.jp(a)
"The growth and yield of crop plants depend on nitrogenous fertilizers. However an excess amount of inorganic nitrogen has been known to cause environmental pollution and ecological imbalance. It is important to achieve efficient yields of crops with a low amount of nitrogenous fertilizers. To examine the effects of genetic modification of nitrogen metabolism on productivity and nitrogen use efficiency (NUE) in crop plants, a fungal NADP-dependent glutamate dehydrogenase (NADP-GDH) gene was introduced into potato and rice plants under the control of the CaMV-35S promoter. The transgenic potato (GDH-potato) plants showed, compared to the control plants, a higher photosynthetic rate, a larger number of stolons and tubers, a higher tuber yield, and a higher NUE for tuber dry weight. The transgenic rice (GDH-rice) plants showed a higher tiller number and a higher spikelet number per panicle, compared to the control plants, resulting in a higher biomass production and a higher grain yield. NUE was also higher in the GDH-rice plants than the control plants. The higher productivity and NUE in the GDH-transgenic plants was more remarkable in soil with lower nitrogen application. Furthermore, 15N labeling experiments, in combination with a potent inhibitor for plant glutamine synthetase, revealed that in the roots of GDH-rice seedlings, ammonium ions were able to be assimilated directly by the introduced NADP-GDH. These results strongly suggest that the transgenic plants over-expressing NADP-GDH activities possess a high NUE, particularly under low nitrogen condition, resulting from a direct effect of introduced NADP-GDH on nitrogen assimilation. This work was supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN). "
(a) The University of Tokyo

P39005 Controlled silencing of 4-Coumarate:Coenzyme A Ligase alters lignocellulose composition.
Yang, Jaemo-presenter jyang@danforthcenter.org(a) Roger, Beachy N (a)
"Lignocellulose is touted as the primary source of renewable energy in the coming years. An important challenge to achieving this goal is that most biomass, such as corn stover, switchgrass, woody plants and other feedstocks, are not easily converted to fermentable substrates. Typically, lignin, a phenolic polymer, impedes the degradation of cell wall polysaccharides to fermentable sugars. In order to reduce lignin levels during or before full maturation we generated transgenic Arabidopsis plants containing genes that confer constitutive or inducible silencing of the 4CL gene that plays a key role in lignin biosynthesis. While the 4CL gene was highly expressed in WT plants, the amount of 4CL mRNA was greatly reduced in transgenic plants in which the 4CL gene was silenced by RNAi. As a result the stems of the transgenic plants exhibited 25% reduction in lignin with concomitant 20% increase in cellulose. To determine if it was possible to alter lignocellulose composition at specific times in plant development we applied an inducible gene expression system to 4CL-silencing. We treated the gene-inducing ligand to three different growing stages: at bolting, in immature stages (5 ~ 7 cm high), and at intermediate stages (10 ~ 15 cm high). The result was similar to the result in plants with constitutive knock-down of 4CL: i.e., the stems of induced plants exhibited increased cellulose content and reduced amount of total lignin when compared with non-induced stems. Interestingly, even the bolting stems from plants induced during the intermediate stage exhibited altered biochemical composition of the cell wall. Our results suggest that it will be possible to alter lignocellulose composition in some plants without affecting normal growth and development. "
(a) Donald Danforth Plant Science Center

P39006 Developing high-phytonutrient potatoes
Navarre, Roy A-presenter roy.navarre@ars.usda.gov(a,b) Zhang, Wentao (b) Holden, Joanne (a) Shakya, Roshani (b) Goyer, Aymeric (b) Miller, Creighton (c) Reddivari, Lavanya (c)
"Only a fraction of the genetic diversity available in potato wild-species has been incorporated into modern cultivars. LCMS analysis of methanolic extracts from diverse potato germplasm was used to better understand the extent of qualitative and quantitative phytonutrient variation among potatoes. Genotypes were identified with total phenolic content exceeding 10 mg/g DWG or with high antioxidant values that surpass the levels of many other vegetables. Extracts from some genotypes were found to have antiproliferative properties when assayed against prostate cancer cells. Phytonutrient concentration was found to vary during tuber development, with younger tubers having greater concentrations of many compounds, including chlorogenic acid (CGA). Silencing the CGA biosynthetic gene hydroxycinnamoyl CoA quinate hydroxycinnamoyl transferase resulted in a marked increase in tuber flavonols. The folate content of over 70 genotypes was examined and about a 3-fold range found. Even greater concentrations of folate were achieved by overexpressing ADCS and GCHI in a tuber specific manner. "
(a) USDA Agricultural Research Service (b) Washington State University (c) Texas A&M University

P39007 Potential targets for seed improvement through bioengineering starch synthesis pathways
Cao, Fangping-presenter cfp_888@126.com(a) Cao, Heping (b)
"Starch is the principle carbohydrate in the food chain and is a renewable and biodegradable polymer widely used in the food, beverage, paper, textile, and livestock feed industries. It is the major component of the harvestable organs in many agronomic plants. The quantity and quality of starch thus affects crop yield and industrial uses. This has generated great interest in understanding starch biosynthesis pathway. According to the current model, the substrate ADP-glucose is mainly produced in the cytosol by ADP-glucose pyrophosphorylase (AGPase, transferred into amyloplast by adenylate translocator (ANT), and utilized in the synthesis of linear chains by starch synthase (SS). Starch structure is further determined by the activities and specificities of branching enzyme (BE) and debranching enzyme (DBE), and by their interactions with SS and other proteins. Each biochemical step is catalyzed by several isoforms. For example, maize seed has at least two forms of AGPase, ANT, and DBE, 3 forms of BE, and five forms of SS. Starch amount is dramatically reduced by down-regulation of AGPase and ANT, as evidenced from the mutations at the bt1, bt2, and sh2 loci. Starch structure is significantly altered by down-regulation of SS, BE, and DBE, as evidenced from the mutations at the du1, wx1, ae1, and su1 loci. Alteration of starch quantity and quality is also observed in transgenic plants with up- or down-regulation of key enzyme activities in the starch synthesis pathway. Since starch constitutes more than 70% of the dry weight in those seeds and starch synthesis is the predominant biochemical event in seed development, the quantity and quality of nutritionally important protein and oil could be improved by directing certain portion of carbons from starch synthesis."
(a) Beijing Forestry University (b) USDA-ARS Southern Regional Research Center

P39008 Promoter Analysis and Endosperm-specific Expression of Rice Phytoene Synthase Genes (*psy*1 and *2*) in Rice
Leung, Chiu Yi-presenter chiuyi_105@yahoo.com.hk(a) Sun, Samuel, Sai Ming (a)
"Phytoene synthase (PSY) was a key enzyme responsible for the carotenoid biosynthetic pathway, and was employed to boosted the β-carotene content up to 37μ;g/g of rice endosperm in the Golden Rice project which aimed to combat vitamin A deficiency (VDA). Early studies have confirmed that there were duplications of *psy* gene during the evolution of different plant species. In maize and tomato, *psy1* were proven to be responsible for carotenoid accumulation in fruit and seed, while *psy2* mainly played their role in leaves due to the difference in membrane association. Three *psy* homologs have been identified in the genome of rice and were shown to have differential expression pattern. While PSY3 was thought to be related

to abiotic stress-induced abscisic acid formation, the roles of PSY1 and PSY2 have not been characterized. In this project, we investigated the tissue-specific activity of rice *psy1* and *2* by constitutively expressing them under the CaMV35S promoter in rice. With different preferences in membrane association, rice *psy1* and *2* would be active in different tissues. Such differences would be determined by monitoring the changes of carotenoid level in different tissues. A boost of β-carotene up to 440μ;g/g of dry rice calli was detected. Furthermore, seven endosperm specific GCN4 motifs (TGAGTCA) were added into the *psy1* promoter to restore its seed-specific activity. The activity of the modified promoter would be determined using β-glucuronidase (GUS) reporter gene."
(a) Department of Biology, The Chinese University of Hong Kong

P39009 Engineering feedback insensitive enzymes in the lysine synthetic pathway of rice
Yu, Wai Han-presenter veronica_yu72@hotmail.com(a) Sun, Sai Ming Samuel (a)
"Rice is nutritionally unbalanced for its deficiency in certain essential amino acids especially lysine. Aspartate kinase (AK) and dihydrodipicolinate synthase (DHPS) are the two key enzymes involved in the synthesis of lysine. Both enzymes are feedback sensitive to lysine. Previous studies have identified AK and DHPS enzymes feedback insensitive to lysine from natural mutants in *Corynebacterium glutamicum*, *Escherichia coli*, barley and maize. However, no such natural mutant has been identified in rice. To enhance the free lysine content in rice through metabolic engineering, we are attempting to modify the lysine binding domain in genes encoding AK and DHPS for insensitivity to lysine feedback inhibition. The modified AK and DHPS genes were introduced into *Japonica* rice under the control of seed-specific Gt1 promoter to generate transgenic rice plants. Free lysine content in transgenic rice seeds will be analyzed and compared with the wild type rice. Progress and results from this study will be presented and discussed (This research has been supported by the UGC-AoE Center for Plant and Agricultural Biotechnology grant and the PVMR project grant of the Bill and Melinda Gates Foundation). "
(a) The Chinese University of Hong Kong

P39010 "Genetic co-activation of C, N and S assimilation pathways in the Dof1 transgenic Arabidopsis plants"
Fujimori, Tamaki (a,b) Nakano, Ryohei (a,b) Sato, Shigeru (a,b) Ishida, Tetsuya (a,b) Yanagisawa, Shuichi-presenter asyanagi@mail.ecc.u-tokyo.ac.jp(a,b)
http://park.itc.u-tokyo.ac.jp/syokuei/
"Nutrient assimilation pathways are the basic building blocks for an integrated substance production system in which the influx and efflux of metabolites link distinct pathways. We previously reported the enhancement of nitrogen assimilation in the transgenic Arabidopsis plants expressing the Dof1 transcriptional activator (1). Here, by targeted metabolome analysis of the transgenic Arabidopsis plants that were grown under various growth conditions, we investigated effects of the enhancement of N assimilation on C and S assimilation. The results indicated the promotion of S assimilation in proportion to the genetic enhancement of N assimilation in the transformants. Furthermore, although effects on CO2 fixation was not evident in the transformants grown under the standard light condition, the enhancement of CO2 fixation was obviously observed in the transformants grown under stronger illumination, accompanied with enlargement of the amounts of carbon metabolites. These results indicated that C, N and S assimilation pathways in the Dof1 transformants are co-activated under particular growth conditions. Such co-activation of three different assimilation pathways was also found to cause the accumulation of total protein and starch in the Dof1 transformants. These results therefore suggest that a single genetic modification can cause co-activation of nutrient assimilation pathways and promote substance production in plants. Moreover, as S assimilation-related genes were upregulated in this modified metabolic state, the modified metabolic balance states were visualized using green fluorescence protein of which expression was under the control of an S assimilation-related gene promoter. (1) Yanagisawa et al., PNAS, 101: 7833-7838, (2004)."
(a) Graduate School of Agricultural and Life Sciences, The University of Tokyo (b) Crest, Jst

P39011 RNAi-mediated elimination of toxic gossypol from cottonseed: a powerful demonstration of the effectiveness of molecular tools to enhance global food security
Rathore, Keerti S.-presenter rathore@tamu.edu(a) Sundaram, Sabarinath (a) Sunilkumar, G. (a) Campbell, LeAnne M. (a) Puckhaber, Lorraine (b) Stipanovic, Stipanovic D. (b)
"Cottonseed, a byproduct of lint production, remains an abundant but greatly underutilized source of protein because of the presence of toxic gossypol. Annual, worldwide production of 44 million metric tons (MMT) of cottonseed contains ~10 MMT of protein, enough to meet the basic protein requirements of 500 million people. We utilized RNA interference to inhibit the expression of the δ-cadinene synthase gene in a seed-specific manner, thereby disrupting a key step in the biosynthesis of gossypol in cotton [Gossypium hirsutum L.; Sunilkumar et al. (2006) Proc. Natl. Acad. Sci., 103: 18054-18059]. Compared to an average gossypol value of 10 μ;g/mg in wild-type seeds, seeds from RNAi lines showed values as low as 0.2 μ;g/mg. Importantly, the levels of gossypol and related terpenoids that are derived from the same pathway were not diminished in the foliage and floral parts of mature plants and thus remain available for plant defense against insects and diseases. The stability of the trait has been confirmed by testing of several lines over five generations in the greenhouse. Further, we found that the germinating, RNAi seedlings are capable of launching terpenoid-based defense pathway when challenged with a pathogen. Thus, the 'silenced state' of the δ-cadinene synthase gene that existed in the seed, does not leave a residual effect that can interfere with the normal functioning of the cotton seedling during germination. Evidence will be presented showing that transgene-encoded RNAi can be restricted in a spatial and temporal manner in cotton plants."
(a) Texas A&M University (b) U.S. Department of Agriculture, Agricultural Research Station

SESSION P40 – MINERAL NUTRITION

P40001 "Functional Characterization of a Novel Iron Transporter, *FEA1*, from *Chlamydomonas reinhardtii* and its Application for Iron-Specific Metal Uptake in Plants"
Narayanan, Narayanan N-presenter nnarayanan@danforthcenter.org(a) Chiu, WaiTing (a) Ihemere, Uzoma (a) Moon, Hangsik (b) Siritunga, Dimuth (c) Singh, Sareena (e) Oda, Saharu (e) Falcao, Vanessa (e) Rajamani , Sathish (d) Sayre, Richard T (a)
"The FEA1 protein is a unique algal soluble protein secreted into the periplasmic space of *Chlamydomonas reinhardtii* under iron deficient conditions or in the presence of toxic concentrations of cadmium. We demonstrate that the FEA1 protein compliments both yeast iron permease (*ftr1*) and uptake mutants (*fet3fet4*), suggesting that the FEA1 protein functions as an iron transporter. Based on protein structural predictions, we hypothesize that the FEA1 protein undergoes a transition from a soluble to a membrane protein following formation of a hairpin structure with two potential transmembrane spanning domains (TMS) that then insert into the membrane. The formation of the hairpin structure is dependent on the formation of either a disulfide or metal thiolate bond between two cysteines located at the periplasmic surface of the two predicted TMS. We provide evidence that this structural transition is necessary for iron transport activity. We show that the polar residues located in the TMS are critical for FEA1 function presumably for formation of an ion channel. A predicted cytoplasmically-localized nucleotide binding motif (P-loop) is also required for function. In

plants, *FEA1* compliments the irt1 iron-uptake mutant of *Arabidopsis* indicating that the FEA1 protein functions in a variety of organisms. Transgenic *FEA1* wild-type plants have three- to four-fold higher root iron levels compared with wild-type plants when grown at high pH (8.5) indicating an enhanced ability to transport iron in calcareous soils. Furthermore, we demonstrate that the FEA1 transporter is Fe+2-specific. These features indicate that algae have efficient mechanisms for transporting iron under a variety of stress conditions that can impair iron-dependent metabolism and growth."
(a) Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132, USA (b) Syngenta Biotechnology, Inc., 3054 E. Cornwallis Road, Research Triangle Park, NC 27709, USA (c) Department of Biology, University of Puerto Rico, Mayaguez, Puerto Rico, 00680, USA (d) Department of Biology, Dartmouth College, Hanover, New Hampshire, 03755, USA (e) Department of Plant Cellular and Molecular Biology, The Ohio State University, Columbus, OH 43210, USA

P40002 Transporters Involved in Uptake and Distribution of Silicon in Rice
Ma, Jian Feng-presenter maj@rib.okayama-u.ac.jp(a) Yamaji, Naoki (a) Mitani, Namiki (a)
"Silicon (Si) is a beneficial element for plant growth. It is especially important for sustainable and high yield of rice, a typical Si-accumulating species. Silicon is taken up and translocated in the form of silicic acid, a non-charged molecule. Recently, we have cloned three genes (Lsi1, Lsi2, and Lsi6) from rice, which are involved in Si uptake and distribution. Lsi1 encodes an influx transporter of Si, while Lsi2 encodes an efflux transporter. Both Lsi1 and 2 are located at the exodermis and endodermis of the roots, but Lsi1 is on the distal side, while Lsi2 is on the proximal side. Knockout of either Lsi1 or Lsi2 resulted in significant decreased Si uptake, accumulation and yield. Therefore, coupling of Lsi1 with Lsi2 is required for efficient uptake of Si in rice. On the other hand, Lsi6, a homolog of Lsi1, was also identified in rice. Different from Lsi1 and Lsi2, Lsi6 is also expressed in the shoots. It is localized at the xylem parenchyma cells of the leaf blade and sheath. Defect of Lsi6 resulted in increased Si in the guttation and disturbed distribution of Si in the shoots. Lsi6 is therefore involved in the unloading of Si from the xylem. Recently, we found that Lsi6 plays an important role in distribution of Si to the panicles at the reproductive stage. Lsi6 is expressed at large vascular bundle of the nodes. Knockout of this gene resulted in decreased Si accumulation of the panicles and increased accumulation in the leaf blades. Detailed data on the expression pattern, cell-specificity localization, phenotype of the knockout lines, etc. will be presented at the meeting."
(a) Research Institute for Bioresources, Okayama University

P40003 Root suberin forms an extracellular barrier that affects water relations and mineral nutrition in Arabidopsis
Hosmani, Prashant S.-presenter phosmani@purdue.edu(a) Baxter, Ivan (b) Rus, Ana (a) Lahner, Brett (a) Borevitz, Justin (c) Muthukumar, B (a) Mickelbart, Michael (a) Schreiber, L (d) Franke, R (d) Salt, David E (a,b)
"Though central to our understanding of how roots perform their vital function of scavenging water and solutes from the soil, no direct genetic evidence currently exists to support the foundational model that suberin acts to form a chemical barrier limiting the extracellular, or apoplastic, transport of water and solutes in plant roots. Using the newly characterized *enhanced suberin1* (*esb1*) mutant, we established a connection in Arabidopsis thaliana between suberin in the root, and both water movement through the plant, and solute accumulation in the shoot. *Esb1* mutants, characterized by increased root suberin, were found to be have reduced day time transpiration rates, and increased water use efficiency during their vegetative growth period. Furthermore, these changes in suberin and water transport were linked to decreases in the accumulation of Ca, Mn and Zn, and increases in the accumulation of S, K, As and Mo in the shoot. These changes in elemental accumulation are interpreted as evidence that a significant component of the radial root transport of Ca, Mn and Zn occurs in the apoplast, whereas radial transport of S, K, As and Mo is insensitive to suberin, and therefore likely to primarily occur via a cell-to-cell transport pathway."
(a) Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN 47907, USA. (b) Bindley Bioscience Center, Purdue University, West Lafayette, IN 47907, USA. (c) Department of Ecology and Evolution, University of Chicago, Chicago, IL 60637, USA. (d) Institute of Cellular and Molecular Botany, University of Bonn, Kirschallee 1, D-53115 Bonn, Germany.

P40004 Identification of mutants with altered tolerance to iron limitation
morrissey, joe b-presenter jbm2@dartmouth.edu(a) salt, david (b) lahner, brett (b) baxter, ivan (b)
"Staple foods lack sufficient levels of bioavailable iron; consequently, much of the developing world suffers from iron deficiency. Understanding how micronutrients such as iron accumulate in seeds is essential to combating this dietary deficiency. We identified two Arabidopsis mutants with altered iron accumulation in the seed from a FN mutagenesis screen. 131:61 suffers from interveinal chlorosis, and dies on alkaline soil. Despite this, it accumulates more iron in its shoots and seeds than wild type when grown on normal soil, likely from an observed constitutive iron-deficiency response. This suggests an inability to efficiently utilize iron, rather than a defect in uptake. A second FN-mutant, 118:65, has similar metal content to wild type when grown on normal soil, but thrives on alkaline soil: it maintains higher chlorophyll levels than wild type, and accumulates significantly more iron in its shoot and seeds. Interestingly, the genes known to be involved in iron uptake from soil are not upregulated. Mapping of these mutations has begun, and will hopefully reveal genes with potential for improving the iron levels of crop seeds. "
(a) Dartmouth College (b) Purdue University

P40005 Nitrogen Partitioning and Remobilization in *Arabidopsis* under Sufficient and Depleted Conditions
Zayed, Adel M-presenter azayed@monsanto.com(a) Crosby, Robert (a) Jones, Lloyd (a) Boyes, Doug C (a) Edwards, Janice W (a)
"Increasing global demand for agricultural products requires our world to identify better ways to get more out of each acre of farmland. Greater grain demand drives the need for higher crop yields. Our research program at Monsanto centers around increasing yields in three large-acre crops (corn, soybeans and cotton) used for food, feed, fiber and fuel. One of our programs with high potential impact is improved nitrogen-utilization corn. Nitrogen fertilizer is one of the most price-sensitive input costs for farmers. We are testing genes for their ability to normalize grain yield in low-nitrogen environments and for higher yields under normal nitrogen conditions. Improving crop nitrogen utilization efficiency will help meet the ever increasing human demand for food and will also lead to a significant reduction in any harmful environmental impact of nitrogen fertilizers. Improving our understanding of nitrogen assimilation and remobilization is crucial for improving crop NUE. We have developed a high throughput hydroponic system for Arabidopsis plants that enables the assessment of multi aspects of nitrogen biology and their impact on seed yield including uptake, partitioning and remobilization. Arabidopsis plants are grown under N-sufficient or various levels of N-depleted conditions and the ability of plants to accumulate and remobilize stored nitrogen is studied and linked to changes in seed yield. Using this approach we demonstrated that plants improve their N partitioning and remobilization to sink tissues under N depleted conditions. Thus, this assay can be used to screen transgenic plants for their improved N remobilization to identify those genes that when expressed in important crops can improve nitrogen utilization and therefore grain yield. "
(a) Monsanto Company

P40006 GEOCHEM-EZ: A Chemical Speciation Program with Greater Power and Flexibility
Shaff, Jon E.-presenter jes8@cornell.edu(a,b) Schultz, Benjamin A (a,b) Craft, Eric J (a) Clark, Randy T (a,c) Kochian, Leon V (a)

"GEOCHEM-EZ is a multi-purpose chemical speciation program, designed to replace GEOCHEM-PC, which can only be used in DOS consoles. GEOCHEM-PC has been widely used in plant nutrition to perform equilibrium speciation computations to estimate the activity of ions in solution and to consider simple complexes and solid phases. It is also important to scientists doing molecular biological research who need to modify their solution composition for phenotyping and screening transformed organisms (e.g., transgenic Arabidopsis plants), who want to control pH, redox conditions, ionic strength (e.g., for protoplast isolations) or are using heterologous systems such as yeast expressing plant genes. To enhance the usability and address several weaknesses in GEOCHEM-PC, we upgraded the program using a Java graphical user interface, improving both the power and function, and allowing it to run on any computer that supports a Windows XP or Vista environment. Included in this program are improvements which would be expected by modern users (interactive and illustrated help files, hierarchical organization of options, logical output, real-time error checking), while maintaining complete backward compatibility to the GEOCHEM-PC format. A customizable database of common salts has been added, which eliminates the need to parse and calculate the concentration of each metal or ligand. This last feature will aid in making data input more rapid and in eliminating the most common user errors. One can instantly preview input and output files and make necessary corrections (e.g. charge balance the solution), something that formerly involved having to save these files and run the calculations a second time. GEOCHEM-EZ is available as a free download from http://www.PlantMineralNutrition.net. "
(a) USDA-ARS, Robert W. Holley Center for Agriculture and Health (b) Department of Plant Biology, Cornell University (c) Department of Biological and Environmental Engineering, Cornell University

P40007 Localization of a novel protein that plays a role in conditional responses of root system architecture to anion availability
Porco, Silvana-presenter silvana_po@hotmail.com(a) Hermans, Christian (b) Bush, Daniel R. (a)
"The development of plant roots is highly responsive to the availability and distribution of mineral nutrients in soil. Previous work in our lab identified *Arabidopsis* mutants altered in their ability to regulate lateral root (LR) development in response to N status. In wild-type plants low nitrate (0.6 mM) supply results in a highly branched root system, while uniformly high nitrate (60 mM) results in a single primary root (PR) and inhibition of the elongation of lateral root primordia before emergence. The *arm* (anion altered root morphology) mutant displays inhibition of PR length, promotion of LR elongation, root swelling and increased root hair density in the presence of high concentrations of nitrate. Further investigation revealed that the response is conditional on monovalent anions, with high chloride generating the same phenotype. Positional cloning of *arm* revealed a point mutation in *AtCTL1*, encoding a putative chitinase-like gene. The goal of this work is to determine the role of *AtCTL1* in root responses to nitrate. The presence of a putative signal secretion sequence at the N terminus of AtCTL1 lead us to examine the subcellular localization of CTL1 by transient expression of a CTL1::GFP fusion in onion cells. In spite of the putative signal, we find the CTL1::GFP chimera in the cytoplasm. To determine if tissue-specific factors play a role in its localization, transgenic *Arabidopsis* expressing CTL1::GFP under its native promoter have been generated and will be examined shortly. Taken together, these data will add to our understanding of CTL1 function in regulating root architecture in response to nutrient availability. "
(a) Colorado State University (CSU) (b) Universite Libre de Bruxelles, Belgium

P40008 Nitrate reductase cycle in pineapple: what is the contribution of roots and shoot to nitrogen assimilation under thermoperiodic culture conditions?
Freschi, Luciano (a) Nievola, Catarina C (b) Rodrigues, Maria A (a) Domingues, Douglas S (a) Sluys, Marie-Anne V (a) Mercier, Helenice-presenter hmercier@usp.br(a)
"In higher plants, nitrate reductase activity (NRA) is regulated by a variety of environmental factors and oscillates with a characteristic diurnal rhythm. Earlier investigations in our laboratory have demonstrated that the diurnal pattern of NRA in pineapple (*Ananas comosus*) can be strongly modified by changes in the day/night temperature regime. Plants cultivated *in vitro* under constant temperature (28^{o}C light/dark) showed the highest levels of NRA in the leaves during the day. Conversely, plants grown under thermoperiodic conditions (28^{o}C light/15^{o}C dark) exhibited the highest NRA levels in the roots and, surprisingly, during the dark period. Since cytokinins are considered the main hormonal class involved in the control of NRA, this study investigated a possible relationship between oscillations in the endogenous cytokinins and the diurnal rhythm of NR expression and activity in pineapple organs. To achieve this, the levels of NR expression, NRA, Z, ZR, iP and iPR were simultaneously analyzed every 2 h throughout the diurnal cycle in leaves and roots of plants cultivated *in vitro* under constant or thermoperiodic conditions. The results demonstrated that transitory increases in the levels of Z, ZR and iPR coincided with the accumulation of NR transcripts and preceded the rise of NR activity, suggesting a role for these cytokinin species in the diurnal regulation of the NR cycle in pineapple. Accordingly, exogenous application of these same cytokinins also induced a significant increase in NR transcription and NR activity of both root and shoot tissues. Altogether, these results provide evidence that oscillations in the endogenous levels of distinct cytokinins may determine the organ (root/leaf) where most of the N assimilation will take place"
(a) University of Sao Paulo (b) Institute of Botany

P40009 Identification of a novel nitrate-responsive cis-element in the nitrite reductase gene promoter from Arabidopsis
Konishi, Mineko-presenter amkonish@mail.ecc.u-tokyo.ac.jp(a,b) Shuichi, Yanagisawa (a,c)
"Nitrate is a major nitrogen source for land plants and also acts as a signal molecule to induce changes in growth and gene expression. The gene encoding nitrite reductase (*NIR*) is a typical nitrate-inducible gene. To identify the *cis*-acting DNA element involved in nitrate induction, we initially generated a reporter construct that contained a 3.1-kb upstream and a 2-kb downstream regions of *NIR* from Arabidopsis and the GUS reporter gene. In the transgenic Arabidopsis plants harboring this reporter construct, both GUS activity and the accumulation of the GUS transcript were specifically induced by nitrate. Furthermore, a low concentration of nitrate (300 μ;M) was effective in inducing expression of the reporter gene, in consistency with the response of endogenous *NIR*. Since it was confirmed that the promoter sequence but not the 3' downstream region was involved in the nitrate inducible expression, we scrutinized the *NIR* promoter. The results indicated that the proximal region (-334 to -1) relative to the translation start) conferred nitrate induction, while replacement of the sequence (from -185 to -1) with the 35S minimal promoter abolished the nitrate responsiveness. Accordingly, the sequence from -185 to -1 was suggested to contain a nitrate-responsive *cis*-element (NRE). By comparison of proximal regions of several *NIR* promoters from various higher plant species, we found a conserved sequence. When the four copies of the sequence were fused to the 35S minimal promoter, the synthetic promoter clearly directed nitrate responsive expression of the GUS reporter gene. These results suggest that a conserved NRE may be involved in nitrate responsive expression of *NIR* in various plants. (This work was supported by PROBRAIN.)"
(a) Graduate School of Agricultural and Life Sciences, The University of Tokyo (b) JSPS Research Fellow (c) The Core Research for Evolutional Science and Technology, JST

P40010 Effect of rhizospheric aluminum on nitrogen metabolism in root of *Quercus serrata* Thunb.
Tomioka, Rie-presenter tomiokar@agr.nagoya-u.ac.jp(a) Takenaka, Chisato (a) Tezuka, Takafumi (b)
"Excess Al in soil solutions is toxic to many plant species at a soil pH of 5 or lower and inhibits nutrient uptake and the growth of roots (Kochian, 1995). Most major crops, such as maze and wheat, are relatively sensitive to Al, and Al toxicity is one of the major factors limiting crop production in

acid soil. However, tree species are more tolerant to Al than crop species (Keltjens and Loenen, 1989), and growth enhancement induced by Al has been reported (Huang and Bachelard, 1993). In our previous experiment using 2-year-old Q. serrata seedlings, the stimulatory effect of Al could be detected after 4 to 9 weeks and 15 months treatment (Tomioka et al., 2005). Furthermore, increase of the rate of NO3- uptake from the medium and the number of primordial of tertiary lateral roots was observed in Q. serrata seedlings treated 1 mM Al (Tomioka et al., 2007). Therefore, we speculate that Al treatment may be related to the activity of basic metabolism in plants. The aim of this study is to clarify the effect of Al on factors of nitrogen metabolism in Q. serrata seedlings, such as nitrate reductase (NR), nitrite reductae (NiR), and glutamic synthase (GS) activities. The seedlings were cultured hydroponically in modified 1/10 Hoagland's No.2 solution with or without 1 mM AlCl3 (pH 4.0) for 21 days. As a result, the length of tap root significantly increased (by 5 %) after Al treatment for 14 days. Al treatment for 7 and 14 days remarkably increased NR and NiR activity in the root, while there was not clear difference between with or without Al treatment in GS activity. Al treatment for 21 days significantly increased NR and GS activity. "
(a) Graduate School of Bioagricultural Sciences Nagoya University (b) School of Health and Human Life, Nagoya Bunri University

P40011 "OsENOD93-1, an early nodulin gene is involved in improving nitrogen use efficiency in rice"
Kant, Surya-presenter skant@uoguelph.ca(a) Bi , Yong-Mei (a) Rothstein, Steven (a)
"Nitrogen (N) is an essential macronutrient required for optimal plant growth and development. Most of the crop plants are able to utilize only 30-40% of this applied N with the remaining N lost to the underground aquifers and to the atmosphere. Hence, there is an urgent need for improving N use efficiency (NUE) to reduce input cost for crop production and to minimize environmental pollution. We conducted a whole genome transcriptional profiling analysis in rice plants grown under different N conditions, and identified genes which might be potentially involved in improving NUE. An early nodulin gene *OsENOD93-1* responded significantly to both N induction and N reduction. Transgenic rice plants over-expressing the *OsENOD93-1* gene had increased shoot dry biomass, number of spikes and spikelets, and seed yield as compared to wild type plants. The *OsENOD93-1* gene was expressed at high levels in roots of wild type plants, especially at the panicle emergence stage. The OsENOD93-1 protein was localized in the mitochondria. Transgenic plants accumulated a higher amount of total amino acids and total N in roots. Also, the content of amino acids in xylem sap was higher and an elevated level of aspartate in shoots was detected in transgenic plants, specifically under low N conditions. In situ hybridization analysis revealed that *OsENOD93-1* is expressed in vascular bundles as well as in epidermis and endodermis, suggesting that *OsENOD93-1* plays a role in the transportation of amino acids. Taken together, the results suggest that the *OsENOD93-1* is an important N-responsive gene and its over-expression improve NUE in rice. A similar approach could be applied to improve NUE of other agronomically important crops."
(a) Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

P40012 "OsCSN6, an up-regulated gene under iron deficiency, promoted rice root elongation related to GA signaling"
Zang, YuePeng (a) Yin, LiPing-presenter yinlp@mail.cnu.edu.cn(a)
"Our group found COP9 signalosome subunit 6 (OsCSN6) as one of 233 up-regulated transcripts in rice root by a microarray assay under iron limiting stress. This result was then confirmed by Real-time PCR. To investigate the effect of OsCSN6 in rice root under iron deficiency stress, we generated sense and anti-sense transgenic rice expressing a fusion protein consisting of OsCSN6 and GFP (OsCSN6-GFP) driven by 35S promoter and analyzed the phenotype of the transformants. Roots of T1 and T2 sense lines were much longer than of WT and anti-sense lines under iron deficiency, which suggested that OsCSN6 promoted transgenic rice root elongation in responding to iron limiting stress. To determined how over-expressed OsCSN6 influenced root elongating, the transformants were planted into different iron condition mediums with 10umol/L PAC (inhibitor of GA signal pathway) and 10umol/L TIBA (inhibitor of auxin polar transportation) respectively. Two weeks after germination, OX transgenic rice roots cultured in both iron conditions medium with PAC showed similar length compared to WT cultured in same mediums, which indicated that OX OsCSN6 promoting root elongation under iron deficiency related to GA regulation pathway. To gain further insight into the function of the fusion protein, we also performed localization detection in the fusion protein transformed BY-2 suspending cells. The analysis revealed that OsCSN6-GFP localized not only in nucleus but also in cytoplasm. Further iron deficient treated to transgenic BY-2 cells showed that the fluorescence of the fusion protein increased with time both in cytoplasm and nucleus and reach the maximum after 24h and 36h respectively."
(a) College of life science, Capital Normal University

P40013 MxIRT1 dynamically localizes in yeast and increases Fe and Zn content in rice seeds
Yang, Guang (a) Yin, LiPing-presenter yinlp@mail.cnu.edu.cn(a)
"IRT is a major iron transporter in Strategy I plants. MxIRT1 was isolated from an iron efficient genotype Malus xiaojinensis Cheng et Jiang. It is strongly enhanced in roots at transcript level under iron deficiency and is able to transport iron, zinc, cadmium but manganese. To investigate the MxIRT1 response to iron states, the localization of GFP-fused protein and microarray analysis were performed in yeast. MxIRT1 was mainly localized on plasma membrane (PM) and vesicles in a dynamic pattern according to iron states. Under -Fe conditions (BPDS or Ferrozine added), members of the iron high-affinity pathway were up-regulated in transformants while the fluorescent vesicles increased at PM without fusion until iron resupply. It is interesting to speculate the role of SNARE proteins in this fusion response because their expression was tightly controlled under -Fe conditions. In 1 mM Fe2+,green fluorescence gradually translocated into vacuoles while total MxIRT1 obviously decreased in Western blot. The chip result also showed the vacuole protease expression increased rather than proteosome's. Finally, an abnormal accumulation of fluorescence in vacuole of yeast mutant pep4 validated the degradation of MxIRT1 is an iron-induced vacuole-dependent process. MxIRT1 driven by 35S promoter was introduced into rice, a staple crop problematic for low iron content in seeds. The transformants showed a robust phenotype including increased leaves chlorophyll content, shoot length and total biomass. The Fe and Zn content of T2 seeds were over 4 fold and 20 fold higher than the nontransformed plants respectively. Together MxIRT1, a best known fruit iron transporter, has the potential for biofortificating rice that are rich in Fe and Zn. "
(a) College of life science, Capital Normal University

P40014 The paradigm of calcium deficiency development in fruit
de Freitas, Sergio T.-presenter stonettodefreitas@yahoo.com.br(a) Mitcham, Elizabeth J. (a)
"Calcium deficiency development in fruit has been studied for more than a hundred years and its mechanisms are still not well understood. Besides its simple definition, Ca^{++} deficiency is a complex process that involves not only the total input of Ca^{++} to the fruit, but also a proper Ca^{++} homeostasis at the cellular level. The symptoms of Ca^{++} deficiency start by plasma membrane breakdown, which is believed to be the result of low levels of free Ca^{++} in the apoplast. Therefore, any mechanism present in the cell that can lower the apoplastic pool of free Ca^{++} and reduce the amount of Ca^{++} that binds to the plasma membrane will eventually trigger Ca^{++} deficiency symptom development. It is known that the vacuole represents about 40% of the total Ca^{++} in the tissue, and most of the remaining 60% is bound to deesterified pectins in the cell wall. Consequently, small changes in the capacity of the vacuole and the cell wall to retain Ca^{++} will potentially affect the level of free Ca^{++} present in the apoplast solution, affecting also plasma membrane structure and integrity. Our studies with tomato and apple fruit provide evidence that Ca^{++} movement into the vacuole and Ca^{++} binding to the cell wall represent important mechanisms involved in free Ca^{++} depletion in the apoplast, resulting in membrane breakdown, and Ca^{++} deficiency symptom development. The results obtained also suggest that these mechanisms may be highly conserved in

different plant species. "
(a) Department of Plant Sciences, University of California, Davis, CA 95616 USA

P40015 "Functional analysis of *MATE* family, citrate transporter genes in rice"
Yokosho, Kengo-presenter dns421418@s.okayama-u.ac.jp(a) Daisei , Ueno (a) Naoki , Yamaji (a) Namiki , Mitani (a) Jian Feng, Ma (a)
"Of all genes in plant decoded genome sequence, about 30% encode for various transporter. Among them, MATE transporters represent a large family. We recently identified an Al-tolerance gene (*HvAACT1*) from barley, which belongs to MATE family. This gene encodes a citrate transporter and the role was citrate secretion for rhyzhosphere. Here, we report the functional analysis of two homolog of this gene in rice (*OsFRDL1* and *OsFRDL4*). We measured the transport activity in *Xenopus oocytes*. The both proteins showed transport activity for citrate. Interestingly, the cellular localization of OsFRDL4 was indicated at almost of all cells in roots and induced by Al. OsFRDL1 was localized at the pericycle cells in roots. Furthermore, Tos17 insertion line of them was observed different phenotypes. Tos17 insertion line of OsFRDL4 showed lower citrate secretion than WT in the presence of Al. And root elongation was slightly inhibited more in the insertion line than in WT. *OsFRDL4* was mainly expressed in the roots and the expression level was induced about 100 times by Al. All results indicate that OsFRDL4 is a citrate transporter, which is involved in the Al-induced citrate secretion in rice. On the other hand, there was no difference in the citrate secretion between the WT and Tos17 insertion lines of OsFRDL1. When these lines were grown in low Fe concentration, chlorosis on the new leaves was observed only in the insertion lines. The concentration of Fe and citrate in the xylem sap was lower and Fe precipitation in the root stele was observed in the insertion line. This gene expression level was not affected by Fe and Al. These results suggest that OsFRDL1 is a citrate transporter, which is necessary for Fe translocation to the shoot. "
(a) Research Institute for Bioresources, Okayama University

P40016 Real-time imaging of ^{32}P in a *Lotus japonicus*.
Kanno, Satomi-presenter aa077012@mail.ecc.u-tokyo.ac.jp(a) Yamawaki, masato (a) Tanoi, Keitaro (a) Nakanishi, Tomoko (a)
"Phosphate requirement is high in young tissue and seeds. During developmental stages, phosphate is known to re-translocate from old tissue to young tissue and seeds, depending on the phosphate condition in the environment. However, molecular mechanism of phosphate movement in plants is still limited. Therefore, to know the real-time movement of phosphate in plant, ^{32}P images from culture solution to the plant were obtained both in control sample and that grown in phosphate deficient condition. Then, mRNA expression of phosphate transporters was analyzed in the tissue, which showed distinctive^{32}P accumulation pattern. Under phosphate deficient condition, ^{32}P uptake speed from root to shoot was shown higher than that of control. There was a difference in phosphate translocation manner in an old leaf, which was derived from separation of phosphate at branching point within the leaf or at an internode. When the sample was transferred to phosphate deficient culture solution after short time ^{32}P phosphate supply, accumulated ^{32}P in old leaves was translocated to the younger leaves within 48 hours. We measured mRNA amount of phosphate transporter (LjPT1, LjPT2) in roots, old leaves and new leaves. Under phosphate deficiency, LjPT1 mRNA expression level was increased 4 times in each tissue compared to that of control sample. In the case of LjPT2, mRNA expression level was increased about 6 times in new leaves. To know expression of transporter more in detail, *in situ* hybridization was performed and it was shown that LjPT2 was localized, especially, in the tissue around the sieve tubes. These results suggested that LjPT1 was active under phosphate deficient condition in many tissues and LjPT2 seems to be active in phosphate translocation, from old leaves to new leaves. "
(a) University of Tokyo, Graduate School of Agricultural and Life Science

P40017 Development of Real-Time Imaging System of Nutrients in a Plant Sample
Nakanishi, Tomoko-presenter atomoko@mail.ecc.u-tokyo.ac.jp(a) Yamawaki, Masato (a) Kanno, Satomi (a) Tanoi, Keitaro (a)
"It is very important to visualize how nutrients are taken up by the plant in a living plant. We developed real-time imaging system using conventional beta-ray emitters, which enabled to analyze the amount or the nutrient from the image. The sample was placed in a box where light was irradiated only at an up-ground part of the plant and the roots were kept in dark. Therefore, it was able to prepare cyclic light and dark condition. With this system the plant was found to be active during 6 days. The beta-rays emitted from the sample were converted to light by a CsI scintilator, which was deposited on FOS (fiber optic plate). After amplification, the light was focused by a lens and was guided to a highly sensitive CCD camera. The image was integrated every 3 minutes, successively. The sensitivity of the imaging was more than ten times higher than that of an imaging plate (IP), with similar resolution. When the chemicals labeled with ^{32}P, ^{35}S, ^{45}Ca, as well as ^{14}C were applied in culture solution, it was able to image how these chemicals were taken up by the root as well as translocation and accumulation manner to the up-ground part. In the case of ^{32}P-phosphate, it was found that there was always a preference of translocation to younger tissues in a soybean, rice and other plants. We present the case of *Lotus Japonicus* when ^{32}P-phosphate was applied from the root in culture solution. It was shown that taken up ^{32}P-phosphate was gradually wet up-ground part and there was a high accumulation of ^{32}P at internodes. In the case of roots, the uptake speed of ^{32}P was higher in dark and lower in light condition, which tendency was reverse to those of leaves. When uptake speed of ^{32}P in leaves was high, the apparent uptake amount of ^{32}P in root was decreased. "
(a) University of Tokyo, Graduate School of Agricultural and Life Science

P40018 Phytochrome-mediated effects on activity and post-translational regulation of nitrate reductase in tomato seedlings
Freschi, Luciano-presenter freschi@usp.br(a) Rodrigues, Maria A. (a) Peres, Lazaro E. P. (b) Mercier, Helenice (a)
"Nitrate reductase (NR) is regulated by a set of mechanisms that leads to changes in transcription, post-translational modification and protein turnover. Light is known to influence NR expression and protein levels through both photosynthesis and phytochrome perception; however, the phytochrome-mediated effects of light on NR post-translational regulation are not entirely clear. Therefore, we have analyzed the possible influence of phytochrome on the NR activation state (NR_{as}), which is an ultimate consequence of post-translational modulation. To achieve this, dark-grown seedlings of wild type tomato (cv. Micro-Tom, MT) and phytochrome-deficient mutants *aurea* (*au*) and *yellow green2* (*yg2*) had their NR_{as} determined upon continuous red light (RL) irradiation. In all cases, NR_{as} was defined as the percent ratio of NR activity in the presence of Mg^{2+} (active NR, NR_{act}) versus activity in the absence of Mg^{2+} (total NR, NR_{tot}). Continuous RL irradiation significantly increased both NR_{tot} and NR_{act} in cotyledons, hypocotyls and roots of MT, but failed to induce changes in the NR_{as} in these tissues. The highest levels of NR_{tot} and NR_{act} in cotyledons and roots of MT were attained at 24 h of RL treatment, before declining gradually during the next 5 days. The RL response of NR_{tot} and NR_{act} was dramatically reduced in *au* and *yg2* cotyledons, hypocotyls and roots when compared to near isogenic wild-type seedlings, demonstrating that phytochrome mediates NR_{tot} and NR_{act} stimulation by RL. Also, the phytochrome-deficient mutants showed no marked changes in the NR_{as} during RL treatment. These results indicated that although phytochrome plays a vital role for the NR_{tot} and NR_{act} induction by RL, this photoreceptor seems not to affect NR post-translational modulation in tomato seedlings."
(a) Universidade de Sao Paulo, Sao Paulo, Brazil (b) Escola Superior de Agricultura Luiz de Queiroz, Universidade de Sao Paulo, Piracicaba, Sao Paulo, Brazil

P40019 The sink and source relationship of nitrogen partitioning in different developmental stages of *Phalaenopsis*
Chang, Yao-Chien Alex-presenter alexchang@ntu.edu.tw(a) Peng, Ying-Chun (a)
"Although *Phalaenopsis* (the moth orchid) has emerged as a major floral crop, we know very little about the partitioning of nutrients in relation to plant development. *Phalaenopsis* responses very slowly to fertilization probably because its thick roots and succulent leaves may have functions as storage organs. In this study, we used stable isotope ^{15}nitrogen as a tracer to document the sink-source relationship between organs and nitrogen partitioning within the plant in different developmental stages. Mature *Phalaenopsis* Sogo Yukidian 'V3' plants were used as plant materials and $K^{15}NO_3$ was used to partially substitute the KNO_3 in modified Johnson's solution. Results indicated that both roots and leaves were capable of absorbing nitrogen, with a higher uptake efficiency found in roots. Growing organs such as new growing leaves in vegetative stage and developing stalks in reproductive stage were major sinks for nitrogen partitioning. The sink strength of leaf decreased as the leaf age increased. Mature leaves and roots, where nitrogen stored, both acted as source organs and were capable of retranslocating nitrogen to sink organs, especially during stalk development or in a nutrient deficiency situation. Therefore, a short term nutrient shortage would not retard the growth and flowering of *Phalaenopsis*. The percentage of nitrogen supply from these two source organs depended on the nutrition status of the plant. In a nutrient abundant environment, 29% of the nitrogen demand for stalk development from spiking to bud visible stage was provided by nitrogen reserves and 71% by recent fertilizer applications."
(a) National Taiwan University

SESSION P41 – MODELING & COMPUTATIONAL BIOLOGY

P41001 Identification Amino Acid Residues of Potato ADP-glucose Pyrophosphorylase Large Subunit That Participate Interaction with Small Subunit
Kavakli, Ibrahim H-presenter hkavakli@ku.edu.tr(a) Tuncel, Aytug (a) Ozber , Natali (a) Baris , Ibrahim (a) Keskin, Ozlem (a)
"ADP-glucose pyrophosphorylase (AGPase), a key allosteric enzyme involved in higher plant starch biosynthesis, is compose of pairs of large (LS) and small subunits (SS).Current evidence indicates that the two subunit types play distinct roles in enzyme function. Recently heterotetrameric structure of potato AGPase has been modeled. In this study we have applied MM-GBSA method to identify amino acid residues of LS subunit that participate interaction with SS during the heterotetrameric structure formation. We have further shown the role of these amino acid in subunit-subunit interaction by yeast two-hybrid and complementation of bacterial AGPase. This study will enable to engineer proteins to obtain better assembled variants of AGPase, which can be used to for the improvement of plant yield."
(a) Koc University, Chemical and Biological Eng., Rumeli Feneri Yolu, Sariyer, Istanbul-TURKEY

P41002 ASLpred: Arabidopsis Subcellular Localization predictor
Kaundal, Rakesh (a) Zhao, Patrick Xuechun-presenter pzhao@noble.org(a)
http://bioinfo.noble.org
"We report that 'organism-specific' subcellular localization prediction methods are superior to those 'general' ones. To demonstrate such, we performed a systematic case study in the model plant, Arabidopsis thaliana, and further developed an integrative support vector machine-based **A**rabidopsis **S**ubcellular **L**ocalization **pred**ictor called **ASLpred** that utilizes the combinatorial presence of diverse protein features such as its amino acid composition, sequence-order effects, terminal information, PSSM and the similarity search-based PSI-BLAST information. When used to predict on seven compartments through 5-fold cross-validation test, our hybrid-based best classifier achieves an overall accuracy of 90.15% with a high confidence precision and MCC values of 90.33% and 0.88, respectively. Benchmarking ASLpred on independent dataset from Swiss-Prot outperformed the 5-fold cross-validation test results by achieving 93.89% overall sensitivity. We also validated its predictions on 83 Arabidopsis proteins with experimental information through GFP-tagging and Mass Spectrometry hosted in SUBA and eSLDB databases as another independent testing. Performance comparison on these two independent testing sets shows a significant improvement using 'genome-specific' approach compared to the widely used 'general' tools such as TargetP, LOCtree, PA-SUB, MultiLoc, WoLF PSORT and Plant-Ploc as well as our newly created 'All-Plant' method. Currently, we are experimentally validating some of our top scoring predictions through GFP fusions in each localization class. The **ASLpred** web server, which is under active development, is freely available at http://bioinfo3.noble.org/ASLpred/."
(a) The Samuel Roberts Noble Foundation

P41003 "High confidence identification of candidate G-protein coupled receptors in Arabidopsis, rice, and poplar using whole proteome bioinformatics and wet-bench analyses"
Gookin, Timothy E.-presenter tegookin@psu.edu(a) Kim, Junhyong (b) Assmann, Sarah M (a)
"The ability to sense the environment and respond is a critical requirement for both cellular and organismal success. The heterotrimeric G-protein signaling cascade provides a mechanism for this ability in metazoa and is readily identifiable in plants. In mammals there are over 850 known or predicted G-protein coupled receptors (GPCRs), which play critical roles in diverse physiological processes. Although GPCRs are evolutionarily conserved, and constitute the largest known protein superfamily, the low sequence homology between GPCR subfamilies has hindered their identification and characterization in plants; only two candidate GPCRs with the canonical 7 transmembrane domain structure, GCR1 and RGS1, have been characterized in the model plant, Arabidopsis, and a ligand has not been identified for either candidate. To circumvent the issue of low GPCR sequence homology and identify plant sequences with the highest likelihood of being GPCRs, we developed a combinatorial approach composed of diverse computational methods. Our stringent whole-proteome analyses of the Arabidopsis, rice, and poplar proteomes allowed the high confidence identification of candidate GPCRs and evolutionarily conserved candidate GPCR families. We validated our method by wet-bench testing half of our highest ranking Arabidopsis candidate GPCRs for interaction with GPA1, the sole prototypical G-alpha subunit in Arabidopsis, and found positive results for seven of the eight proteins tested. We are investigating the biochemical and physiological roles of these candidate GPCRs. Potential signaling roles related to ABA will be discussed."
(a) Department of Biology, The Pennsylvania State University (b) Department of Biology and Penn Genome Frontiers Institute, University of Pennsylvania

P41004 Computer vision analysis identifies a role for the Glutamate Receptor-like 3.3 Ca^{2+} channel
Miller, Nathan D (a) Durham, Tessa L (a) Spalding, Edgar P.-presenter spalding@wisc.edu(a)
"Computer vision has advanced the study of plant growth and development by increasing measurement precision and speed relative to manual methods. Developmental details can now be algorithmically extracted from time series of electronic images. Gravitropism, a model stimulus-response system and the subject of this work, lends itself well to this experimental approach. Images of the Arabidopsis seedling root undergoing gravitropism were acquired every 2 min during a 10 h period with 5 µ;m resolution. Tip angle, rate of root elongation, and root width as a function of time were automatically quantified. Automation enabled a systematic grid of conditions (seedling age, media composition, size of seed) to be explored with

many hundreds of trials. Principal component analysis of the tip angle values showed trends in the gravitropic response across the condition grid. K-means clustering separated the population of responses into three classes with distinct time courses that also showed trends across the condition grid. Wavelet analysis was used to calculate the velocity and acceleration of the tip angle for each of the hundreds of trials. Patterns and relationships among the measured parameters resulted in a rich description of the wild-type response and its plasticity. Next, this approach was used to compare the wild-type response with that of a mutant lacking the Glutamate Receptor-like 3.3 molecule. This mutant displays defective ligand-gated Ca^{2+} fluxes in its root cells but no growth or development phenotype had been detected before the present study found heritable root tip acceleration phenotypes in some but not all conditions. Computer vision promises to enable functional genomics by finding, quantifying, and relating subtle developmental phenotypes."
(a) Department of Botany, University of Wisconsin

P41005 How to effectively use the tools and resources at TAIR to enhance your research
Lamesch, Philippe (a) Li, Donghui-presenter donghui@stanford.edu(a) Karthikeyan, A.S. (a)
http://www.arabidopsis.org
"TAIR (www.arabidopsis.org) is a community database for Arabidopsis thaliana. This workshop is designed for users who wish to more effectively utilize the curated data and software resources provided by TAIR. We will address curation of three major data types: gene structure, gene function and metabolic pathway. We will also teach effective search strategies and highlight some important data sets available at TAIR. Both beginning and experienced users will undoubtedly learn new tricks for getting the information they need and are likely to discover new types of data housed at TAIR that can enhance their research efforts. In the Gene Structure Annotation section, we will give details on the recent TAIR9 genome release. We will explain how we used a variety of experimental data types to update gene structures. We will talk about upcoming projects aimed to further improve existing annotations and add missing genes. An overview of how to search for gene structure related data in TAIR will also be given. In the Gene Function section, we will describe the process of annotating from the literature using GO and PO controlled vocabularies, and then demonstrate how controlled vocabularies allow for standardization of annotation, assist in comparative genomics and can be used to classify large data sets. The Metabolic Pathway section will explain the process of pathway curation and methods of assigning genes to pathways. We will demonstrate how to query and retrieve information on pathways, enzymes, genes and metabolites. We will also discuss the use of tools for displaying and analyzing microarray, proteomic and metabolomic data. Finally we will also explain how the user community can actively participate in the ongoing process of improving database contents at TAIR."
(a) TAIR, Carnegie Institution, Stanford

P41006 Reverse-engineering Arabidopsis transcriptional regulatory network via co-expression network-based clustering and motif analysis.
Hernandez, Brian S-presenter vds090@my.utsa.edu(a) Perez, Joseph (a,b) Sponsel, Valerie M (a) Ruan, Jianhua (b)
"Gene co-expression in plants can be determined with ease through microarray data analysis. However, one cannot extrapolate gene regulation from co-expression alone. The aim of this project was to determine the regulation of co-expressed genes in Arabidopsis by integrating microarray data and promoter analyses. We first constructed a gene co-expression network using the large microarray compendium from AtGenExpress. Applying a network clustering tool, we identified 1254 gene clusters, most of which contain highly significant Gene Ontology terms. The promoter region of each gene, defined as 1000bp upstream from the transcription start site, was scanned with ~500 known cis-regulatory motifs in PLACE, allowing for imperfect matches. For each motif, we counted the number of matched genes in each cluster and the entire genome, respectively, and computed a p-value to test whether the motif occurs in a cluster more frequently than would be expected. Overall, 250 motifs were found to be statistically significant in at least one cluster. Finally, we used the motif matches to construct a co-regulation network, in which functional relationships were assessed between motifs and gene clusters using information from the PLACE and Gene Ontology databases. Based on literature, the functions of many motifs corresponded very well to the functions of the gene clusters in which they were found, indicating the significance of our results. We are currently applying de novo motif finding tools to identify possible novel motifs, and are preparing to construct a website for visualizing gene co-expression and co-regulation, which we believe will be a useful resource to further understand complex gene regulation pathways in Arabidopsis as well as other plants. [Funded by NSF UMB0634588]"
(a) University Of Texas at San Antonio, Dept. Biology, One UTSA Circle, San Antonio, 78249 (b) University Of Texas at San Antonio, Dept. Computer Science, One UTSA Circle, San Antonio, 78249

SESSION P42 – ORGANELLE BIOLOGY

P42001 Novel termination-dependent translation in chloroplasts
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"The chloroplast genome of higher plants contains ca. 80 protein-coding genes. Translational control is the major step of chloroplast gene expression, and it is important for the stoichiometric production of individual subunits in photosynthetic complexes. To study mechanisms of translation unique to chloroplasts, we have developed an *in vitro* system from tobacco chloroplasts. This system is highly active enough to measure the relative rate of translation. The *ndhC* and *ndhK* genes are partially overlapped and cotranscribed in many land plants. The downstream *ndhK* mRNA possesses 4 possible AUG initiation codons in many dicot plants. Using our *in vitro* system, we defined that the major initiation site of tobacco *ndhK* mRNAs is the third AUG that is located 4 nt upstream from the *ndhC* stop codon. Mutation of the *ndhC* stop codon arrested translation of the *ndhK* cistron. Frameshift of the *ndhC*-coding strand inhibited also *ndhK* translation. The results indicated that *ndhK* translation depends on termination of the preceding cistron, namely translational coupling. Surprisingly, removal of the *ndhC* 5'-UTR and its AUG still supported substantial translation of the *ndhK* cistron. This translation was abolished again by removing the *ndhC* stop codon. Although translation of the downstream cistron of an overlapping mRNA is generally very low, we found that the *ndhC/K* mRNA produces NdhK and NdhC in similar amounts. As the stoichiometry of NdhK and NdhC is suggested to be 1:1, the *ndhC/K* mRNA is translated not only by a translational coupling event but also by a novel termination-dependent pathway. For the latter pathway, free ribosomes are likely to be loaded on the *ndhC* coding region, migrate to the *ndhC* stop codon and start to translate the *ndhK* cistron."
(a) Graduate School of Natural Sciences, Nagoya City University (b) Sugiyama Human Research Center, Sugiyama Jogakuen University

P42002 Developmental changes and organelle biogenesis in the reproductive organs of thermogenic skunk cabbage
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http://news7a1.atm.iwate-u.ac.jp/zazensou/nedo/
"The sex-related thermogenesis, during the reproductive organ development in the inflorescence, is a unique feature among some protogynous arum species. One of such plants, skunk cabbage can produce a massive heat during the female stage, but not during the subsequent male stage in which

the stamen completes the anther dehisces and pollen release. Although recent studies have identified the spadix as the thermogenic organ, it remains entirely unclear how individual tissues or intracellular structures are correlated with thermogenesis. In this study, we examined reproductive organ development and organelle dynamics during the development of thermogenic stages. During the female stage, the stamens exhibit extensive structural changes and organelle dynamics especially in anther. They accumulate high level of mitochondrial proteins, including possible thermogenic factors, AOX and UCP. In contrast, the petals and pistils seem to be less dynamic during the female stage. However, they contain larger amount of mitochondria in the female stage than those in the male stage in which they increase large cytoplasmic vacuoles. Comparative study between female and male spadices suggests that mitochondrial amount rather than their function is correlated with the thermogenesis. However, their spadices even in male contain larger amount of mitochondria that had greater oxygen consumption, compared with non-thermogenic plants examined. Taken together, our data suggest that extensive maturation process in stamens may produce a massive heat by their increased metabolic activities, in addition, petals and pistils may act synergistically with the stamens by regulating the mitochondrial amount rather than their function."
(a) Cryobiofrontier Research Center, Iwate University (b) The 21st Century Centers of Excellence Program, Iwate University (c) Graduate School of Life Sciences, Tohoku University

P42003 A DEAD-box RNA helicase RH39 is required for chloroplast rRNA processing in *Arabidopsis thaliana*.
Nishimura, Kenji-presenter k-nishim@bs.naist.jp(a) Ogawa, Taro (a) Ashida, Hiroki (a) Yokota, Akiho (a)
"Chloroplasts possess their own genome and prokaryotic-type protein synthesis apparatus. Recent advances in molecular genetics have revealed that nuclear-encoded factors contribute to regulation of chloroplast protein synthesis or construction of the translation machinery. To gain insight into the chloroplast protein synthesis, we have characterized the *nara12-1* (*the gene necessary for the achievement of RuBisCO accumulation*) mutant of Arabidopsis with impaired chloroplast translation. Positional cloning of *NARA12* identified a DEAD-box RNA helicase RH39. DEAD-box family proteins are conserved in various organisms and play a crucial role in RNA metabolism, but there is little knowledge of the DEAD-box proteins in higher plants, and in particular the physiological functions in chloroplasts have never been examined. Transient expression of the *N*-terminus of the RH39-GFP fusion protein in tobacco leaves demonstrated that RH39 is localized in chloroplasts. RNA gel blot analyses showed that chloroplast 23S rRNA processing is defective in *nara12-1*. Association of mRNAs with ribosomes was normal in this mutant, while not only mature 23S rRNAs but also the precursors were observed in polysome fractions, implying that the relative amount of functional ribosomes is reduced in the mutant polysomes. Furthermore, T-DNA insertion allele *nara12-2* was embryonic lethal due to abnormal seed formation. Our results suggest that RH39 is essential for chloroplast rRNA maturation, translation of plastid-encoded genes and normal plant development."
(a) Grad. Sch. Biol. Sci., Nara Inst. Sci. Technol.

P42004 Plastid-targeting of rice alpha-amylase glycoprotein from the Golgi apparatus through the secretory pathway
Kitajima, Aya (a) Asatsuma, Satoru (b) Okada, Hisao (a) Hamada, Yuki (a) Kaneko, Kentaro (a) Toyooka, Kiminori (c) Matsuoka, Ken (b,c) Nakano, Akihiko (d,e) Mitsui, Toshiaki-presenter t.mitsui@agr.niigata-u.ac.jp(a)
"The well-characterized secretory glycoprotein, rice alpha-amylase isoform I-1 (AmyI-1), was localized within the plastids, and proved to be involved in the degradation of starch granules in the organelles of rice cells. In addition, a large portion of AmyI-1 fused to green fluorescent protein (AmyI-1-GFP), expressed transiently, also co-localized with a simultaneously-expressed fluorescent plastid marker in onion epidermal cells. The plastid targeting of AmyI-1 was inhibited by both dominant negative and constitutive active mutants of AtARF1 and AtSAR1, which arrest the ER-to-Golgi traffic. In cells expressing fluorescent trans-Golgi and plastid markers, they frequently co-localized when co-expressed with AmyI-1. Three-dimensional time-lapse imaging and electron microscopy of high-pressure frozen/freeze-substituted cells clearly demonstrated that contact and subsequent absorption of the Golgi-derived membrane vesicles with cargo into plastids occur within the cells. The transient expression of a series of carboxy-terminal truncated AmyI-1-GFP fusion proteins in the onion cell system showed that the region from Trp301 to Gln369 is necessary for plastid targeting of AmyI-1. Furthermore, the results obtained by site-directed mutations for Trp302 and Gly354, located on surface opposite sides of AmyI-1 protein, suggest that multiple surface regions are necessary for plastid targeting. Based on these results, we propose that Golgi-to-plastid traffic is involved in the transport of glycoproteins to plastids, and that plastid targeting is accomplished in a sorting signal-dependent manner."
(a) Niigata University (b) Kyushu University (c) RIKEN Plant Science Center (d) RIKEN Advanced Science Instititute (e) University of Tokyo

P42005 "Chloroplast-Nuclear signaling: A Tale of Phosphatases, Gene silencing and Histone Modifications"
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"Chloroplast-nuclear signaling involves retrograde signals from the chloroplast to the nucleus and the opposite process of nuclear modifications altering chloroplast function is referred to an anterograde signaling. We have demonstrated that high light-mediated retrograde signaling requires the nucleotidase/phosphatase, SAL1 (1). Knockouts in Arabidopsis of SAL1, *alx8* and *fry1-1*, have elevated transcripts of *APX2*, *ZAT10* and *DREB2A* (2). In addition, the plants have lower levels of H_2O_2, enhanced tolerance to drought and altered leaf morphology. SAL1 either breakdowns IP3 and/or 3'(2')-phosphoadenosine 5'- phosphate (PAP). Two studies have implicated PAP activity as critical for the leaf phenotype due to PAP inhibition of exoribonucleases, which in turn inhibit gene silencing (3, 4). The implications of gene silencing for the chloroplast-nuclear signaling functions of SAL1 will be discussed. With respect to anterograde signalling, the chloroplast and carotenoid regulation mutant, *ccr1*, demonstrated that the histone methyltransferase, SDG8, is required for expression of the *CAROTENOID ISOMERASE* gene. The ccr1 knockout of SDG8 has altered histone methylation, reduced *CRTISO* mRNA, reduced lutein and increased shoot branching (5). The altered carotenoid profile may partially affect shoot branching, potentially by perturbed biosynthesis of the carotenoid substrates of strigolactones. Thus, lutein, a carotenoid critical for photosynthesis and photoprotection, appears to be regulated by a chromatin modifying enzyme. 1. Wilson*, Estavillo* et al (2009) Plant J. online 2. Rossel et al (2006) Plant Cell Environ. 29: 269-281 3. Gy et al (2007) Plant Cell 19: 3451-3461 4. Kim and von Arnim (2009) Plant J. online 5. Cazzonelli*, Cuttriss* et al. (2009) Plant Cell online "
(a) ARC Centre of Excellence in Plant Energy Biology, Australian National University

P42006 A comparative genomics approach identifies RARE1: a pentatricopeptide repeat protein mediating chloroplast *accD* transcript editing
Heller, Wade P.-presenter wph7@cornell.edu(a) Robbins, John C. (a) Hanson, Maureen R. (a)
"Proper chloroplast function depends on regulated interactions between nuclear and plastid genes and their products. Nuclear-encoded proteins affect nearly every aspect of chloroplast gene expression--from transcription to proteolysis. The molecular apparatus responsible for C-to-U RNA editing in chloroplasts is encoded by nuclear genes. Recognition of the correct C target of editing is directed by site-specific trans-factors that interact with sequences 5' of edited nucleotides. A few such trans-factors have been identified in Arabidopsis mutants lacking RNA editing of particular Cs, and all are members of the pentatricopeptide-repeat (PPR) protein family. The Arabidopsis PPR family comprises over 450 proteins, some members of which are known to be mediate aspects of organellar expression in addition to RNA editing, including polycistron cleavage, splicing and translation.

PPR proteins contain degenerate 35aa repeats, some having additional C-terminal motifs, including the 'E' (extended) domain, and the 'DYW' domain, which is believed to have catalytic activity. At present there are 4 known editing factors, including RARE1, that have DYW domains in addition to the PPR and E domains. *RARE1* was identified by a comparative genomics analysis of Arabidopsis and rice PPR genes in combination with virus-induced gene silencing. Assays of editing in *rare1* insertional mutants confirmed the *accD* transcript editing defect of *RARE1* silenced plants. Wild-type plants edit *accD* transcripts to restore an evolutionarily conserved leucine residue in the encoded protein, β-carboxyltransferase. Despite a complete lack of *accD* editing, homozygous *rare1* mutants are unexpectedly robust and produce abundant progeny."
(a) Department of Molecular Biology & Genetics, Cornell University

P42007 Studies on mitochondrial DNA amount and mitochondrial morphology in rice egg cell
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"In plant vegetative cells, mitochondria are usually small grain-shaped. On the other hand, it was reported that unusually shaped giant mitochondria (large cup-shaped or long stretched rod-shaped) appeared in the egg cells of geranium, maize, ipomoea nil, and bracken. In this study, to characterize egg cell mitochondria of rice, we isolated unfertilized egg cells of rice by means of non-enzymatic manual dissection and observed the egg cell mitochondria and mitochondrial DNA (mtDNA) simultaneously. These observations showed that mitochondria in rice egg cell were small grain-shaped, unlike geranium, maize, ipomoea nil, and bracken. The double staining of mitochondria and mitochondrial DNA by MitoTracker and SYBR Green I showed that mitochondria in the rice egg cell had a large amount of mtDNA compared with that of the rice root protoplast. To see the mtDNA amount in the rice egg cell more quantitatively, real-time PCR analysis was performed. We have quantified the copy numbers of four mitochondrial genes per single rice egg cell and rice leaf protoplast. Real-time PCR analysis revealed that an egg cell had more than 10-fold copy numbers of all of four genes encoded in the mitochondrial genome, compared with those of a leaf protoplast."
(a) Graduate School of Agricultural and Life Sciences, The University of Tokyo (b) Department of Biological Sciences, Tokyo Metropolitan University

P42008 Second Site Suppressors of *pex6-1* Bypass Fatty Acid β-Oxidation Requirement During Seedling Establishment
Ratzel, Sarah-presenter sratzel@rice.edu(a) Rasbery, Jeanne (a) Bartel, Bonnie (a)
"Peroxisomes are eukaryotic organelles that house a variety of important metabolic reactions. In plants, these reactions include fatty acid beta-oxidation to utilize seed lipid stores for energy and the related process of indole-3-butryic (IBA) beta-oxidation to form the active hormone indole-3-acetic acid (IAA). Because peroxisomes lack genetic material, all enzymes required for these functions are imported through the action of PEX proteins. PEX5 is a receptor that binds proteins destined for the peroxisomal matrix, and PEX6 is an ATPase needed to recycle PEX5 from the peroxisome for further cycles of import. The Arabidopsis *pex6-1* mutant is IBA resistant and requires exogenous sucrose for hypocotyl elongation when grown in the dark, indicating peroxisomal defects. *pex6-1* can be partially rescued by over-expressing PEX5, suggesting that PEX6 is involved in peroxisomal receptor recycling (Zolman and Bartel, 2004, PNAS 101: 1786). We are investigating *pex6-1* interactions using directed reverse genetic approaches and by conducting a suppressor screen in the *pex6-1* background. We identified several second site suppressors that can be divided into two groups, those that restore *pex6-1* matrix protein import defects and those that maintain these defects. Most of the suppressors have restored sucrose independence but maintain IBA resistance, suggesting a mechanism that can bypasses the fatty acid beta-oxidation requirement during seedling establishment. Currently, we are using positional cloning to identify the genes that are defective in these mutants to better understand the role of peroxisomal import and receptor recycling in fatty acid beta-oxidation and phytohormone metabolism."
(a) Rice University

P42009 Interdependence of the PEX5 and PEX7 peroxisome-targeting receptors in *Arabidopsis thaliana*
Martinez, Naxhiely-presenter nax@rice.edu(a) Woodward, Andrew (b) Bartel, Bonnie (a)
"Peroxisomes are single membrane-bound organelles that function to compartmentalize certain metabolic reactions critical to plant and animal development. The import of proteins from the cytoplasm into the organelle matrix depends on more than a dozen peroxin (PEX) proteins, with PEX5 and PEX7 serving as receptors that shuttle proteins bearing a peroxisome targeting sequence (PTS) into the organelle. PEX5 is the PTS1 receptor, PEX7 is the PTS2 receptor, and in both plants and mammals, PEX7 depends upon PEX5 binding to deliver PTS2 cargo into the peroxisome. In this study, we characterized *Arabidopsis thaliana pex7* mutants isolated through forward and reverse genetic screens in physiological and biochemical assays. We found a *pex7* missense mutation, *pex7-2*, that disrupts PEX7-cargo binding and PEX7-PEX5 interactions in yeast, as well as PEX7 accumulation in plants. Moreover, we observed an unexpected decrease in PEX5 accumulation in *pex7* mutants. We examined localization of various peroxisomally targeted GFP derivatives in the *pex7* mutants and, surprisingly, we observed defects not only in PTS2 import, but also in PTS1 protein import. Together, our data suggest that PEX5 and PTS1 import depend on PEX7 for function in *Arabidopsis*. This work was supported by the NSF (MCB-0745122) and by an NIH predoctoral fellowship (F31-GM081911)."
(a) Rice University (b) Southwestern University

P42010 Initiation of chloroplast biogenesis by activation tagging
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"During the de-differentiation and regeneration process, plastids are converted from chloroplasts to proplastids and are reconverted into chloroplasts again. The gene for hygromycin B phosphotransferase and β-glucuronidase (GUS) placed under control of the *RBCS* (the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase) promoter were introduced into Arabidopsis genome. White calli induced from roots of transgenic Arabidopsis were used for mutant screening by activation tagging with quadruply repeated enhancers derived from the CaMV 35S promoter. Transformed calli were subjected to screening for possible hygromycin B resistance resulting from the expression of hygromycin B phosphotransferase driven by the *RBCS* promoter. Three mutant lines, *ces101* to *ces103* (callus expression of RBCS), were obtained from approximately 4,000 transformed calli. The active transcription driven by the *RBCS* promoter in all *ces* mutants was confirmed by the expression of both the GUS reporter gene and the endogenous *RBCS* gene. Chlorophyll and carotenoid were detected in all *ces* mutants, as was light-dependent O_2 evolution. The integrated loci of T-DNA were determined by TAIL (thermal asymmetric interlaced)-PCR. The candidate genes for *ces* mutants were selected according to their expression profiles. Introduction of a DNA fragment harboring the gene for receptor-like kinase and small peptide into the parental line reproduced the phenotype of *ces* mutants. We thus conclud that CES101 is a receptor-like kinase and CES102 is a small peptide."
(a) University of Shizuoka

P42011 Comparison of mesophyll and bundle sheath chloroplast envelopes reveals novel transmembrane proteins with differential expression
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"The chloroplast envelope represents the interface between the cytosol and the plastid. Yet only a few metabolite transport proteins have been characterized at the molecular level. These would be of particular importance in the case of the C4 plant maize where most transport proteins necessary for the CO2 accumulation in the bundle sheath as well as other C4-specific metabolic fluxes are still unknown. We hypothesized that a comparative proteomics of Zea mays mesophyll and bundle sheath chloroplasts will reveal proteins that are differentially expressed in BS and MS cells, including candidate proteins that control metabolite fluxes between and within the cells. We were able to identify over 200 proteins from maize chloroplast envelopes. 70% of those contain transmembrane regions and 45% are known chloroplast envelope proteins. However, 25% have not been previously characterized. YFP labelling has so far confirmed the chloroplast localization of eight of these novel proteins. A semi-quantitative analysis based on peptide counts of the proteome of Z. m. mesophyll and bundle sheath chloroplast envelopes has revealed 23 differentially expressed proteins. RT-PCR from mRNA from bundle sheath and mesophyll protoplasts has so far corroborated the expression patterns identified through proteomics. We are continuing to confirm localization and expression patterns of those novel proteins throughout the plants. We are also analyzing Arabidopsis knock-out mutants of some of the candidate genes. One of the candidates is Mep1 (Maize envelope protein 1), one of the most abundant proteins in maize mesophyll chloroplast envelopes. Metabolite analysis suggests a possible role for Mep1 as monocarboxylate transporter. This project was funded by NSF grant 0548610 to SHB and APMW. "
(a) Department of Biochemistry and Molecular Biology, Michigan State University (b) Independent Study Michigan State University (c) Heinrich Heine Universitaet, Duesseldorf, Germany

P42012 A bioinformatics/genetic approach to identify chloroplast proteases that degrade chloroplast proteins during leaf senescence in Arabidopsis
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"Chloroplast protein degradation during leaf senescence is a major contributor to nitrogen allocation since chloroplast proteins comprise 75% of cellular nitrogen. We have demonstrated that chloroplasts remain distinct organelles late into the senescence process, and thus some protein degradation within the chloroplast, independent of SAVs and RCBs, is likely. Two bioinformatics approaches were used to identify 18 chloroplast proteases that have increased expression during senescence. One approach started with all protease genes (MEROPS) while the other (Virtual Plant) started with all genes up-regulated in senescent tissue. To determine if these genes play a role in leaf senescence, a three-pronged approach is being taken. First, protease-green fluorescent protein (GFP) fusions are being expressed in plants. For the first four proteases studied, three were shown to be in the chloroplast. Second, real-time qPCR is being used to confirm up-regulation of protease mRNAs during senescence. Data for the first seven proteases indicate that expression is increased in older leaf tissue. The third approach is to isolate Arabidopsis lines with T-DNA insertions in protease genes. These mutants are currently being analyzed for protein content during senescence using immunoblots. Rubisco large subunit was quantified relative to Lhcb1 midway through senescence in WT and mutant lines. For the first seven mutant lines tested, two serine proteases, *s41-3* and *s33a-1* and one metalloprotease, *m41a-2*, displayed increased Rubisco LSU protein content (120-130%) during senescence when compared to WT. These results suggest that some of the chloroplast proteases identified using bioinformatics approaches are contributing to chloroplast protein degradation during leaf senescence."
(a) Department of Biological Sciences, California State University Long Beach

P42013 The impact of photo-oxidation on chloroplast DNA: mutational lesions and recombinational repair
Sears, Barbara B.-presenter sears@msu.edu(a) Nguyen, Ngoc (a) Enell, Matthew (a) Blaine, Allison (a) Nagori, Ashita (a) Mayle, Ryan (a) Raehtz, Kevin (a)
"In saturating light, photosynthetic electron transfer results in the production of reactive oxygen species (ROS). Reactive oxygen can damage lipids and proteins of the chloroplast and consequently reduce cell viability. Although ROS is capable of damaging DNA, the impact of photo-oxidation on chloroplast DNA (cpDNA) has not been carefully investigated. To examine whether cpDNA is as susceptible to oxidative damage as are the lipid and protein components of the chloroplast, we grew the unicellular green alga *Chlamydomonas reinhardti* under different light conditions, and in the presence of the singlet oxygen-generating chemical Rose Bengal and the superoxide-generating chemical methyl viologen. To quantify the frequency of spontaneous mutation of cpDNA, cells were plated on the antibiotic spectinomycin, which targets the chloroplast ribosome. SpecR mutations are known to be due to base substitutions in the 16S rRNA gene, which is encoded in the cpDNA. We found that the mutation frequency increased when cells were exposed to higher intensity light, as well as Rose Bengal and methyl viologen, allowing us to conclude that cpDNA is indeed susceptible to ROS damage. We hypothesize that the polyploidy of cpDNA serves as a buffer against the reactive oxygen produced within the chloroplast, by providing many templates for recombinational repair. To test this theory, we have examined whether cell lines that overexpress either the wild-type *E. coli RecA* gene or a dominant negative *recA* variant within the chloroplast show altered vulnerability to the photosensitizing agents."
(a) Michigan State University

P42014 Characterization of the *Arabidopsis* PEROXIN5 receptor
Khan, Bibi R.-presenter Miz_Khan@yahoo.com(a) Zolman, Bethany (a)
"Plant peroxisomes are the primary site for fatty acid beta-oxidation. Peroxisomes play a critical role during germination and early seedling establishment in oilseed plants. Prior to photosynthesis, stored lipids are beta-oxidized in peroxisomes to provide the necessary energy for development. Peroxisome mutants that are defective in fatty acid metabolism are dependent on exogenous sucrose for germination and early development. Besides fatty acid beta-oxidation, peroxisomal proteins also function in degradation of branched chain amino acid, photorespiration, embryogenesis, jasmonic acid synthesis and conversion of indole-3-butyric acid (IBA) to indole-3-acetic acid (IAA). Peroxisomes lack genetic material; proteins required for peroxisomal processes are imported posttranslationally into the matrix. In plants the PEX5 receptor is required for matrix protein import. Here we examine the phenotype of *Arabidopsis* plants in which the PEX5 receptor was mutated. This *pex5-10* mutant has a T-DNA insertion in exon 5 of the *PEX5* gene. Sequencing results showed that exon 5 along with the T-DNA is removed in this mutant, resulting in a truncated PEX5 protein. The *pex5-10* mutant is defective in germination and is completely dependent on sucrose for early seedling establishment. This mutant also displays delayed seedling development and is resistant to IBA. However, the mutant displays no defects in photorespiration, or reproduction; rather adult *pex5-10* plants display wild-type phenotypes. The PEX5 protein is highly expressed in seedlings and decreases as the plant ages, consistent with the mutant phenotype. "
(a) University Of Missouri-St. Louis

P42015 Movement and remodeling of the higher plant endoplasmic reticulum.
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"Using a novel analytical tool, this study investigates the relative roles of actin, myosin and Golgi bodies on form and movement of the endoplasmic

reticulum (ER) in tobacco leaf epidermal cells. Expression of a truncated myosin-XI and drug-induced actin depolymerisation produce a more persistent network of ER tubules and larger persistent cisternae. The treatments differentially affect two persistent size classes of cortical ER cisternae, those greater than 0.3 μ;m^2, and those smaller, called punctae. The punctae are not Golgi and ER remodeling occurs in the absence of Golgi bodies. The treatments diminish the mobile fraction of ER membrane proteins, but not the flow of mobile membrane proteins. The results support a new model whereby ER network remodeling is uncoupled from membrane surface flow, and the punctae are network nodes that act as foci of actin polymerization, regulating network remodeling through exploratory tubule growth and myosin-mediated shrinkage. "
(a) Texas A&M University (b) Oxford Brookes University

P42016 "Towards understanding the molecular function of an essential protein in the plastid outer envelope, OEP80"
Hsu, Shih-Chi-presenter hschsu@ucdavis.edu(a) Inoue, Kentaro (a)
"Protein import is a prerequisite for plastid biogenesis. Toc75 plays an essential role for this process and co-exists with its paralog OEP80 in the outer membrane of the plastid envelope. While Toc75 has been defined as a protein conducting channel, the function of OEP80 remains elusive. The gene duplication giving rise to these two proteins from the common ancestor with the extant cyanobacteria appears to have occurred early during the endosymbiosis, leading to the establishment of the protein import apparatus in the plastid. To further our understanding of the endosymbiotic event, we began to define the function of OEP80. By a genetic study using *Arabidopsis thaliana*, we found that OEP80 becomes essential after the globular stage of embryo development, whereas previous studies showed that *toc75*-null embryos cannot pass the preglobular stage. We were able to rescue the embryo-lethal *oep80*-null plants by expression of an OEP80 cDNA with a constitutive promoter, but not by that of a Toc75 cDNA. These results suggest that both Toc75 and OEP80 are essential for embryogenesis, but may have distinct functions in plastids. *In silico* analyses of nuclear genes encoding plastidic proteins indispensable for various stages of embryogenesis have led to a speculation that OEP80 may be involved in transport of components necessary for plastidic gene expression. We also found that i) the main form of OEP80 protein is ca. 10-kD smaller than the predicted size by immunoblotting and immunoprecipitation with an antiserum against bacteria-produced OEP80 protein followed by LC-MS/MS, and ii) the first 156-bp of the originally-annotated coding sequence are dispensable for genetic complementation of *oep80*-null plants. These data suggest non-canonical translation initiation of OEP80."
(a) Department of Plant Sciences, University of California, Davis

P42017 Plant cells without detectable plastids are generated in the *crumpled leaf* mutant of *Arabidopsis thaliana*
Yoshioka, Yasushi-presenter yoshioka@bio.nagoya-u.ac.jp(a) Chen, Yulin (a,b) Asano, Tomoya (c) Fujiwara, Makoto T. (d) Yoshida, Shigeo (e) Machida, Yasunori (a)
"Plastids are maintained in cells by proliferating prior to cell division and being partitioned to each daughter cell during cell division. It is, however, unclear whether cells without plastids are generated when plastid division is suppressed. The *crumpled leaf* (*crl*) mutant of *Arabidopsis thaliana* is a plastid division mutant that displays severe abnormalities in plastid division and plant development. We show that the *crl* mutant contains cells lacking detectable plastids; this situation probably results from an unequal partitioning of plastids to each daughter cell. Our results suggest that *crl* has a partial defect in plastid expansion, which is suggested to be important in the partitioning of plastids to daughter cells when plastid division is suppressed. The absence of cells without detectable plastids in the *accumulation and replication of chloroplasts 6* (*arc6*) mutant, another plastid division mutant of *A. thaliana* having no significant defects in plant morphology, suggests that the generation of cells without detectable plastids is one of the causes of the developmental abnormalities seen in *crl* plants. We also demonstrate that plastids with trace or undetectable amounts of chlorophyll are generated from enlarged plastids by a nonbinary fission mode of plastid replication in both *crl* and *arc6*."
(a) Division of Biological Science, Graduate School of Science, Nagoya University (b) College of Life Science, Hebei Normal University (c) Advanced Science Research Center, Kanazawa University (d) Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo (e) Plant Science Center, RIKEN

P42018 Bright bulbs lighted up on continuous the vacuolar membrane - seeking for its characteristics and biological significance -
Saito, Chieko-presenter chiezo@riken.jp(a) Awai, Chie (a) Tominaga, Motoki (a) Uemura, Tomohiro (b) Ebine, Kazuo (b) Ito, Jun (c) Abe, Hiroshi (a) Ueda, Takashi (b) Morita, Miyo T (c) Nakano, Akihiko (a,b)
"The plant vacuole fulfills a variety of functions and is essential for plant growth and development. The vacuolar membrane has very complex three-dimensional configuration and dynamic features. We have previously identified bulb, a complex and mobile structure on the continuous vacuolar membrane (Saito et al. 2002). To reveal its biological functions, we have carried out several approaches to obtain further structural evidence, evidence for a differentiated subregion of the vacuole and clues of its functions. We have obtained data, using transgenic Arabidopsis lines expressing soluble-GFP, YFP-2xFYVE, and own-promoter-driven GFP fusions of SNARE molecules. We have succeeded in visualizing bulb-like structures by expressing non-membrane proteins and found that bulbs are formed in more tissues than we already reported. We also tried to envisage qualitative differences of the continuous vacuolar membrane by multi-color live imaging. We have isolated two bulb-less mutants among those that are known to be defective in shoot gravitropism or in morphology of the vacuolar membranes in endodermal cells in the flowering stem. "
(a) Riken (b) The University of Tokyo (c) Naist

P42019 In vivo effects of NbSiR silencing on chloroplast development in Nicotiana benthamiana
Ahn, Eu-Ree-presenter sweater83@gmail.com(a) Kang, Yong-Won (a) Lee, Jae-Yong (a) Pai, Hyun-Sook (a)
"Sulfite reductase (SiR) performs dual functions, acting as a sulfur assimilation enzyme and as a chloroplast (cp-) nucleoid binding protein. In this study, we examined *in vivo* effects of SiR deficiency in plants. Virus-induced gene silencing of *NbSiR* encoding a *Nicotiana benthamiana* sulfite reductase resulted in leaf yellowing and growth retardation phenotypes, and cysteine supplementation could not rescue the phenotypes. NbSiR:GFP fusion protein was targeted to chloroplasts and colocalized with cp-nucleoids. The recombinant proteins of the full-length NbSiR and its C-terminal half had cp-DNA compaction activities *in vitro*, and the induced expression of the full-length NbSiR condensed genomic DNA in *E. coli.* In earlier stages, the *NbSiR*-silenced plants showed significant defects in PEP (plastid-encoded multimeric RNA polymerase)-dependent transcript accumulation. In later stages, depletion of NbSiR resulted in chloroplast ablation. In *NbSiR*-silenced plants, enlarged cp-nucleoids containing a large amount of cp-DNAs were observed in the middle of the abnormal chloroplasts, and the cp-DNAs are predominantly in subgenomic sizes based on pulse field gel electrophoresis. The abnormal chloroplasts developed prolamellar body (PLB)-like cubic lipid structures in the light without accumulating POR proteins. Further analyses revealed that sulfur depletion induced the PLB-like membrane organization. These results suggest that NbSiR plays a role in cp-nucleoid metabolism and thylakoid membrane development."
(a) Department of Biology, Yonsei University

P42020 Silencing of *Nicotiana benthamiana* TAC10 caused defects in PEP-dependent transcript accumulation and chloroplast biogenesis

Jeon, Young-presenter planty@daum.net(a) Pai, Hyun-Sook (a)
"pTAC10 was identified as a component of the plastid transcriptionally active chromosome (pTAC) in *Arabidopsis*, but its function in chloroplasts has not been characterized. In this study, we investigated in vivo functions of pTAC10 using gene silencing and overexpression technology in *Nicotiana benthamiana*. NbTAC10, *Nicotiana benthamiana* TAC10, contains a S1 domain that is involved in nucleic acid binding. Confocal microscopy indicated that NbTAC10:GFP fusion protein was targeted to the chloroplasts and colocalized with the chloroplast (cp)-nucleoids. Induced expression of the recombinant proteins of the full-length NbTAC10 or its S1 domain condensed genomic DNA in E. coli, and arrested the bacterial growth. Interestingly, the NbTAC10:GFP proteins were localized in the vicinity of the condensed nucleoids in the growth-arrested E. coli cells. Silencing of NbTAC10 by using virus-induced gene silencing (VIGS) or cosuppression resulted in leaf yellowing and moderate plant growth defects. Based on northern blot analyses, NbTAC10 deficiency significantly perturbed the PEP (plastid-encoded multimeric RNA polymerase)-dependent transcript accumulation but not the NEP (nucleus-encoded phage-type RNA polymerases)-dependent transcript accumulation. Prolonged depletion of NbTAC10 resulted in chloroplast ablation with drastic size reduction and thylakoid degeneration. In contrast, NbTAC10-overexpressing plants showed no visible plant phenotypes, and their plastid transcription profiles and chloroplast biogenesis were also normal. These results and the previous finding suggest that TAC10 plays a critical role in PEP-dependent chloroplast transcription and biogenesis within the plastid transcriptionally active chromosome complex."
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P42021 Amyloplast division mechanism in the endosperm of rice
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"Although significant progress has been made in our understanding of chloroplast division and development in the Arabidopsis leaf, little is known about the molecular mechanisms that control plastid division processes in cereals such as rice (*Oryza sativa*). In this study, we characterized in detail an *arc5* mutant of rice, and compared the roles of *ARC5*, a member of the dynamin superfamily, in chloroplast division in the leaf and amyloplast division in the endosperm. Unlike the binary fission of chloroplasts, amyloplasts divide at multiple sites, generating a beads-on-a-string structure. In addition, large amyloplasts divide by budding-type division, giving rise to small amyloplasts attached to their surfaces. Fluorescent protein, fused to either the wild type ARC5 or a dominant negative form ARC5(K60A), was targeted to the constriction sites in dividing amyloplasts. The loss of function of *ARC5* and overexpression of *DsRed-ARC5* in the endosperm similarly inhibited amyloplast division, which resulted in fused amyloplasts with thick connections. These results suggest that a productive complex of the division machinery is formed only when the stoichiometry of ARC5 relative to other factors is in a suitable range. Furthermore, the size of starch granules was, on average, smaller when amyloplast division was inhibited, suggesting that amyloplast division processes have a significant effect on starch granule synthesis in the rice endosperm. Consistently, the gelatinization peak temperature of *arc5* starch was significantly higher than for wild-type starch, indicating that amylopectin synthesized in *arc5* is structurally different from that in the wild type."
(a) National Institute of Agrobiological Sciences

P42022 Mutations in *ClpR4* suppress the leaf variegation phenotype of *thf1*
Wu, Wenjuan (a) Huang, Jirong-presenter huangjr@sibs.ac.cn(a)
"Thylakoid Formation 1 (THF1) is highly conserved in all oxygenic photosynthetic organisms. Loss-of-function mutants of *thf1* result in leaf variegation and low activity of photosystem II (PSII) in high light conditions. However, molecular mechanism underlying *thf1*-induced phenotypes remains unclear. We screened suppressors for the leaf-variegated phenotype of *thf1* mutants from EMS mutagenized plants, and obtained 8 independent lines with the rescued phenotype. One of the suppressors turns out to be a mutation in *ClpR4*, which encode a subunit of the chloroplast ClpP/R protease, through the map-based cloning approach. In the *clpr4* mutant, the splicing site of the second intron is mutated and leads to generation of three transcripts from *ClpR4*. Both *clpr4* and *clpr4 thf1* showed virescence and serrated leaves, which is consistent with the reported *clpr2* and *clpr1* mutants. *clpr1* has been shown to suppress the leaf variegation phenotype of *var2*. Interestingly, our data showed that *clpr4* also suppresses *var2* variegation. Currently, we are studying mechanism how reduced activity of Clp protease can suppress the leaf variegation phenotype. References: Koussevitzky, S *et al.*, (2006). *Plant Mol. Biol.*63:85-96. Miura, E *et al.*, (2007). *Plant Cell* 19:1313-1328. Park, S., and Rodermel, S.R. (2004). *PNAS* 101:12765-12770. Rudella, A *et al.*, (2006) *Plant Cell* 18:1704-1721. Yu, F *et al.*, (2008). *Plant Cell* 20:1786-1804. "
(a) SIPPE,Shanghai institutes for biological sciences,CAS

P42023 Towards developing a method for the isolation of plastids from soybean somatic embryos
Clark, Karen R. (a) Sparace, Salvatore A.-presenter smsprc@clemson.edu(a)
"The plastid plays a major role in the metabolic processing of carbon and other resources for the synthesis of seed storage reserves such as oil and starch. We have initiated a line of research designed to help better understand how plastids from developing soybean (*Glycine max* L.) embryos regulate the allocation of metabolic resources into the various seed storage components. However, a reliable method for the routine isolation of large quantities of physiologically active plastids from these embryos is currently not available. Based on methods developed for other plant tissues, and using de novo fatty acid biosynthesis as a marker for physiological functionality, we have made progress towards developing a method that can be used for the isolation of plastids from developing soybean somatic embryos. The greatest rates of fatty acid biosynthesis (nmoles acetate/h) were recovered in the 500 x g and then the 3000 x g pellet fractions, with essentially no activity occurring in the 3000 x g supernatant. Similarly, rates of fatty acid biosynthesis were greatest in plastids isolated early in embryo development, corresponding to 25 days in culture. Crude plastids of the 3000 x g pellet could be further purified by centrifugation through various concentrations of percoll or on percoll step gradients, but with considerable loss of total activity. Fatty acid biosynthesis by 3000 x g plastids was linear with up to 100 µ;L of plastids(equivalent to 19 µ;g chlorophyll) in one-hour reactions, but gradually decreased over 6 hours. Our observations indicate that plastids isolated from developing soybean somatic embryos are similar, but relatively unique in comparison to other plastids. This work was supported by Grant no. 8233 from the United Soybean Board to S.A. Sparace."
(a) Clemson University

P42024 Rice *OGR1* Encodes a Pentatricopeptide Repeat-DYW Protein and Is Essential for RNA Editing in Mitochondria
Kim, Sung-Ryul-presenter breeder@postech.ac.kr(a) Yang, Jung-Il (a) Moon, Sunok (a) Ryu, Choong-Hwan (a) An, Kyungsook (a) Kim, Kyung-Me (a) Yim, Jieun (a) An, Gynheung (a)
"RNA editing is the alteration of RNA sequences via insertion, deletion, and conversion of nucleotides. In flowering plants, specific cytidine residues of RNA transcribed from organellar genomes are converted into uridines. About 35 editing sites are present in the chloroplasts of higher plants; six pentatricopeptide repeat (PPR) genes involved in RNA editing have been identified from Arabidopsis. Although ~500 editing sites are found in mitochondrial RNAs of flowering plants, only one gene has been reported from *Arabidopsis* that is involved in such editing. Here, we identified rice mutants that are defective in seven specific RNA editing sites on five mitochondrial transcripts. Their various phenotypes include delayed seed germination, retarded growth, dwarfism, and sterility. Mutant seeds from heterozygous plants are opaque. This mutation, named *opaque and growth retardation 1* (*ogr1*), was generated by T-DNA insertion into the gene that encodes a PPR protein containing the DYW motif. The OGR1:GFP fusion

protein is localized to mitochondria. Ectopic expression of *OGR1* in the mutant complements the altered phenotypes. We conclude that OGR1 is essential for RNA editing in rice mitochondria and is required for normal growth and development."
(a) Department of Integrative Bioscience and Biotechnology, Pohang University of Science and Technology (POSTECH)

P42025 Functional characterization of an Arabidopsis C-terminal tail-anchored protein in peroxisome and mitochondrial division
Aung, Kyaw-presenter aungkyaw@msu.edu(a) Hu, Jianping (a)
"Peroxisomes and mitochondria are ubiquitous and essential organelles in most of the eukaryotic cells. The division of peroxisomes and mitochondria is important for the maintenance cellular homeostasis, responses to environmental cues, and organelle inheritance. In Arabidopsis, DYNAMIN-RELATED PROTEIN3 (DRP3) and FISSION1 (FIS1) play conserved roles across eukaryotes in the division of both peroxisomes and mitochondria. Here, we present another putative component of the division machineries shared by these two types of organelles, peroxisome and mitochondrial division factor1 (PMD1). PMD1 is targeted to the surface of both peroxisomes and mitochondria as fluorescent protein fusions. Biochemical analyses suggest that PMD1 is a C-terminal tail-anchored protein on the peroxisomal membrane and possibly the mitochondrial membrane as well. The loss-of-function mutant *pmd1-1* exhibits enlarged peroxisomes and elongated mitochondria, whereas overexpression of *PMD1* leads to aggregation of numerous minute peroxisomes and mitochondria. Furthermore, the aggregated peroxisomes and mitochondria form heterocomplexes. Collectively, these findings suggest that PMD1 has a crucial role in the division and/or positioning of peroxisomes and mitochondria. "
(a) Department of Energy Plant Research Laboratory and Plant Biology Department, Michigan State University

P42026 Arabidopsis VAC1 protein is involved in the regulation of chloroplast gene expression
Hsieh, Ming-Hsiun-presenter ming@gate.sinica.edu.tw(a) Tseng, Ching-Chih (a) Sung, Tzu-Ying (a) Li, Yi-Chiou (a) Lin, Chien-Li (a) Hsu, Shih-Jui (a)
"We have isolated an Arabidopsis albino mutant, vanilla cream1 (vac1), which is caused by a T-DNA insertion in a pentatricopeptide repeat (PPR) gene. Protoplast transient expression assay revealed that the VAC1-GFP fusion protein is localized to the chloroplast. Northern blot analysis indicated that steady-state levels of chloroplast rRNA transcripts were significantly decreased in the vac1 albino mutant. This may lead to a global defect in the expression of chloroplast genes. There are two types of RNA polymerase involved in transcribing chloroplast genes: a plastid-encoded multimeric RNA polymerase (PEP) and a nucleus-encoded RNA polymerase (NEP). These RNA polymerases are responsible for the transcription of distinct types of plastid genes. In general, PEP is involved in the transcription of photosynthesis genes and NEP preferentially transcribes housekeeping genes. We used northern blot analysis to examine the expression of chloroplast genes in the vac1 mutant. Interestingly, the expression of PEP transcribed genes is down-regulated, whereas the expression of several NEP transcribed genes is up-regulated in the vac1 mutant. These molecular phenotypes, e.g. global defects in chloroplast gene expression, are similar to those of ribosome- or PEP-deficient mutants. These results suggest that the VAC1 PPR protein may be involved in regulating ribosome or PEP activities. Alternatively, the VAC1 PPR protein may play a role in coordinating the expression of chloroplast genes transcribed by PEP and NEP. "
(a) Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan

P42027 "pfkB-type carbohydrate kinase family protein, NARA5, is essential for the active expression of plastid-encoded photosynthetic genes in *Arabidopsis thaliana*"
Ogawa, Taro-presenter ta-oga@bs.naist.jp(a) Nishimura, Kenji (a) Aoki, Takehiko (a) Ashida , Hiroki (a) Yokota, Akiho (a)
"The active expression of photosynthetic genes in chloroplasts depends on many nucleus-encoded factors. To understand the molecular mechanism involved in the expression of photosynthetic genes, we have screened Arabidopsis mutants which shows markedly lower levels of plastid-encoded photosynthetic proteins including RuBisCO. Map-based cloning of the mutated gene in the *nara5-1* mutant, revealed that *NARA5* (*At4g27600*) encodes a phosphofructokinase B (pfkB)-type carbohydrate kinase family protein of unknown function. NARA5 fused to green fluorescence protein is localized in chloroplasts. The T-DNA insertion mutant, *nara5-2*, is unable to grow on soil, indicating that NARA5 is essential for autotrophic growth of Arabidopsis. Quantitative RT-PCR analysis of photosynthetic genes during light-induced greening of etiolated seedlings of *nara5* alleles shows that transcripts of plastid-encoded photosynthetic genes are severely decreased, particularly that of RuBisCO large subunit. Recently, two pfkB-type carbohydrate kinase family proteins were identified together with plastid-encoded RNA polymerase (PEP) subunits in a transcriptionally active chromosome complex from Arabidopsis and mustard chloroplasts (Pfalz *et al.* 2006). These suggest the functional significance of pfkB-type carbohydrate kinase family proteins in the transcription of photosynthetic genes in chloroplasts."
(a) Grad. Sch. Biol. Sci., Nara Inst. Sci. Technol.

P42028 Proteomic identification and functional characterization of peroxisomal histidine triad family proteins in Arabidopsis
Yang, Pingfang-presenter yangpf@msu.edu(a) Hu, Jianping (a,b)
" Peroxisomes are essential organelles in plant growth and developments and are involved in numerous metabolic processes such as β-oxidation, photorespiration, and reactive oxygen species (ROS) scavenging. Exploring the protein constituents of the peroxisome will deepen our understanding about this single-membrane-bound organelle. Using a proteomic approach followed by in vivo targeting verifications, we identified a number of novel proteins from Arabidopsis leaf peroxisiomes (Reumann et al., *Plant Physiology* 2009). Among these proteins, there are three members of the Histidine triad (HIT) family, named HIT1, HIT2 and HIT3, all of which contain peroxisome targeting signals type 1 or 2. The HIT family proteins contain a conserve HΦHΦH (Φ stands for hydrophobic amino acid) motif. Its members in yeast and mammals can hydrolyzes the compounds Ap_nX (n=1 to 5; X stands for the group like NH_2, lysine, adenosine, glucose) into Adenosine monophosphate (AMP). Although HIT family proteins ubiquitously exist in various organisms, their physiological functions in plant are poorly understood. Each of the three Arabidopsis HIT proteins has homologs in other plant species. Microarray and semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) data show that all three genes are constitutively expressed in different tissues and throughout development in Arabidopsis. Furthermore, yeast two-hybrid analysis revealed that HIT2 and HIT3 can form homo-dimers and that all of these three HIT proteins can hetero-dimerize with each other. To uncover the physiological functions these three HIT proteins conduct in plant peroxisomes, ongoing experiments are aimed at determining the enzyme activity and substrates for these HIT proteins."
(a) Department of Energy-Plant Research Laboratory, Michigan State University (b) Plant Biology Department, Michigan State University

P42029 "PARC6, a Novel Chloroplast Division Factor, Influences FtsZ Assembly and is Required for Recruitment of PDV1 During Chloroplast Division in *Arabidopsis*"
Yang, Yue (a) Glynn, Jonathan M. (a) Vitha, Stanislav (a,b) Schmitz, Aaron J. (a) Hemmes, Mia (a) Miyagishima, Shin-ya (a,c) Osteryoung, Katherine W.-presenter osteryou@msu.edu(a)
"Chloroplast division in plant cells is accomplished through the coordinated action of the tubulin-like FtsZ ring inside the organelle and the dynamin-like ARC5 ring outside the organelle. This coordination is facilitated by ARC6, an inner envelope protein required for both FtsZ assembly and ARC5

recruitment. Recently, we showed that ARC6 specifies the mid-plastid positioning of the outer envelope proteins PDV1 and PDV2, which have parallel functions in dynamin recruitment. PDV2 positioning involves direct ARC6-PDV2 interaction but PDV1 and ARC6 do not interact, indicating an additional factor functions downstream of ARC6 to position PDV1 (Glynn et al. 2008, Plant Cell 20: 2460-2470). Here, we show that PARC6 (Paralogue of ARC6), an ARC6-like protein unique to vascular plants, fulfills this role. Like ARC6, PARC6 is an inner envelope protein with its N-terminus exposed to the stroma and *Arabidopsis parc6* mutants exhibit chloroplast and FtsZ filament morphology defects. However, whereas ARC6 promotes FtsZ assembly, PARC6 appears to inhibit FtsZ assembly, suggesting ARC6 and PARC6 function as antagonistic regulators of FtsZ dynamics. The FtsZ inhibitory activity of PARC6 may involve its interaction with the FtsZ-positioning factor ARC3. A PARC6-GFP fusion protein localizes both to the mid-plastid and to a single spot at one pole, reminiscent of the localization of ARC3, PDV1 and ARC5. Although PARC6 localizes PDV1 it is not required for PDV2 localization or ARC5 recruitment. Our findings indicate that PARC6, like ARC6, plays a role in coordinating the internal and external components of the chloroplast division complex, but that PARC6 has evolved distinct functions in the division process. "
(a) Michigan State University (b) Texas A&M University (c) RIKEN, Japan

P42030 Integrity of the plant Golgi apparatus
Chen, Yani-presenter yani@msu.edu(a) Faso, Carmen (a) Kentaro , Tamura (b) aurelia , boulaflous (a) Federica, Brandizzi (a)
"How the endoplasmic reticulum (ER) and the Golgi apparatus maintain their morphological and functional identity while they work in concert to ensure the production of biomolecules necessary for the cell's survival is a fundamental question that remains largely unanswered, especially in plants. Here, we report on the isolation of Arabidopsis mutants identified through a forward genetics screen specifically designed for the plant Golgi [Boulaflous et al. (2008) From imaging to genes. Traffic 9: 1613-7]. The mutant phenotypes suggest that we have identified key-players of the trafficking machinery that controls membrane export from and import into the Golgi. In support of this, our preliminary analyses based on membrane traffic assays show that we have identified novel mutations that are responsible for the maintenance of the architecture and membrane steady-state distribution at the plant Golgi. Here we will present our latest results on this project. "
(a) Department of Energy Plant Research Laboratory, Michigan State University (b) Department of Botany, Graduate School of Science, Kyoto University

P42031 "Behaviour of chloroplasts in leaves of succulent C_3, C_4, and CAM plants under drought conditions"
Kondo, Ayumu-presenter ayumu@ccmfs.meijo-u.ac.jp(a) Terashima, Tomonori (a) Funaguma, Toru (a)
"In leaves of some succulent crassulacean-acid-metabolism (CAM) plants, including *Kalanchoe* species, we previously discovered the phenomenon of chloroplast clumping. A combination of light and water stress induced chloroplasts to form spherical bodies within mesophyll cells. We have suggested that this phenomenon in succulent plants is a morphological mechanism that protects against light stress intensified by a severe water deficiency. The spherically clumping of chloroplasts was only found in the leaves of succulent CAM plants and not in leaves of other non-succulent C_3, C_4 plants. Therefore, it is unclear whether the phenomenon is attributed to the structure of succulent leaf or the photosynthetic mechanism. In this study, we examined about the anatomical and biochemical features involved in the phenomenon of chloroplasts clumping by used 5 succulent species, such as *Mesembryanthemum spectabilis* (C_3), *Portulaca grandiflora*, *P. oleracea* (C_4), *Kalanchoe blossfeldiana*, *Zygocactus truncates* (CAM). When subjected to drought stress, chloroplasts became densely clumped in one area of the mesophyll cells in *K. blossfeldiana* and *Z. truncates*. In two species in the *Portulaca*, only mesophyll chloroplasts moved and gathered centripetally close to the bundle sheath cells. In *M. spectabilis*, although no specific arrangement of chloroplasts were observed in mesophyll cells, the number of chloroplasts in a mesophyll cell were decreased significantly as compared with its under well-water conditions. These findings suggest that the chloroplast behaviour patterns against drought stress differ depending on the photosynthetic type. "
(a) Meijo University

P42032 Inside the peroxisome.
Barlow, Robert-presenter rbwtb@umsl.edu(a) Khan, B. Rafeiza (a) Zolman, Bethany K (a)
"Peroxisomes contain hundreds of enzymes that act in numerous metabolic pathways. Fatty acid beta-oxidation is essential for early seedling development in Arabidopsis and other oilseed plants. Plant peroxisomes also are implicated in photomorphogenesis, lateral root formation, and jasmonic acid synthesis required for wounding responses. Genetic evidence suggests that indole-3-butyric acid (IBA) is converted to the active auxin indole-3-acetic acid (IAA) in a peroxisomal process similar to fatty acid beta-oxidation. Our recent studies have focused on the molecular mechanism of IBA beta-oxidation. *ibr3* has altered IBA responses. *IBR3* encodes a peroxisomal enzyme with aminoglycoside phosphotransferase and acyl-CoA dehydrogenase/oxidase domains, making IBR3 a candidate enzyme for acting in IBA oxidation. However, mutants defective in peroxisomal fatty acid beta-oxidation enzymes also show IBA-resistant phenotypes. For instance, five members of the acyl-CoA oxidase (ACX) family of enzymes influence IBA responses; studies of other phenotypes in *acx* mutants show similar redundancy. Therefore, multiple enzymes may be acting in IBA metabolism or, more likely, disruptions in individual peroxisomal pathways can affect other processes. We are doing biochemical and genetic tests to examine the activity and specificity of IBR3 and ACX proteins in peroxisomal processes and to determine the roles of each protein in plant growth and development. "
(a) University of Missouri Saint Louis

SESSION P43 – ORGANISMAL EVOLUTION

P43001 "Selection, gene flow, and the formation of range boundaries in *Clarkia xantiana* (Onagraceae)"
Gould, Billie A-presenter bag59@cornell.edu(a) Fabio, Eric (a) Geber, Monica (a)
"Determining why species have restricted natural geographic ranges is a fundamental question in ecology and evolutionary biology. Here we present results of a study of the contribution of adaptive genetic differentiation to the formation of range boundaries in the annual wildflower *Clarkia xantiana ssp. xantiana*. This species is endemic to the mountains of California and occurs across a steep east/west moisture gradient. Population local adaptation to different water availability conditions across the range may influence the formation of the range boundaries of this species through the flow of maladaptive alleles into edge populations. Here we present data on adaptive trait differentiation among 15 *Clarkia* populations grown in a common garden, including traits such as flowering time, reproductive effort, vegetative structure, and specific leaf area. We contrast these results with results from a survey of 30 populations in the field. We also present preliminary measurements of both adaptive (Q_{st}) and neutral (F_{st}) genetic differentiation across the range and compare this with estimates of gene flow and population structure derived from microsatellite marker diversity in this species. We are thus able to infer the relative importance of both selection and gene flow in restricting the natural range of this species."
(a) Cornell University, Department of Ecology and Evolutionary Biology

P43002 "Introgression in a native tree species, and conservation implications"
Hoban, Sean M-presenter shoban@nd.edu(a) Tim, McCleary (a) Jeanne, Romero-Severson (a)
http://www.nd.edu/~treedna1/
"Hybridization of introduced species with native species is increasingly recognized as an important conservation problem. Investigations in short lived species have shown that the consequences of genetic invasion can be rapid, extensive, and evolutionarily significant. However, our knowledge of natural hybridization and hybrid persistence in trees is limited. Hybrid dynamics may be different in trees, which have unique population and reproductive biology, and undergo selection over a long time scale with a wide variance in microclimate, disease pressure, and competition. We investigate the question of naturally occurring interspecific hybrids between two forest trees: the native North American butternut (Juglans cinerea L.) and the introduced Japanese walnut (Juglans ailantifolia Carriere). Using nuclear and chloroplast DNA markers, we provide evidence of 29 probable F1 and 22 probable advanced generation hybrids in seven locations (N=187) across the eastern range of the native species. Two locations show extensive admixture (95% J. ailantifolia and hybrids). Although hybridization was highest in fragmented, semi-rural landscapes, we also found probable hybrids in three National Forests. Hybridization appears to be asymmetrical with 90.1% of hybrids having J. ailantifolia as the maternal parent, which may indicate partial one way intrinsic incompatibility. This is the first genetic data supporting natural hybridization in our system, and among the first reports of natural hybridization between native and introduced forest trees. If further investigation reveals that introgression is as widespread as our study indicates, hybridization could substantially alter the genetics of J. cinerea, even in protected areas, with potentially cascading ecosystem consequences. "
(a) University of Notre Dame

P43003 Genetic Diversity and a Haplotype Analysis of *Brasenia schreberi* (Cabombaceae) in East Asia based on Chloroplast DNA
Choi, Hong-Keun-presenter hkchoi@ajou.ac.kr(a,b) Kim, Changkyun (a,c) Na, Hye Ryun (a,d)
"We investigated the nucleotide variation of chloroplast DNA (cp DNA) trnL-F intergenic spacer region to infer their evolutionary relationships and to examine the level of genetic diversity and population differentiation among fifteen populations of *Brasenia schreberi* distributed in South Korea, Japan, and China. The trnL-F intergenic spacer region of cp DNA in *B. schreberi* varied from 1,416 to 1,419 bp and thirty two sites were variable after multiple alignments. In total, twenty two haplotypes were identified from three hundreds individuals of *B. schreberi. A relatively low level of haplotype diversity (h >/i> = 0.079) and nucleotide diversity (pi = 0.00009) were detected in B. schreberi . Pairwise comparisons of Fst deduced from cp DNA variation suggested no significant genetic differentiation between populations of B. schreberi . Low genetic differentiation among populations was consistently indicated by both hierarchical analyses of molecular data (AMOVA) and the structure of a neighbor-joining dendrogram. Furthermore, the distribution of haplotypes was not geographically confined within each population. Haplotype A was widely distributed over the all sampled populations. In the minimum spanning network of cp DNA haplotyes in B. schreberi , interior position coupled with high frequency indicated the ancestry haplotype was type A. Lacking of population differentiation among populations in terms of geographic distances of B. schreberi* may be due to the effects of lower substitution rates or lineage sorting. On the basis of the minimum spanning network in cp DNA, we suggest the haplotype A was an ancestral type in *B. schreberi* population differentiation because the A haplotype is in the central position of the network with the highest frequency. "
(a) Ajou University (b) Professor (c) Research Associate (d) Graduate Student

P43004 Characterization of eukaryotic translation initiation factor 6 (eIF6) genes in plants
Kato, Yuki-presenter theair_23@yahoo.co.jp(a,b) Konishi, Mineko (a,b) Shigyo, Mikao (a) Yoneyama, Tadakatsu (a) Yanagisawa, Shuichi (a,b)
"Eukaryotic translation initiation factor 6 (eIF6) is an evolutionary conserved protein among eukaryotes. This protein is involved in ribosome biogenesis and essential for cell growth and viability in yeast. It is also known that the *eIF6* mRNA level is modulated in several animal species, depending on the growth stages and the circumstances. In spite of the evidently critical role of eIF6 in eukaryotic cells, plant *eIF6* genes have not been characterized yet. Here we show the first characterization of plant *eIF6* genes. Despite that a single gene encodes eIF6 in yeast and animals, two copies of eIF6-like genes, which were referred to as Os-*eIF6;1* and Os-*eIF6;2*, and At-*eIF6;1* and At-*eIF6;2*, were identified in rice and *Arabidopsis* genomes. The expression levels of these genes were differentially affected by supply of ammonium nitrate. Ammonium nitrate induced expression of Os-*eIF6;2* but not that of Os-*eIF6;1* in rice, whereas this compound affected neither expression of At-*eIF6;1* nor that of At-*eIF6;2* in *Arabidopsis*. The insertion of T-DNA into At-*eIF6;1* was found to cause embryonic lethal while disruption of At-*eIF6;2* did not induce any apparent phenotype. At-eIF6;1 therefore appeared to play a dominant role in *Arabidopsis*, in consistency with the dominant expression of At-*eIF6;1*. Furthermore, we show that, since expression of At-eIF6;1 rescued the eIF6 defective phenotype in yeast, plant eIF6s may also be involved in ribosome biogenesis. Based on these findings, we will discuss the possibility that two copies of *eIF6* genes are present in each plant genome and utilized in different manners in distinct plant species. Further characterization of plant eIF6s, including subcellular localization analysis and expression pattern analysis with GUS reporter gene, will be presented."
(a) The University of Tokyo (b) Core Research for Evolutional Science and Technology, JST

P43005 Contrasting phylogeographical patterns of *Toxicodendron radicans* in East Asia
Chiang, Yu-Chung -presenter yuchung@mail.npust.edu.tw(a) Shih, Huei-Chuan (b) Hsu, Tsai-Wen (c) Tsai, Chi-Chu (d) Chiang, Tzen-Yuh (e)
"In this study, maternally transmitted chloroplast DNA (cpDNA), mitochondrial DNA (mtDNA) and paternally ISSR codominant markers were used to investigate the genetic diversity, phylogeographic pattern and possible refugium within and among natural populations on the two subspecies of *Toxicodendron radicans* in Asia. *T. radicans* in Asia, known as the relative or complex of North American poison ivy is a perennial climbing deciduous woody shrub. Populations are distributed in the monsoon Asia, including Southwest China, Taiwan and Japan, where the geological history is largely affected by plate tectonics, island formation, and Pleistocene glaciations. Gillis (1975) reclassified *Rhus ambigua* to *T. radicans* and devided to two subspecies. One subspecies, *T. radicans* ssp. *hispidum*, is distributed in Southwest China, while another one, ssp. *orientale*, is distributed in Japan. In this study, we cloned and sequenced the *rbcL-atpB* spacer of chloroplast genome, *nad1* intron of mitochondrial genome and analyses 10 ISSR codominate makers for 504 individuals including populations collected from Taiwna, Japan, mainland China and America. Based on the statistics for population differentiation among two Asian subspecies and origin species was found high signification exist, showing the speciation process effected in two sub species and origin species in different geographical area. "
(a) Department of Life Science, National Pingtung University of Science and Technology (b) School of Nursing, Mei-Ho Institute of Technology (c) Endemic Species Research Institute (d) Kaohsiung District Agricultural Improvement Station (e) Department of Life Sciences, Cheng Kung University

P43006 A preliminary phylogeny of Caribbean *Tabebuia* based on the nrDNA ITS region.
Martinez, Nirzka-presenter nirzka@yahoo.com(a,b) Salazar, Jackeline (c) Caraballo, Marcos (b) Santiago, Eugenio (a,b) Mateo, Amelia (c)

"*Tabebuia* Gomes ex DC. is an extremely captivating Neotropical plant genus in the Bignoniaceae family. The genus is considerable diverse, comprising approximately 100 taxa distributed from Mexico to Argentina. The majority of the described species are concentrated in the Greater Antilles (~60), especially in the islands of Cuba and Hispaniola. Not only there is a high level of endemism in the region, as well as endemism per island, but in addition the genus is extremely versatile in terms of morphological variation and adaptations to a wide range of ecological conditions (e.g., life zones, soil, and altitude). Although other researchers previously have found that the genus *Tabebuia* seems to be paraphyletic, the evolutionary relationships among the species in the Caribbean Islands have still not been clearly elucidated. The purpose of the study is to test the monophyly of the Caribbean group and evaluate the evolutionary relationships between the species and species complexes, assess patterns of morphological evolution and adaptive-ecological diversification among species, and the evaluation of geographical relationships within the group. Preliminary phylogenetic reconstruction using a partial group of species and nucleotide sequences from the internal transcribed spacer (ITS) suggests a closer relationship between Caribbean Tabebuia and continental *T. rosea* and *T. aurea*. It also confirms the paraphyly of the genus with the presence of members of the genera *Ekmanianthe* and *Crescentia*. Final results will not only clarify the evolutionary relationships among taxa, but will also help in answering important biogeographical questions pertaining to their origin and diversification patterns. "
(a) Department of Biology, University of Puerto Rico, Rio Piedras Campus (b) Botanical Garden Herbarium, University of Puerto Rico (c) Biology School, Autonomous University of Santo Domingo (UASD)

SESSION P44 – PHOTORECEPTORS, LIGT SIGNALING & PHOTOMORPHOGENESIS

P44003 "HEMERA, an essential regulator linking phytochrome nuclear bodies and light signaling in *Arabidopsis*"
Chen, Meng-presenter chen.meng@duke.edu(a,b) Galvao, Rafaelo M (a) Li, Meina (a) Burger, Brian (b) Bugea, Jane (b) Chory, Joanne (b,c)
http://www.plasticgenome.org/
"Phytochromes are red and far-red photoreceptors regulating every facet of plant development and growth. Two early phytochrome signaling events have been described: (A) Light directly regulates the relocation of phytochrome A (phyA) and phytochrome B (phyB) from the cytoplasm to the nucleus, where they interact and colocalize with a group of bHLH transcription factors (PIFs) on discrete subnuclear foci called phytochrome nuclear bodies and regulate transcription; (B) Light triggers rapid degradation of phyA and some of the PIFs. The function of phytochrome nuclear bodies in relationship to phytochrome signaling events is unknown. We carried out a unique genetic screen looking for phyB:GFP mislocalization mutants. This screen identified a novel photomorphogenetic mutant, *hemera* (*hmr*). Strikingly, besides defects in phyB:GFP nuclear body formation, the *hmr* mutant is impaired in all phytochrome responses examined, including chloroplast biogenesis and phyA degradation, suggesting that HMR is an essential regulator linking phytochrome nuclear body formation and light signaling. The tall and albino phenotypes of *hmr* make it the founding member of a new class of photomorphogenetic mutant. In addition, the *hmr* mutant is the first phytochrome signaling mutant defective in phyA proteolysis, which suggests a biochemical role of HMR and phytochrome nuclear bodies in protein degradation. Further characterization of HMR will likely to provide great insight into the mechanistic link between phytochrome nuclear bodies and early phytochrome signaling events."
(a) Department of Biology, Duke University, Durham, NC 27708, USA (b) Plant Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 29037, USA (c) Howard Hughes Medical Institute, The Salk Institute for Biological Studies, La Jolla, CA 92037, USA

P44004 Blue light-specific regulation of CIB1 protein expression in Arabidopsis
Liu, Hongtao-presenter liuhongtao76@gmail.com(a) Lin, Chentao (a)
"Cryptochromes (CRY) are photolyase-like blue-light receptors that mediate light responses in plants and animals. How plant cryptochromes act in response to blue light is not well understood. We have recently reported the identification and characterization of the Arabidopsis CIB1 (cryptochrome-interacting basic-helix-loop-helix). CIB1 interacts with CRY2 (cryptochrome 2) in a blue light-specific manner, and it acts with additional CIB1-related proteins to promote CRY2-dependent activation of FT gene expression and floral initiation and. CIB1 binds to the FT protmoter in vivo. We proposed that the blue light-dependent interaction of cryptochrome(s) with CIB1 and CIB1-related proteins represents an early photoreceptor signaling mechanism in plants. Consistent with our hypothesis that CIB1 is specifically involved in signaling of blue light receptor CRY2, we discovered that CIB1 protein expression is regulated specifically by blue light. CIB1 protein is degraded in the absence of blue light. CIB1 is degraded, via a ubiquitin/proteasome pathway, in the dark, red, and FR light. CIB1 degradation is suppressed in blue light, resulting in accumulation of CIB1 in blue light. Possible molecules associated with CIB1 expression will be discussed"
(a) MCDB,University of California, Los Angeles

P44005 The role of phytochrome in the regulation of the ethylene production in *Arabidopsis*
Kim, Soon Young-presenter kimsy@andong.ac.kr(a) Kwak, Eun Hee (a) Kim, Jong Sik (a) Park, Ji Hea (a) Hangarter, Roger (b)
"Phytochrome is involved in the control of many major processed during plant development, including seed germination, leaf development, and flowering. Previous work in our laboratory has demonstrated the pattern of ethylene production of phytochrome mutants (*phy A*, *phy B*, and *phyAB*) of *Arbidopsis*. The results suggested that ethylene production is inhibited in the phytochrome mutants compared to the wild type in the light-grown condition. This inhibition was due to the lower level of ACC synthase activity in the mutants. This study examines the effect of light condition such as dark, red light or far red light-grown condition in the ethylene production in these mutants. These results will be discussed in the relation to the level of ACC synthase and ACC oxidase activity in phytochrome mutants of *Arabidopsis*. Supported by Korea Research Foundation grants 2008-521-C00240
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(a) Andong National University (b) Indiana University

P44006 Photoreceptor systems for light-dependent intracellular positioning of mitochondria in Arabidopsis thaliana
Islam, Md. Sayeedul-presenter islam@bio.sci.osaka-u.ac.jp(a) Niwa, Yasuo (b) Takagi, Shingo (a)
"While mitochondria movement in plant cells is actin- and/or microtubule-dependent, light-dependent intracellular positioning and movement of mitochondria remain to be elucidated. We asked whether mitochondria in leaf palisade cells of Arabidopsis thaliana stably expressing mitochondria-targeted GFP occupy different intracellular positions under different light conditions. The pattern of light-dependent redistribution of mitochondria was essentially identical to that of chloroplasts, namely, mitochondria occupy the periclinal regions under weak blue light (wBL; 470 nm, 4 µ;mol m-2s-1) and the anticlinal regions under strong blue light (sBL; 100 µ;mol m-2s-1), respectively. Strong red light (660 nm, 100 µ;mol m-2s-1) had a small effect to induce accumulation of mitochondria on the inner periclinal regions. We semi-quantitatively analyzed the mode of movement of individual mitochondria along the outer periclinal walls. Within 30 min of BL illumination, mitochondria movement was acclerated in a fluence-rate dependent manner, whereas it was gradually decelerated resulting in co-localization with chloroplasts. In the presence of a photosynthetic inhibitor DCMU, the normal accumulation and avoidance responses of chloroplasts were impaired. In addition, mitochondria no longer took any specific positions and were distributed all over the cytoplasm regardless of fluence rate of BL. These results strongly suggest the involvement of different

photoreceptors in the regulation of mitochondria movement, namely, phototropins for the early acceleration and photosynthesis for the late deceleration. "
(a) Osaka University (b) Shizuoka University

P44007 Making the most of what you got- regulating branching in the competitive environment.
Finlayson, Scott A.-presenter sfinlayson@tamu.edu(a) Su, Hongwen (a) Krishna Reddy, Srirama R. (a) Abernathy, Scott D. (a) Chen, Shaorong (a) Holalu, Srinidhi V. (a) White, Richard H. (a)
"Branching is an important parameter that contributes to plant architecture, affecting plant fitness in natural environments, and productivity in agricultural crops and pastures. Much work has demonstrated that intrinsic genetic programs are major determinants of branching, but it is also known that environmental signals regulate this important aspect of plant form. Among these environmental signals is the red light: far red light (R:FR) which is perceived by the phytochromes. Reduced R:FR signals impending competition from neighboring plants and elicits the shade avoidance response, which includes decreased branching. In natural environments decreased branching may confer a competitive advantage to plants able to mount an early response, but in the agricultural milieu decreased branching may be undesirable. As with other shade avoidance responses, phyB appears to play the major role in modulating R:FR effects on branching, however in Arabidopsis, other phytochromes contribute as well. In sorghum, loss of phyB function or low R:FR results in a strong arrest of axillary bud outgrowth, while in Arabidopsis, the effects are more complex. Depending on the branching parameters considered, phyB and low R:FR can act either negatively or positively. The most pronounced effect of R:FR on branch development in Arabidopsis appears to involve the control of the correlative inhibition of branch outgrowth. The roles of various light signaling, hormonal and downstream bud autonomous pathways are being discovered using genetic, molecular and physiological methodologies. As our understanding of the environmental regulation of branching expands, we may find opportunities to genetically modify branching responses to R:FR to increase crop productivity at high planting density."
(a) Department of Soil and Crop Sciences, Texas A & M University, Texas AgriLife Research

P44008 Integration of light and plastid signals
Ruckle, Michael E. (a,b) Larkin, Robert M.-presenter larkinr@msu.edu(a,b)
http://www.prl.msu.edu/Facultypages/larkin.html
"Light and plastid signals promote chloroplast biogenesis and are among the most potent inducers and repressors of photosynthesis-related gene expression, respectively. These signals can be likened to a gas and brake system that promotes efficient chloroplast biogenesis and function. To learn more about the regulation of photosynthesis-related gene expression by plastid signals we performed a new *genomes uncoupled* (*gun*) mutant screen in *Arabidopsis thaliana*. The expression of genes that encode proteins active in photosynthesis is uncoupled from chloroplast function in *gun* mutants, and the expression of the nuclear and the plastid genomes is uncoupled in particular *gun* mutants. To identify mutants with defects in both light and plastid signals, we screened this new collection of *gun* mutants for photomorphogenic phenotypes and found that *cryptochrome 1* (*cry1*) mutants are *gun* mutants. Our subsequent genetic analysis of plastid and light signaling mutants indicates that a plastid signal can rewire a light signaling network, converting it from a positive to a negative regulator of photosynthesis-related genes such as those that encode the light-harvesting chlorophyll *a*/*b*-binding proteins and the small subunit of Rubisco. We found that these interactions contribute to chloroplast biogenesis, especially in high-intensity light conditions. To test these ideas further, we are screening for factors that contribute to this integration of light and plastid signals. The current status of the work will be presented."
(a) Michigan State University Department of Energy Plant Research Laboratory, Michigan State University (b) Department of Biochemistry and Molecular Biology, Michigan State University

P44009 Characterization of Cryptochrome Function in Strawberry (*Fragaria spp.*)
Chatterjee, Mithu (a) Ricaurte, Sasha A-presenter sa.ricaurte@gmail.com(a) Kumar, Dibyendu (b) Folta, Kevin M (a)
"Cryptochromes are blue light and UV-A sensing photoreceptors found both in plants and animals. Cryptochromes regulate diverse developmental processes in plants, such as inhibition of hypocotyl elongation, anthocyanin accumulation, cotyledon and leaf expansion, and flowering time. Genetic manipulation of *CRY2* in commercially important fruit crops such as strawberry may be of immense significance in regulating traits like flowering date; a critical consideration in strawberry production. Thus, we have made an attempt to functionally characterize cryptochrome2 from *Fragaria spp.*. Full-length *Fragaria vesca* CRY2 (*FvCRY2*) cDNA was obtained by screening a *Fragaria vesca* seedling library with a partial *FvCRY2* as probe. *FvCRY2* codes for protein of 645 amino acid residues and bears typical type I photolyase signature in the N-terminal region and a conserved DQXVP-acidic-STAES (DAS) domain towards the C-terminal. Phylogenetic analysis grouped FvCRY2 under eudicot CRY2 class and showed maximum resemblance with grape CRY2. *FvCRY2* mRNA is ubiquitously expressed in all the tissues tested. A complete photophysiological assessment of *FvCRY2* effects on physiological processes in strawberry has been performed in over-expression and antisense transgenic Arabidopsis and strawberry plants. These studies delineate the role for this important blue light sensor in horticulturally-relevant processes in strawberry. These findings likely apply to other members of the Rosaceae family."
(a) Horticultural Sciences Department, University of Florida, Gainesville FL (b) Interdisciplinary Center for Biotechnology Resources, University of Florida, Gainesville FL

P44010 MAX2 Regulates Seed Germination and Early Seedling De-etiolation by Modulating Multiple Hormone Levels in *Arabidopsis*
Zhu, Ling-presenter lingzhu@mail.utexas.edu(a) Shen, Hui (a) John, Andrea (a) Huq, Enamul (a)
"Ubiquitin-mediated protein degradation has been shown play central roles in light and hormone-regulated plant growth and development. Previously, we have shown that MAX2, an F-box protein, positively regulates facets of photomorphogenic development, including seed germination and seedling de-etiolation in response to light. However, how MAX2 controls these responses is still unknown. Here we show that MAX2 oppositely regulates GA and ABA biosynthesis to optimize seed germination in response to light. Dose-response curves showed that *max2* seeds are hyposensitive to GA-induced seed germination compared to wild type seeds. RT-PCR assays demonstrated that the expression of GA biosynthetic genes are down-regulated, while the expression of the ABA biosynthetic genes are up-regulated in the *max2* seeds compared to the wild type seeds. Treatment with an auxin transport inhibitor, NPA, showed that increased auxin transport in *max2* seedlings contributes to the long hypocotyl phenotype of the *max2* seedlings under red, far-red and blue light conditions. Moreover, these phenotypes are specific to max2, as the biosynthetic mutants in the newly discovered strigolactone pathway, *max3* and *max4* did not display any defects in seed germination and seedling de-etiolation compared to wild type. Taken together, these data suggest that strigolactone does not regulate *Arabidopsis* seed germination and seedling deetiolation. However, MAX2 regulates seed germination and early seedling de-etiolation by modulating multiple hormone levels in a strigolactone-independent manner in *Arabidopsis*."
(a) Section of Molecular Cell and Developmental Biology and The Institute for Cellular and Molecular Biology, University of Texas at Austin

P44011 HY5 activity is partially compromised in *ted3* plants
Desai, Mintu-presenter desaim@msu.edu(a) Hu, Jianping (a)
http://www.prl.msu.edu/Facultypages/Hu.html
"Arabidopsis plants display two contrasting developmental growth forms; skotomorphogenesis in dark and photomorphogeneis in light. The phenotype in dark germinated seedlings is due to the concerted role of COP/DET/FUS proteins, which form a protein complex that targets the positive regulators of photomorphogenesis eg. HY5, HYH, LAF1 for proteolysis *via* the proteasome. The *det1* plants have a photomorphogenic phenotype in the dark, suggesting its inability to tag the positive regulators of photomorphogenesis for degradation. The (reversal of *det1*) mutant was identified as a suppressor of *det1*. It contains a transition from G to A in the PEX2/TED3 gene and results in a missense mutation of a valine to methionine substitution in front of the C-terminal RING finger domain of this peroxisomal membrane protein. We postulated that a truncated PEX2 protein which is only comprised of the RING finger domain may be generated in the *ted3* and is involved in degradation of HY5 *via* the proteasome. We show here that the PEX2 RING finger domain by itself localizes to the nucleus and interacts with HY5 *in vivo*. We also demonstrate that overexpressing the PEX2 RING finger domain in *det1* plants leads to a partial *ted3* phenotype, indicating a role of this domain in suppressing the *det1* phenotype. In addition, the expression of some HY5 target genes is decreased in the *ted3* plant and the HY5 protein level is significantly decreased in the *ted3* plants compared to the *det1* and wild type plants. These observations together suggest that HY5 activity in the *ted3* plants is partially compromised and that this alteration of HY5 activity may have been responsible, at least in part, for the reversal of the *det1* phenotype."
(a) Michigan State University, Plant Research Laboratory

P44012 *In planta* Magnesium Chelatase studies: focus on the CHLI and CHLD subunits
Farmer, Phyllis R-presenter prf42002@yahoo.com(a) Willows, Robert D. (a)
"Mg Chelatase is a multi-subunit enzyme situated at a crucial point in tetrapyrrole biosynthesis. Positioned at the pathway branch where heme and chlorophyll biosynthesis diverge, it functions to insert Mg^{2+} into substrate Protoporphyrin IX (Proto IX), committing the intermediate into the chlorophyll biosynthetic branch. Though this enzyme has been under intense scrutiny for decades, its mechanism of assembly and its stoichiometry have yet to be demonstrated. Previous work focused on mutant studies and in-vitro analysis of subunits from *Rhodobacter* and *Synechocystis*, resulting in the current theory wherein subunit I functions as the ATP motor for the enzyme, D serves as a platform for enzyme assembly, and H binds the substrate Proto IX. Biochemical analyses of recombinant proteins revealed that subunit H binds Proto IX non-covalently, suggesting that H may be the catalytic subunit. However, recent studies have shown that BchH behaves as a substrate for the I:D complex, and suggest that enzyme control occurs via the D-subunit. Our studies aim to investigate assembly and action of Mg-Chelatase in Planta. In plants, chelation may involve elements such as Gun4, a cofactor necessary for chlorophyll accumulation. We endeavored to circumvent the issue of a heretofore undiscovered constituent, using *Agrobacterium* to transiently express wild type and mutant Mg Chelatase subunits in *N benthamiana* leaves. In a multidirectional approach, the tissue was then assayed for changes in chelatase activity, product accumulaton and gene expression. We are currently employing iTRAQ to investigate changes in the protein profile of infiltrated tissue.This work explores the action of Mg chelatase in an environment that we hope will expand our understanding of the higher plant enzyme."
(a) Macquarie University

P44013 ATOMIC PERSPECTIVES ON PHYTOCHROME PHOTOCHEMISTRY
Vierstra, Richard D-presenter vierstra@wisc.edu(a) Ulijasz, Andrew T (a) Li, Huilin (d) Wagner, Jeremiah R (a) Zhang, Junrui (a) Forest, Katrina T (a) Cornilescu, Gabriel (b) Markley, John L (a,b) von Stetten, David (c) Hildebrandt, Peter (c) Li, Hua (d)
"A complex array of photoreceptors coordinates the response of both prokaryotes and eukaryotes to their ambient light environment. One of the most influential is the phytochrome (Phy) superfamily, a large and diverse group of photochromic photoreceptors that use a bilin chromophore for light detection. These biliproteins sense red (R) and far-red light (FR) through two relatively stable conformations, a R-absorbing Pr form and a FR-absorbing Pfr form. By photointerconverting between Pr and Pfr, Phys act as light-regulated switches in various signaling cascades. Phy-type photoreceptors were first discovered in higher plants by their ability to direct numerous R/FR photoresponses critical for agricultural productivity. More recently, they were also shown to exist in various cyanobacteria, proteobacteria, actinobacteria, fungi, and slime molds. Despite their agricultural importance and evolutionary conservation, we still do not fully understand at the atomic level how Phys photoconvert between Pr and Pfr and how Pfr is then perceived. In the past few years, great strides have been made in determining how Phys function at the molecular level. Key was solving the first 3-D structures of the chromophore-binding domain (CBD) as Pr. These structures conclusively determined the conformation of the bilin linked to the apoprotein, revealed how the bilin is deeply buried in the CBD, showed that the CBD is uniquely folded into a rare figure-of-eight knot, identified a heretofore unknown dimerization contact in the CBD, and provided important clues for how plant Phys arose from their microbial progenitors. Current studies are using x-ray crystallography, NMR spectroscopy, resonance Raman, and Single-Particle EM combined with structure-guided mutagenesis to decipher the events required to generate Pfr from Pr and how Pfr then triggers associated signaling cascades. Especially useful was the discovery of novel thermostable Phys amenable to solving the solution structure of the Pr and Pfr forms. Eventually this work will provide a framework to redesign Phys for agricultural benefit. "
(a) University of Wisconsin (b) NMR Facility at Madison (c) Technische Universitat-Berlin (d) Brookhaven National Laboratory

P44014 Improved elongation of Scots pine seedlings under blue light depletion is not dependent on resource acquisition
Sarala, Marian-presenter marian.sarala@oulu.fi(a) Taulavuori, Erja (a) Karhu, Jouni (b) Savonen, Eira-Maija (c) Laine, Kari (d) Kubin, Eero (b) Taulavuori, Kari (a)
"Blue light (400-500 nm) removal induced shoot elongation of two-year-old Scots pine (*Pinus sylvestris* L.) seedlings were related to resource acquisition and frost hardening in Northern Finland (64^{o}N). The seedlings were grown in plexiglass chambers, either orange in colour or transparent. The orange chamber removed the blue wavelengths. Blue light inhibits elongation in plants. Resources are here defined as the storage compounds (carbohydrates, C/N ratio and soluble proteins) that build up in the plants and can be mobilised in the future to support biosynthesis. Conifers store a great amount of starch in the needles during spring and early summer. The carbon utilised to elongation, however, are mainly from newly stored resources. The removal of blue light did not affect the gas exchange and accumulation of growth resources. The results suggest that the increasing effect of blue light removal on Scots pines elongation is probably a photomorphogenic regulation response of metabolism, i.e. it works through the non-photosynthetic pigments (phytochrome, cryptochrome and phototropin) regulating the metabolic pathways leading to a better utilization of available stores. This is additionally supported by the findings that the removal of blue did not affect the physiological parameters (pigment composition, chlorophyll fluorescence and lipid peroxidation) measured during the preparation for winter. These parameters should be influenced if the blue light removal affects through the resource acquisition, since cold hardening in autumn is depended on cryoprotectants and thereby photosynthetic products. Therefore, the regulation effect of blue light leading to an inhibition of growth is obviously in blocking the full utilization of growth resources."
(a) University of Oulu, Department of Biology (b) Finnish Forest Research Institute, Muhos Research Unit (c) Finnish Forest Research Institute, Parkano Research Unit (d) University of Oulu, Thule-Institute

P44015 Special Dimerization Pattern of *Deinococcus radiodurans* Bacteriophytochome Photoreceptor Revealed by Single-Particle Electron Microscopy

Zhang, Junrui-presenter zhang23@wisc.edu(a) Li, Hua (b) Li, Huilin (b) Vierstra, Richard D (a)

"As light is an essential signal regulating most aspects of a plant's life, plant has evolved several groups of photoreceptors to monitor the ambient light environment. Among them, phytochromes (Phys) are dimeric red/far-red photosensing proteins with a bilin chromophore covalently attached to the N-terminal region. In plant Phys, the C-terminal histidine kinase (HK) related domain is dimeric, while the N-terminal photosensing region is monomeric. This dimeric feature is necessary for correct Phy functions. However, recent structural information on several bacteriophytochome photoreceptors (BphPs) indicates that the N-photosensing region of Phys can form dimers: independent of the C-terminal region. Here, we generated 3-D structure of full-length *Deinococcus radiodurans* (*Dr*) BphP using Single Particle Electron Cryo-Microscopy (SPEM) which is a very direct method of macromolecule structure determination. This 8.5 Angstrom EM structure aligns perfectly with the previous BphP X-ray crystallographic structures, confirming the existence of a large dimerization interface spanning a large portion of the N-terminal half. Unlike the plant Phys, the C-terminal HK domain of *Dr*BphP is not visible in the EM image. Further investigation reveals that the HK domain is very flexible, which questions the assumption that HK domain is the dimerization region in BphP. "

(a) University of Wisconsin, Laboratory of Genetics (b) Brookhaven National Laboratory

P44016 THF1 interact with PsbS and affect transient NPQ of Arabidopsis thaliana

Zhang, Lingang-presenter zhanglg@sippe.ac.cn(a) Huang, Jirong (a)

"Nonphotochemical quenching of chlorophyll fluorescence (NPQ) is always regarded as an efficient method to dissipate the excess energy and protect the photosyntem II (PSII) reaction center from photooxidative stress. Transient NPQ (qETR) is a kind of NPQ that transiently generated during transition of dark-adapted plants to nonsaturating light. It has been proposed that transient NPQ represents an initial energy quenching state of PSII. Just as stay-state NPQ formed at saturating light intensities, it is strictly dependent on the presence of PsbS and a transthylakoid pH gradient and is modulated dependent on the amount of depoxidized xanthophylls. THF1 (Thylakoid Formation Factor 1), 26.8KDa, is nucleus-encoded protein that is localized in chloroplast. The gradual increase in 683nm fluorescence in thf1 mutant implies that uncoupled antenna proteins of PSII are increased in the mutants under high light intensity. In this experiment, we have extended previous works on the THF1 localization and its effect on the photosynthesis of Arabidopsis thaliana. THF1 can interact with PsbS and both co-localize with light harvesting complex II (LHCII). Deletion of THF1 can increase the amount of PsbS and affect its distribution among PSII supercomplexes, which can increase the transient NPQ under normal light conditions."

(a) Shanghai Institutes for Biological Science, Chinese Academy of Sciences

P44017 The role of DNA damage and repair in the UV-B response of etiolated *Arabidopsis thaliana*

Biever, Jessica J-presenter bieve010@umn.edu(a) Gardner, Gary (a) Brinkman, Doug (a)

"Etiolated *Arabidopsis thaliana* seedlings, when irradiated with UV-B light (280-320 nm), show an inhibition of hypocotyl elongation. The extent of inhibition increases as UV-B fluence increases and occurs in mutants deficient in known photoreceptors including phytochrome, cryptochrome, and phototropin. DNA absorbs strongly in the 240-310 nm range, suggesting that DNA could serve as part of the UV-B perception mechanism. The two main damage products formed when DNA absorbs UV-B radiation are cyclobutane pyrimidine dimers (CPDs) and pyrimidine-6,4-pyrimidinone dimers (6,4PPs). After formation, these lesions stimulate DNA repair mechanisms, further indicating the possibility of some sensory role for UV-B absorption by DNA. Recent work in our laboratory has shown that the allelic nucleotide excision repair mutants *uvr1-1* and *uvh3* are both hypersensitive to UV-B, even at very low fluences. To determine whether DNA absorption of UV-B is related to the inhibition of hypocotyl elongation, DNA damage was measured in wild type and DNA repair mutants of *A. thaliana* over a broad UV-B fluence range. Using monoclonal antibodies specific for either CPDs or 6,4PPs, the amounts of both damage products were determined. Generally, DNA damage increased as UV-B fluence increased in both wild type Arabidopsis, *uvr1-1*, and *uvh3*, suggesting that photodimer formation may be related to the inhibition of hypocotyl elongation observed in etiolated seedlings irradiated with UV-B. Expression of chalcone synthase (*CHS*), known to be induced in *A. thaliana* after UV-B irradiation, was also determined using real-time PCR (RT-PCR) in wild type Arabidopsis, *uvr1-1*, and *uvh3* to determine any relationship *CHS* induction may have with inhibition of hypocotyl elongation and formation of DNA lesions."

(a) University of Minnesota, Department of Horticultural Science

P44018 Functional characterization of *Os*PIF1 in rice

Lee, Yongju (a) An, Gynheung (b) Park, Phun Bum-presenter pbpark@suwon.ac.kr(a)

"Phytochromes are red and far-red absorbing photoreceptors that provide plants with circadian, seasonal, and positional information which is critical for the control of germination, seedling development,shade avoidance, reproduction, dormancy, flowering, and sleep movement. Phytochromes are unique among the plant photoreceptors in their capacity to interconvert between a red-absorbing form (Pr form) which is biologically inactive and a far-red absorbing form (Pfr form) which is biologically active. Phytochromes regulate various light responses through interacting with signalling proteins such as PIFs (phytochrome interacting factors) and PILs (PIF like factors) in Arabidopsis thaliana. We have identified and characterized a PIF in rice (*Oryza sativa*) for the first time. We named it as a *Os*PIF1. *Os*PIF1 is one of the bHLH (basic helix-loop-helix) transcription factors. We have compared the phenotype of T-DNA insertional mutant of *Os*PIF1, activation tagging line of *Os*PIF1, and wild type. The coleoptile length of activation tagging line was much shorter than the wild type in dark grown seedlings, and the coleoptile length of the knockout mutant is longer than the wild type in the red light grown seedlings. The germination rate of the knockout mutant in dark and far-red light condition is less than the wild type. The amounts anthocyanin of knockout mutant were more than the those of wild type in the far-red light seedlings. For functional studies of OsPIF1, the microarray will be investigated afterward. "

(a) Department of Bioscience and BIotechnology, The University of Suwon (b) Division of Molecular Life Sicnece, POSTECH

P44019 Inducing phytochrome B signaling without activation of other phytochromes

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http://www.mcb.ucdavis.edu/faculty-labs/lagarias/index.html

"Phytochrome B (phyB) plays a dominant role in red light sensing in plants, with its regulatory roles redundantly shared and modulated by other members of the phytochrome protein family. This redundancy has made it difficult to identify genes and gene products specific to the phyB signaling pathway. Recently, our laboratory identified a class of dominant, constitutively active mutant alleles of phyB that faithfully recapitulate phyB-regulated gene expression networks in a light-independent manner (Su, Y.S., and Lagarias, J.C. 2007 Plant Cell 19, 2124-2139; Hu, W., Su, Y.S., and Lagarias, J.C. 2009 Mol. Plant 2, 166-182). By exploiting the chromophore-dependent activity of the Y276H allele (YHB) of Arabidopsis phyB, we have developed a bilin-inducible system to manipulate phyB signaling. This system not only permits investigation of phyB signaling in darkness without activation of other phytochromes, but also offers the potential to study phyB-specific signaling pathways in light-grown plants under conditions where

all other phytochromes have been inactivated. The research is funded in part by NIH grant RO1-GM068552. "
(a) Department of Molecular and Cellular Biology, College of Biological Sciences, University Of California

P44020 "Irradiating different combinations of blue, UV-A, and UV-B lights induced distinct patterns of anthocyanin accumulation at different parts of hypocotyls in tomato seedlings"
Kawabata, Saneyuki-presenter ayuki@mail.ecc.u-tokyo.ac.jp(a) Kouno, Yukiko (a) Li, Yuhua (b) Zhou, Bo (b)
"Effects of the irradiation of blue, UV-A, UV-B, blue + UV-A, blue + UV-B, and UV-A + UV-B on anthocyanin accumulation at different positions of hypocotyls were investigated in seedlings of tomato 'Money Maker' (MM) and the cryptochrome 1 mutant (*cry1*). In MM, higher accumulation of anthocyanin was observed at 1) the upper to middle part of hypocotyls by blue, 2) the middle part by UV-B, and 3) the middle to lower part by UV-A. In *cry1*, anthocyanin accumulation by blue almost completely disappeared, suggesting that the response to blue was mediated by cryptochrome 1. In contrast, the accumulation by UV-A was reduced in *cry1* as compared with MM but significant accumulation remained at the middle to lower part. Moreover, the accumulation pattern induced by UV-A differed from that of UV-B. These results suggested that there exists the UV-A specific induction of anthocyanin biosynthesis that is not mediated by cryptochrome 1 and UV-B photoreceptors. In *cry1*, synergistic effects were observed upon blue + UV-B and UV-A + UV-B irradiation at the middle part of the hypocotyls. Overall, three types of responses were observed: 1) blue/UV-A response mediated by cryptochrome 1, 2) UV-A specific response, 3) UV-B specific response, 4) interaction between blue and UV-B, 5) interaction between UV-A and UV-B."
(a) Graduate School of Agricultural and Life Sciences, University of Tokyo (b) College of Life Sciences, Northeast Forestry University

P44021 A Glutathione S-transferase Functions in the Integration of Light and Abscisic Acid Signaling Pathways to Regulate Hypocotyl and Root Elongation in *Arabidopsis*
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"Previous studies showed that a glutathione S-transferase can interact with far-red insensitive 219 (FIN219/JAR1) to regulate hypocotyl elongation in continuous far-red light (cFR). Here, we report on our isolation by RT-PCR of another plant glutathione S-transferase, *AtGSTU17*, from *Arabidopsis thaliana* that exhibited its enzymatic activities involving glutathione (GSH) or 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. *AtGSTU17* transcripts were regulated by multiple photoreceptors, especially phytochrome A (phyA) under all light conditions. Its loss-of-function mutants displayed a long hypocotyl phenotype under cFR and affected light responsive genes such as *CHS*, auxin responsive genes, and several transcription factors, which is further supported by hypophotomorphogenic responses, including less accumulation of anthocyanins and a defect in FR-mediated inhibition of greening. In contrast, ectopic expression of *AtGSTU17* resulted in an opposite effect on photomorphogenic development. Moreover, *AtGSTU17* transcripts were induced by ABA, auxin and jasmonate in a light-dependent manner. Intriguingly, both *atgstu17* and *phyA* mutants displayed insensitivity to ABA-mediated inhibition of root elongation. Taken together, these data indicate that AtGSTU17 plays a vital role in the integration of FR and ABA signalings to regulate elongation of hypocotyls and roots in *Arabidopsis*."
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P44022 Photo-physical and Biochemical Properties of the Pumping Rhodopsins in *Acetabularia acetabulum*
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"Microbial rhodopsins are retinal-binding proteins which function as ion pumps and photosensory receptors. We obtained two opsin genes from juvenile specific cDNA library of *Acetabularia acetabulum* which is a giant unicellular green alga (kindly provided by Professor Dina Mandoli). Surprisingly, there is no intron even though it is eukaryotic gene. The genes of *Acetabularia* rhodopsins (ARI & ARII) encode polypeptide of 253 and 254 amino acid residues, respectively. Interestingly, *Acetabularia* codes TAA and TAG to glutamate and use TGA as only stop codon, so we changed these sequences to CAA (Glu) for the expression. The *Acetabularia* opsin genes were expressed in *E. coli* UT5600 with endogenous retinal biosynthesis system and MISTIC (110-amino acid, an acronym for membrane-integration sequence for translation of IM protein constructs) sequences. We measured the absorption spectra and light-induced difference spectra. When it is purified, unmisticated ARI showed pink color and its absorption maximum was around 523 nm. The pKa of ARI was measured and tested the proton pumping outward activity at pH 9. Two of photointermediates of ARI have been detected including M & O that have absorption maximum at 392 and 580 nm, respectively. And also we could detect the mRNA of *Acetabularia* rhodopsin in the *A. acetabulum* whole cell by RT-PCR (reverse transcription polymerase chain reaction). (This work was supported by grant from the 21C Frontier Microbial Genomics and Application Program) "
(a) Dept of Life Science and Interdisciplinary Program of Integrated Biotechnology, Sogang University, Seoul Korea

P44023 "Gaining insight to phototropism using the gain of function mutant, *hph*"
Holland, Jennifer J-presenter jenniferjholland@gmail.com(a) Celaya, R. Brandon (b,a) Galen, Candace (a) Liscum, Emmanuel (a)
"While insights have been made relative to the molecular components to the phototropic response, several questions remain. Specifically, we were interested in using the hyper-phototropic mutant,*hph*, which is a physiological gain-of-function phot1 mutant to better understand the events associated with photoperception and events immediately following. Previous work on this mutant has shown that the *hph* mutant phenotype results from expression of both a wild-type and mutant protein, the latter, designated phot1^{hph}, being truncated directly after the LOV2 domain. This phenotype has been recapitulated by generating a double homozygous transgenic line in the *phot1-5* null mutant background that expresses both full-length phot1-GFP and truncated phot1^{hph}-mCherry. Using these double transgenic lines, the protein-protein interactions between the truncated phot1hph and full-length phot1 have been investigated, as well as the sub-cellular localization of the two phot1 protein types. The effect of phot1^{hph} on the overall water use efficiency of the plant has also been investigated, and these results have suggested an potential link between drought tolerance and blue light signaling. "
(a) University of Missouri, Division of Biological Sciences (b) University of California- Los Angeles, Department of Molecular, Cell and Developmental Biology

P44024 Role of CRL3^{NPH3} and monoubiquitylation in phototropin-mediated phototropism
Roberts, Diana R.-presenter drr6d5@mizzou.edu(a) Pedmale, Ullas V. (a,b) Liscum, Emmanuel (a)
"Plants are sessile by nature and because of this characteristic they have evolved many strategies to cope with the diversity of environmental stimuli they encounter in nature. One such strategy is the phototropic response by which plants reorient the aerial organs toward and roots away from direction blue light. The blue light response can be broken down into three general steps: perception of light, signaling to establish a lateral redistribution of auxin, and initiation of a differential growth response resulting in phototropic curvatures. Loss-of-function genetic screens have identified several key proteins involved in phototropic signal-response, including the primary photoreceptors, phototropins 1 and 2 (phot1 and phot2), and the phot1-interacting BTB protein, NPH3. NPH3 has also been shown to interact with Cullin 3 (Cul3) as a likely substrate adapter in a Cul3-based E3 ubiquitin ligase designated CRL3^{NPH3}. Recent studies in our laboratory have shown the phot1 is monoubiquitylated *in planta* in a blue light-

dependent manner suggesting that, as a NPH3-interacting protein, it is a substrate for the $CRL3^{NPH3}$ complex. Interestingly phot1 is degraded under high light conditions (120 µ;mol m^2 s^{-1}) and is targeted for internalization (and apparent recycling) under low light conditions (0.1-10 µ;mol m^2 s^{-1}). Monoubiquitylation appears to be important for both of these processes. We therefore propose that phot-dependent phototropism is modulated in large part through monoubiquitylation of phot1 by $CRL3^{NPH3}$. These ubiquitylation responses represent the blue light-induced signal transduction events occurring prior to (and presumably required for) auxin redistribution, auxin-dependent changes in transcription, and development of differential growth."
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P44025 Identification of bZIP Proteins Interacting with CONSTANS of Arabidopsis using Yeast two hybrid screen
Shin, Su Young -presenter 337ssy@hanmail.net(a) Kim, Hye Jin (a) Sim, Soon Ae (a) Jeon, Su Jeong (a) Song, Young Hun (a) Hong, Jong Chan (a)
" In Arabidopsis, the transcription factor COSTANS (CO) promotes flowering by directly activating expression of the FlOWERING LOCUS T (FT) in long-day conditions and the expression of CO is regulated by the circadian clock. The CO function has been well documented by genetic and expression analysis. However, the molecular mechanisms how CO activates FT genes at the promoter region is largely unknown. This requires identification and extensive analyses of proteins interacting with CO and the FT promoter at the protein-protein and protein-DNA level. Arbidopsis thaliana bZIP (AtbZIP) factors regulate various plant cellular processes including pathogen defence, light and stress signalling, seed maturation and flower development. Previous work has shown that OBF4, a member of bZIP family, interact with CONSTANS in yeast and in vitro (Song et al, 2008). In this study, we analyzed protein-protein interactions between CO protein and Arabidopsis bZIP proteins using yeast two hybrid screen. We identified six bZIP transcription factors that interact with CO among 65 Arabidopsis bZIP factor tested. We have studied expression of each bZIP protein gene and found that at least two bZIP proteins showed diurnal expression pattern under both long-day (LD) and short-day (SD) condition. We also found that these proteins localized to the nucleus and interact with FT promoter. This study suggests that CO-interacting bZIP proteins may play an important role in the control of flowering time in CO-mediated photoperiodic flowering pathway in Arabidopsis. (Supported by BK21 program, EBRC and the grant from KOSEF (R01-2007-000-11232-0), MOST(KRF-2008-314-C00362 * corresponding author: Tel. 055-751-5960, E-mail: jchong@gnu.ac.kr "
(a) Gyeongsang National University

P44026 Isolation of the suppressor mutants of closed stomata phenotype in phot1 phot2 double mutant
Kinoshita, Toshinori-presenter kinoshita@bio.nagoya-u.ac.jp(a) Morimoto, Sayuri (a) Ono, Natsuko (b) Soda, Midori (a) Hayashi, Yuhki (a) Nakamura, Suguru (a) Nakano, Takeshi (c) Inoue, Shin-ichiro (b) Shimazaki, Ken-ichiro (b)
"Phototropins (phot1 and phot2) act as blue light receptors for phototropism, chloroplast relocation, stomatal opening and leaf flattening. In the blue light-induced stomatal opening, phototropins induce activation of the plasma membrane H^+-ATPase, which provides driving force for stomatal opening, through the phosphorylation of the C-terminus with subsequent binding of the 14-3-3 protein in response to blue light in guard cells. However, signaling pathway between phototropins and the H^+-ATPase is largely unknown. In this study, we performed the mutant screening focused on curled leaf and closed stomata phenotypes in phot1 phot2 double mutant of Arabidopsis. One of the isolated mutants, named *scs1* (suppressor of closed stomata phenotype in phot1 phot2), showed leaf flattening and open stomata phenotypes, but this mutant was sensitive to a phytohormone abscisic acid. Interestingly, the plasma membrane H^+-ATPase in guard cells of *scs1* was constitutively activated, therefore, this mutant is likely to show the open stomata phenotype. Now, we are trying to identify *SCS1* locus by map-based cloning."
(a) Nagoya University (b) Kyushu University (c) Riken

P44027 Construction of Arabidopsis Transcription Factor ORFeome Library and Identification of Multiple HY5 Interacting Proteins by High Throughput Yeast Two-Hybrid (Y2H) Screen
Kim, HyeJin-presenter eve322@hotmail.com(a) Shin, SuYong (a) Sim, SoonAe (a) Son, Geon Hui (a) Jeon , Sujeong (a) Song, YoungHun (a) Yun, Daejin (a) Hong, JongChan (a)
" Understanding the complex mechanism of transcription regulation and biological processes requires information about protein-protein and protein-DNA interaction networks of transcription factors (TF). In this study, we report the construction of Arabidopsis TFs ORFeome library for high throughput yeast two-hybrid (Y2H) screen and protein-protein interaction network. Arabidopsis HY5 protein that play an important role in photomorphogenic development was used as a bait to identify multiple target proteins to construct photomorphogenic regulatory network. First, We have constructed expression library for yeast two-hybrid screen carrying 1,404 TFs in a correct reading frame to the prey vector pDEST22 (Invitrogen). Second, we have developmed high throughput Y2H screening method to test interaction to the bait TF by individual transformation. Using the HY5 protein as a bait we have isolated 30 different TFs, which include 6 B-box zinc finger factors, 4 bZIP factors, 3 C2H2, 3 TCP proteins, and others. Each TF was tested for binding to the several truncated form of HY5 to find the specificity of interaction and the binding domain. 15 potential target proteins showed specific binding to the HY5 protein in vitro. This study suggests that HY5 protein mediates expression of multiple downstream genes by complex interactions with multiple regulatory factor of different classes. Furthermore, the newly generated yeast AD-ORFeome library will be a valuable tool in isolating many novel interacting proteins to the regulatory protein of interest. (Supported by KOSEF (R01-2007-000-11232-0), MOST (KRF-2008-314-C00362), BK21 program)"
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SESSION P45 – PHOTOSYNTHESIS & RESPIRATION

P45001 Synergistic effects of elevated CO_2 and fertilization on net CO_2 uptake and growth of the CAM plant *Hylocereus undatus*
Weiss, Israel-presenter weissis@bgu.ac.il(a) Mizrahi, Yosef (a) Rave, Eran (b)
" This study examined the response of the commercial CAM vine-cactus fruit crop *Hylocereus undatus* to elevated CO_2 (1,000 µ;mol mol $^{-1}$ *vs*. control of 380 µ;mol mol $^{-1}$) under high and low fertilization regimes (0.5- and 0.1-strength Hoagland's solution, respectively). Elevated CO_2 increased total daily net CO_2 uptake, nocturnal acid accumulation, shoot elongation, and total dry mass by 39, 24, 14, and 6%, respectively, *vs*. ambient CO_2. Plants exposed to high fertilization showed 36, 21, 198, and 79% increases in total daily net CO_2 uptake, nocturnal acid accumulation, stem elongation, and total dry mass, respectively, *vs*. those receiving the low-fertilization regime. However, plants exposed to both high fertilization and elevated CO_2 demonstrated 108, 77, 264 and 111% increases in total daily net CO_2 uptake, nocturnal acid accumulation, stem elongation and total dry mass, respectively. This response was 25-71 % higher than the summed effects of the separate responses to each factor, indicating a synergistic effect of elevated CO_2 and high fertilization. In conclusion, highly fertilized CAM crops may benefit from elevated CO_2 to a greater extent than CAM plants grown under a low fertilization regime. "
(a) Department of Life Sciences, Ben-Gurion University of the Negev (b) Gilat Research Center, Agricultural Research Organization, Ministry of Agriculture, Isarel

P45002 Unveiling novel features of the *Methanococcoides burtonii* form III Rubisco
Alonso, Hernan-presenter hernan.alonso@anu.edu.au(a) Whitney, Spencer (a)
"*Methanococcoides burtonii* is a methanogenic archaea isolated from Ace Lake, Antarctica, that lives in anoxic conditions and average temperatures of 1-2°C. Its full genome sequence identified a putative 53 kDa Rubisco large (L) subunit that shows higher sequence homology to the dimeric (L_2) bacterial Form II than the Form III Rubiscos associated with archaea. In these organisms, the Form III typically serves to remove ribulose-P_2 produced during purine/pyrimidine metabolism and contrasts with all other Rubisco Forms that otherwise initiate primary carbon assimilation. Here we present the biochemical characterization of *M. burtonii* Rubisco (MBR) and confirm its classification as a Form III Rubisco. The putative MBR gene (*RbcL*) was cloned and stably expressed in tobacco chloroplasts by substituting it for the plastome-encoded tobacco *rbcL* gene. Like Form II Rubiscos, the assembly requirements of MBR were readily met by the tobacco chloroplast chaperone network, where it accumulated to 7-10% (w/w) of the leaf soluble protein in tissue-culture-grown plants. ESI-mass spectrometry and size exclusion chromatography showed that the purified MBR is a decamer of L-subunits, as seen for the $(L_2)_5$ Form III Rubisco from *Thermococcus kodakaraensis*. Catalytically, the MBR Rubisco could carboxylate ribulose-P_2 at room temperature under air levels of O_2 despite having a high affinity for O_2, with a K_O of 2.5 µ;M and a CO_2/O_2 specificity of 1.8. Like other form III Rubiscos, MBR has a high affinity for ribulose-P_2 ($K_{ribulose\text{-}P2}$= 0.13 µ;M), consistent with its putative metabolic role in secondary salvage pathways. Further detailed study of MBR, which also expresses well in *E. coli*, should provide interesting insights into the evolution of the complex catalytic chemistry of Rubisco."
(a) Research School of Biological Sciences, The Australian National University

P45003 Salinity Tolerance in the Single-cell C_4 Species *Bienertia sinuspersici* (Chenopodiaceae)
Leisner, Courtney P.-presenter courtney_leisner@wsu.edu(a) Offermann, Sascha (a) Cousins, Asaph (a) Edwards, Gerald E (a)
"Beginning in 2001, research on the photosynthetic mechanisms of plant species in the Chenopodiaceae family revealed that these plants can carry out C_4 photosynthesis within individual photosynthetic cells, through the development of two cytoplasmic domains having dimorphic chloroplasts. This dispelled the 35 year paradigm that Kranz-type anatomy is required for C_4 photosynthesis. The single-cell C_4 species being studied are halophytes, but the effect of high salt levels on the developmental transition from C_3 to C_4 remains to be determined. We are currently investigating the response of these single-cell C_4 species to increasing salinity and the salt levels for optimal growth, using microscopic, biochemical and physiological techniques. Members of subfamily Suaedoideae, were selected, including the single-cell C_4 species *Bienertia sinuspersici*. Species are being grown in growth chambers in an aerated hydroponic system with modified Hoagland's solution, adapted from a method used with *Arabidopsis*, and are exposed to varying levels of NaCl.[1] Salinity treatments (0 to 500 mM NaCl) show *B. sinuspersici* has high tolerance to salt (up to 200 mM NaCl), while growth is severely impaired in 500 mM NaCl (eq. to sea water). Under all salt levels mature leaves develop the C_4 type of chlorenchyma cells. Differences were observed in chlorenchyma cell morphology, protein and chlorophyll content g fresh wt^{-1}, $\delta^{13}C$ values as a measure of C_4 efficiency, total cell count per leaf, and rates of carbon assimilation m^{-2} leaf area. Funded by NSF IBN-0641232. 1- Arabidopsis (Tocquin P et al. 2006 Physiol Plant 128:677-688) "
(a) Washington State University

P45004 A coupled model for respiration and photosynthesis in the light and dark
Buckley, Thomas N-presenter tom.buckley@sonoma.edu(a,c) Adams, Mark A (b,c)
"Most, if not all, current models of carbon flux treat respiration simplistically. As a first step towards a process-based representation, we tackled several salient questions about leaf respiration. In particular, how to represent the relative supply and demand for energy, reductant and biosynthetic products, and how these are co-determined by respiratory and photosynthetic carbon and energy flows. For example, the partial suppression of respiratory CO_2 release in the light may involve changes in the supply and demand of photoreductant, photorespiratory NADH production, anabolic carbon flows and the coupling among these processes. Similarly, differences in respiratory rate and temperature dependency among tissue types and ages, and among species, likely result from differences in redox states of anabolic products. We present a model designed to enable generation of formal and testable hypotheses concerning the stoichiometric and regulatory basis of these processes. The model, which is analytically soluble, assumes that flows of reductant, energy and carbon are regulated to satisfy specified demands, subject to flexible hypotheses concerning the degree of coupling among subcellular reductant pools. The model is coupled to the photosynthesis model of Farquhar, von Caemmerer and Berry (1980). We discuss the model's predictions about the light suppression of dark respiration, the Kok effect and the engagement of alternative electron sinks in mitochondria. "
(a) Sonoma State University, Biology Department (b) University of New South Wales, Faculty of Agriculture, Food & Natural Resources (c) Bushfire Cooperative Research Centre

P45005 Modification of the thylakoid photosystem II in Cyanobacteria for building a photocell
Schuster, Gadi-presenter gadis@tx.technion.ac.il(a) Larum, Shirly (a) Salama, Faris (a) Adir, Noam (a)
"Living organisms such as plants, algae and certain bacteria efficiently use light and water to produce both storable chemical energy and reducing power via the photosynthesis process. The aim of this project is to construct a novel, sustainable energy production system. A model system is developed that enables the production of reducing power by a crude photosynthetic extract of Cyanobacteria, incubated in the light with a protein electron carrier linked to electrode elements. In our hybrid system, we make use of the photosystem II complex (PSII) that is embedded in the

photosynthetic membranes. The PSII protein D1 has being modified by site directed mutagenisis in such a way that a small artificial soluble protein can transiently bind to PSII and abstract the light driven pumped electrons. The now reduced soluble electron carrier protein serves as an electron conduit with the in-organic system. The system will be engineered such that the electron source (water) will be replenished continually, and the only reaction by-product, molecular oxygen and hydrogen, will be removed and collected. Therefore, this photocell will produce electricity and hydrogen, both extremely valuable resources of energy. The planned system will be completely green in function, with absolutely no production of chemical pollutants. "
(a) Technion - Israel Institute of Technology

P45006 NDF6; A thylakoid protein specific to land plants is essential for activity of chloroplastic NAD(P)H dehydrogenase in Arabidopsis.
Ishikawa, Noriko-presenter n-ishikawa@lif.kyoto-u.ac.jp(a) Takabayashi, Atsushi (a) Ishida, Satoshi (a) Hano, Yasushi (a) Sato, Fumihiko (a) Endo, Tsuyoshi (a)
"Chloroplastic NAD(P)H dehydrogenase (NDH) mediates one of the two pathways of cyclic electron flow around photosystem I (CEF I) and play a role in ATP synthesis under stress condition (e.g. high light, draught and high and low temperature). Although the function of NDH pathway has been studied intensively, its molecular structure remains to be clarified. In previous report, we developed an *in silico* method to screen Arabidopsis putative nuclear genes which highly co-express with the known NDH genes as candidates of unidentified NDH subunit genes, and three of them, *NDF*1 (*NDH-Dependent cyclic electron Flow* 1), *NDF*2 and *NDF*4 proved to encode novel NDH subunits (Takabayashi et al. 2009 and a presentation in this meeting). The homologous gene of *NDF*2 is also suggested to encode an NDH subunit (Ishida et al. 2009). In this report, we modified our screening criteria by normalizing Pearson's correlation coefficient (r) which indicates the co-expression degree between the known NDH genes and candidates, and identified a novel gene *NDF*6 (At1g18730) which encodes a protein that is essential for NDH activity. In Arabidopsis *NDF*6 defective mutant, a post-illumination increase in chlorophyll fluorescence which is an indicator of NDH activity is lacked and accumulation of NDH-H is decreased to 25% of that in wild type. We raised antibodies against NDF6 and indicated that NDF6 is localized in the thylakoid membrane fraction, where NDH complex localizes. NDF6 is unstable in neither an ndhB-less Arabidopsis nor an ndhB-lacking Tobacco. Homologous genes encoding NDF6 were found in land plants but not in cyanobacteria. These results suggest that NDF6 is a novel subunit of chloroplastic NDH complex that was acquired to land plants during evolution."
(a) Graduate School of Biostudies Kyoto Univerisity

P45007 Estimation and characterization of a quantitative trait locus on chromosome 4 for leaf photosynthesis in paddy rice
Asanuma, Shunsuke-presenter s_asanuma_agr@yahoo.co.jp(a) Tsuru, Yukiko (a) Sueyoshi, Tomohiro (a) Murata, Kazumasa (b) Yamamoto, Toshio (c) Ebitani, Takeshi (b) Ookawa, Taiichiro (a) Hirasawa, Tadashi (a)
"To further increase dry matter production and grain yield of paddy rice, improvements in the rate of photosynthesis of each leaf within the canopy are necessary. A high-yielding indica cultivar, Habataki, shows a higher photosynthetic rate in young expanded leaves under light saturated conditions than japonica cv. Koshihikari. This study was conducted to clarify the causes of the varietal difference in the rate of photosynthesis, and to estimate and characterize a quantitative trait locus (QTL) for the rate of leaf photosynthesis in paddy rice. The higher photosynthetic rate in Habataki was caused by both the higher nitrogen content and higher diffusive conductance of leaves. The plant resistance to water transport was larger in Koshihikari than Habataki. The rate of photosynthesis in Koshihikari increased to that of Habataki within a few minutes after releasing hydrostatic pressure in the leaf xylem by excising a leaf at its base. These observations indicate that the leaves of Koshihikari suffered from water stress under light saturated conditions even with a small vapour pressure deficit compared to Habataki. In the preliminary study using the progeny population (BC_5F_2) derived from a Koshihikari/Habataki cross, a QTL for enhancing the rate of photosynthesis was detected in the vicinity of DNA marker RM3836. Using a series of homozygous recombinant lines in which a small part of the chromosome from Habataki around the region with the marker was substituted into the genetic background of Koshihikari, the QTL region for increasing the photosynthetic rate was narrowed to a 2.13 Mbp interval on the long arm of chromosome 4. This QTL region contributed to increased leaf nitrogen content and to decreased plant resistance to water transport."
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P45008 Novel subunits of chloroplastic NAD(P)H dehydrogenase complex identified by bioinformatic screenings
Takabayashi, Atsushi (a,b) Ishida, Satoshi (a) Ishikawa, Noriko (a) Hano, Yasushi (a) Sato, Fumihiko (a) Endo, Tsuyoshi-presenter tuendo@kais.kyoto-u.ac.jp(a)
"Chloroplastic NAD(P)H dehydrogenase (NDH) plays a role in cyclic electron flow around photosystem I to produce ATP. Chloroplast genome encoded 11 subunits that are homologous to prokaryotic NADH:ubiquinone oxidoreductase (NDH-1) in respiratory electron transport. Recent genetic and biochemical studies indicated that the chloroplastic NDH have unique subunits that are encoded in nuclear genome. To find the unidentified subunits, we have conducted an *in silico* screening based on co-expression analysis and phylogenetic profiling with which we identified potential candidates for NDH subunit genes. Further characterization of Arabidopsis T-DNA insertion mutants among these *ndh* gene candidates indicated that five novel *ndf* (NDH-Dependent cyclic electron Flow) mutants (*ndf1, ndf2, ndf4, ndf5 and ndf6*) had impaired NDH activity as estimated by a post-illumination increase in chlorophyll fluorescence. Detailed analyses of *ndf6* is presented by Ishikawa et al. in this meeting. The amounts of NDH-H subunit were greatly decreased in these mutants, suggesting that the loss of NDH activity was caused by the defect in the accumulation of the NDH complex. The NDF1, NDF2, NDF4 and NDF6 proteins were co-migrated with the NDH-H subunit by blue native electrophoresis, suggesting that NDF proteins are the novel subunits of NDH complex. The NDF proteins were unstable in the background of mutants lacking hydrophobic subunits of the NDH complex, while they were stable in mutants lacking hydrophilic subunits. These results indicate that hydrophobic subcomplex binding NDF proteins can exist stably without hydrophilic subcomplex. Phylogenic analyses indicate that the NDF-containing subcomplex of NDH is restricted to land plants."
(a) Graduate School of Biostudies, Kyoto University (b) Center for Gene Research, Nagoya University

P45009 Estimation and characterization of a quantitative trait locus on chromosome 8 for leaf photosynthesis in paddy rice
Tsuru, Yukiko-presenter 50008531019@st.tuat.ac.jp(a) Asanuma, Shunsuke (a) Murata, Kazumasa (b) Ebitani, Takeshi (b) Ookawa, Taiichiro (a) Hirasawa, tadashi (a)
"To further increase dry matter production and grain yield of paddy rice, improvements in the rate of photosynthesis of each leaf within the canopy are necessary. DNA marker-assisted selection is promising for efficiently improving the leaf capacity of photosynthesis in breeding. Habataki, a high-yielding indica cultivar, shows a higher rate of photosynthesis than a common commercial japonica cultivar, Koshihikari. The higher rate of photosynthesis in Habataki was caused by both a higher nitrogen content and higher diffusive conductance of leaves. In a preliminary study using Koshihikari/Habataki BC_5F_2 progeny population, a QTL region for photosynthetic rate was estimated to be located on chromosome 8 in the vicinity of

SSR marker RM8019. In this study, we created a series of homozygous recombinant lines, in which a small part of the chromosome from Habataki around the region with the marker was substituted into the genetic background of Koshihikari. We then conducted experiments to confirm the existence of the QTL for increased rate of photosynthesis at the expected region on chromosome 8, in order to narrow down the QTL region and to clarify the function of this QTL. The QTL region for increasing photosynthetic rate was detected on the expected region of chromosome 8 and located on a 1.45 Mbp interval on the short arm. Both the higher leaf nitrogen content and higher diffusive conductance were responsible for the higher photosynthetic rate in the lines with this QTL. In addition, the higher diffusive conductance in the lines with this QTL was estimated to have resulted from the smaller plant resistance to water transport."
(a) Graduate School of Agriculture, Tokyo University of Agriculture and Technology (b) Toyama Prefectural Agricultural, Forestry & Fisheries Research Center

P45010 Knockdown of Limiting-CO_2-induced Gene HLA3 Decreases HCO_3- Transport and Photosynthetic Ci-affinity in *Chlamydomonas reinhardtii*
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"The CO_2-concentrating mechanism (CCM) of *Chlamydomonas reinhardtii* and other microalgal species is essential for photosynthetic growth in most natural settings. A great deal has been learned regarding the CCM in cyanobacteria, including identification of inorganic carbon (Ci; CO_2 and HCO_3-) transporters, while specific knowledge of analogous transporters has remained elusive in eukaryotic microalgae such as *C. reinhardtii*. Here, we have investigated whether the limiting-CO_2-inducible, putative ABC-type transporter, HLA3 might function as a HCO_3- transporter by evaluating the effect of pH on growth, photosynthetic Ci affinity and [^{14}C]-Ci uptake in very low CO_2 conditions following RNA interference (RNAi) knockdown of HLA3 mRNA levels in wild-type and mutant cells. Although knockdown of HLA3 mRNA alone resulted in only modest but high-pH-dependent decreases in photosynthetic Ci affinity and Ci uptake, the combination of nearly complete knockdown of HLA3 mRNA with mutations in LCIB (encodes limiting-Ci inducible, plastid localized protein required for normal Ci uptake or accumulation in low CO_2 conditions) and/or simultaneous, apparently off-target knockdown of LCIA mRNA (encodes limiting-Ci inducible, plastid envelope protein reported to transport HCO_3-) resulted in dramatic decreases in growth, Ci uptake and photosynthetic Ci affinity, especially at pH 9.0, where HCO_3- is the predominant form of available Ci. Collectively, the data presented here provide compelling evidence that HLA3 is directly or indirectly involved in HCO_3- transport and provide additional evidence supporting a role for LCIA in chloroplast envelope HCO3- transport and for LCIB in chloroplast Ci accumulation."
(a) Iowa State University Department of Genetics Development and Cell Biology (b) University of Nebraska Department of Biochemistry

P45011 Solar-to-product energy conversion efficiency in photosynthesis
Melis, Anastasios-presenter melis@nature.berkeley.edu(a) Mitra, Mautusi (a) Kirst, Henning (a)
http://epmb.berkeley.edu/facPage/dispFP.php?I=25
"Under limiting-irradiance conditions, oxygenic photosynthesis can operate with a solar-to-biomass energy conversion efficiency of nearly 10%. Under bright sunlight, this efficiency drops to less than 1% due to light-saturation of photosynthesis and wasteful dissipation of excess absorbed irradiance. Research seeks to alter the optical characteristics of the photosynthetic apparatus in order to improve the energy conversion efficiency in mass culture under bright sunlight conditions. The work aims to identify novel genes that determine the chlorophyll antenna size in photosynthetic organisms, and demonstrate that a truncated Chl antenna size minimizes absorption and wasteful dissipation of sunlight by individual cells, resulting in better sunlight penetration, increased solar utilization efficiency, and greater photosynthetic productivity under mass culture conditions. To achieve these objectives, DNA insertional mutagenesis, screening, biochemical and molecular analyses were employed for the isolation of tagged truncated Chl antenna size strains in the model green microalga Chlamydomonas reinhardtii. A novel nuclear-encoded gene conferring the Truncated Light-harvesting chlorophyll Antenna size (TLA) property was isolated. Genetic, molecular, biochemical and phenotypic properties of the TLA1 gene will be discussed in this presentation. The truncated Chl antenna size property confers a greater solar-to-product conversion efficiency in photosynthesis, resulting in a greater productivity of the microalgae under mass culture and bright sunlight conditions. In sum, the TLA1 gene, and the property it confers, may find application in the commercial exploitation of microalgae for the generation of biomass, biofuels, or feedstock for the chemical and nutraceutical sectors. "
(a) University of California, Berkeley

P45012 Application of a novel 3D imaging system for non-destructive growth analysis of the C_4 plant *Flaveria bidentis*.
Pengelly, Jasper J L-presenter jasper.pengelly@anu.edu.au(a) Sirault, Xavier R R (b) Furbank, Robert T (b) von Caemmerer, Susanne (a)
"The growth of a plant can be calculated experimentally by conducting a series of destructive harvests of a population of the plant over a given growth period. Measurement of the average increase in weight, leaf area or volume of the groups of plants harvested at each time-point can be used to determine the relative growth rate (RGR). This technique requires a large initial population of plants, and is based on the assumption that data derived from each harvest point is an accurate estimate of the population at that time. This limits its effectiveness in the analysis of segregating transgenic plant populations in which mutation-linked growth changes may be missed if plants at each harvest do not express a mutation equally. We adapted a novel real-time imaging technique to measure plant growth in a non-destructive manner. Successive multi-angle photographic images of individual plants over a time period are used to generate a RGR based on mathematically derived plant volumes at each imaging time. A pilot study using the C_4 dicotyledon *Flaveria bidentis* showed that plant volumes obtained from imaging correlated well with dry weight measurements in determining plant growth. Central to the C_4 photosynthetic pathway in *F. bidentis* is the cytosolic enzyme phosphoenolpyruvate carboxylase (PEPC), which is activated through a light-dependant phosphorylation by a specific protein kinase (PEPC-PK). Here we present data on the effects of low (150 µ;mol quanta m^{-2} s^{-1}) and high (500 µ;mol quanta m^{-2} s^{-1}) light on the growth and photosynthetic efficiency of individual *F. bidentis* wild type (WT) and anti-sense PEPC-PK mutants over a 6-week time period. Parallel destructive harvests were carried out on WT plants to validate growth data obtained from imaging. "
(a) Reseach School of Biological Sciences, Australian National University (b) CSIRO Plant Industry, Black Mountain

P45013 Energy partitioning in absorbed light by photosystem II in flag leaves of rice (*Oryza sativa* L.) plants grown under nitrogen-sufficient and deficient conditions
Etsushi, Kumagai-presenter ekumagai@agr.kyushu-u.ac.jp(a) Takuya, Araki (b) Osamu, Ueno (b)
"The responses of the energy partitioning of absorbed light by photosystem II (PSII) antenna and the photoprotection to nitrogen (N)-deficiency were investigated in flag leaves of rice plants. Gross photosynthetic rate and the contents of chlorophyll and ribulose-1,5-bisphosphate carboxylase/oxygenase significantly decreased under N-deficiency. In contrast, there was no significant difference in maximum quantum yield of PSII between the N-sufficient (NS) and N-deficient (ND) plants, indicating that the flag leaves of the ND plants were well protected from photooxidative damage. N-deficiency caused a decrease in the energy flux via linear electron transport (J_{PSII}) and an increase in the energy flux via light-dependent thermal dissipation. Thus, trans-thylakoid pH gradient (δpH)- and xanthophyll cycle-dependent thermal dissipation increased, as excess energies

were accumulated in the ND leaves. In contrast, the energy flux via fluorescence and light-independent constitutive thermal dissipation did not change with N-deficiency. The energy flux via photorespiration (J_o) was significantly decreased under N-deficiency, which was associated with a decrease in catalase activity. Furthermore, no significant difference in J_o/J_{PSII} was observed between the NS and ND plants, indicating that photorespiration was down-regulated in response to N-deficiency. The energy flux via alternative electron flow was significantly increased with N-deficiency, which was accompanied by enhanced activity of superoxide dismutase. Our result suggests that enhanced δpH- and xanthophyll cycle-dependent thermal dissipation and the water-water cycle coordinately protect flag leaves of rice plants from photooxidative damage under N-deficiency."
(a) Graduate School of Bioresource and Bioenviromental Sciences, Kyushu University (b) Facuty of Agriculture, Kyushu University

P45014 The role of a previously poorly annotated gene in Photosynthesis II electron transport under high light
Lu, Yan-presenter luy@msu.edu(a) Imre, Kathleen M (a) Kim, Eunha (a) Hall, David A (a) Last, Robert L (a)
http://www.plastid.msu.edu
"As the site of photosynthesis, the chloroplast has several thousand proteins that are targeted to or synthesized within it. To understand the functions of plastid-targeted proteins, including those that regulate photosynthetic capacity, ~3000 *Arabidopsis thaliana* homozygous T-DNA lines were analyzed with morphological, chemical and physiological assays (www.plastid.msu.edu). More than 100 putative mutants were identified based on alterations in chlorophyll fluorescence. Parallel mutant screening with 80 other phenotypic traits helped to identify novel components regulating photosynthesis and to develop functional hypotheses for under-characterized genes. For example, two alleles of a zinc finger domain-containing protein in the thylakoid membrane were found to have lower maximum photochemical efficiency of PSII (*Fv/Fm*) after high-light treatment. Analysis of publicly available microarray data sets showed that the gene strongly co-expressed with photosynthesis-related genes under a variety of stress conditions. PSII electron transport rate was reduced in both mutants, which dissipated less energy via PSII photochemistry, and more energy via non-regulated non-photochemical pathway than wild type plants. The steady-state levels of *in vivo* phosphorylation of the PSII complex proteins CP43, D1/D2 and H appeared to be changed in both mutants. These results suggested that the zinc finger domain-containing protein may protect PSII from photoinhibition by impacting the phosphorylation status of PSII complex proteins."
(a) Michigan State University

P45015 "REP27, a thylakoid membrane protein functioning in the D1/32 kD reaction center protein turnover and PSII repair from photodamage"
Park, Sungsoon-presenter sungsoon@nature.berkeley.edu(a) Dewez, David (a) Garcia-Cerdan, Jose (a) Lindberg, Pia (a) Melis, Anastasios (a)
"The goal of the research is to identify genes and proteins required for the photosystem-II (PSII) repair mechanism in oxygenic photosynthesis. Via DNA insertional mutagenesis in *Chlamydomonas reinhardtii*, *rep27*, a PSII repair aberrant strain was isolated. Molecular analysis of the *rep27* strain resulted in the cloning of REP27, a nuclear gene encoding a chloroplast-targeted protein containing two tetratricopeptide repeat motifs (TPR1 & 2), two transmembrane domains, and an extended C-terminal region. Cell fractionation and Western blot analysis localized the REP27 in the *C. reinhardtii* chloroplast thylakoids. A folding model for REP27 suggested N- and C-termini exposed to the chloroplast stroma. Truncated REP27 cDNA constructs were made for complementation of the *rep27* mutant, whereby TPR1, TPR2, both TPR1 and TPR2, or the C-terminal domain were deleted. *rep27*-complemented strains with the REP27 minus the TPR motifs showed elevated levels of D1, comparable to those in the wild type, but the PSII photochemical efficiency of these complemented strains was not restored, suggesting that the functionality of the PSII reaction center was not recovered. It is suggested that TPR motifs play a role in the functional activation of the newly integrated D1 protein in the PSII reaction center. *rep27*-complemented strains missing the REP27 C-terminal domain showed low levels of D1 protein, as well as low PSII photochemical efficiency, comparable to those in the *rep27* mutant. Therefore, the C-terminal domain is needed for a *de novo* D1 biosynthesis and/or assembly of D1 in the PSII template. We conclude that REP27 plays a dual role in the regulation of D1 protein turnover by facilitating co-translational biosynthesis-insertion and activation of the nascent D1 in the PSII repair process."
(a) University of California Berkeley

P45016 The functional anatomy of rice leaves: implications for refixation of photorespiratory CO2 and efforts to engineer C4 photosynthesis into rice.
Sage, Tammy L-presenter tammy.sage@utoronto.ca(a) Sage, Rowan R (a)
"One mechanism to radically enhance global food stocks is to introduce C4 photosynthesis into C3 crops from warm climates, notably rice. To accomplish this, an understanding of leaf structure and function is essential. Chlorenchyma cell structure of rice and related warm-climate C3 grasses is distinct from cool temperate C3 grasses. In temperate C3 grasses, vacuoles occupy the majority of the cell, while chloroplasts, peroxisomes and mitochondria are pressed against the cell periphery. In rice, 66% of protoplast volume is occupied by chloroplasts, and chloroplasts/stromules cover >95% of the cell periphery. Mitochondria and peroxisomes occur in the cell interior and are intimately associated with chloroplasts/stromules. We hypothesize that the chlorenchyma architecture of rice enhances diffusive CO2 conductance and maximizes scavenging of photorespired CO2. The extensive chloroplast/stromule sheath forces photorespired CO2 to exit cells via the stroma, where it can be refixed by Rubisco. Deep cell lobing and small cell size, coupled with chloroplast sheaths, creates high surface area exposure of stroma to intercellular spaces thereby enhancing mesophyll transfer conductance. In support of this, rice exhibits higher mesophyll transfer conductance, greater stromal CO2 content, lower CO2 compensation points at warm temperature, and less oxygen sensitivity of photosynthesis than cool temperate grasses. Rice vein length per leaf, mesophyll thickness, and intercellular space volume are intermediate between most C3 and C4 grasses, indicating the introduction of Kranz anatomy into rice may not require radical changes in leaf anatomy; however, deep lobing of chlorenchyma cells may constrain efforts to engineer C4 photosynthesis into rice."
(a) University of Toronto

P45017 A proteomics approach to analyze differential protein accumulation in the dimorphic chloroplasts of the single-cell C_4 species *Bienertia sinuspersici* (Chenopodiaceae)
Offermann, Sascha-presenter soffermann@wsu.edu(a) Doroshenk, Kelly A. (b) Okita, Thomas W. (b) van Wijk, Klaas J. (c) Edwards, Gerald E. (a)
"The recent discovery of C_4 photosynthesis in terrestrial plants without Kranz anatomy raised interest in the biochemical and physiological properties, as well as, the molecular requirements for this unique form of C_4. *B. sinuspersici*, a halophytic species adapted to arid conditions, performs C_4 photosynthesis within a single cell through location of dimorphic chloroplasts in separate compartments. They are proposed to function analogous to mesophyll and bundle sheath chloroplasts in Kranz-type C_4. The CO_2 concentrating mechanism in C_4 requires the accumulation of a distinct set of photosynthetic enzymes and transporters in the specialized chloroplast types. Previous immunolocalization studies show the dimorphic chloroplasts differentially accumulate pyruvate,Pi dikinase of the C_4 cycle and Rubisco of the C_3 cycle. To characterize the degree of chloroplast differentiation,

and the molecular basis for single-cell C_4 photosynthesis, we are currently undertaking a proteomics study to identify metabolic functions which are selectively compartmentalized between the two chloroplasts. A protocol has been developed based on cell fractionation, and differential and density centrifugation methods, to purify the two different chloroplast types. Western analyzes have shown near exclusive separate localization of Rubisco and PPDK between to the two types of purified chloroplasts. Purified chloroplasts are currently being analyzed via two-dimensional differential gel electrophoresis (2D-DIGE), which shows a unique pattern for each chloroplast type. Mass spectroscopy based methods will be used to identify differentially localized proteins in order to define the molecular basis for single-cell C_4 photosynthesis, and the cooperative functions of these chloroplasts. NSF IBN-0236959 "
(a) School of Biological Sciences, Washington State University, Pullman (b) Institute of Biological Chemistry, Washington State University, Pullman (c) Department of Plant Biology, Cornell University, Ithaca, New York

P45018 The effect of temperature on the limiting mechanisms of photosynthesis in sweet potato
Busch, Florian-presenter florian.busch@utoronto.ca(a) Sage, Rowan F (a)
"We examined the limitations of photosynthesis over a range of temperatures in sweet potato (*Ipomoea batatas*) using gas exchange, chlorophyll a fluorescence and theoretical modeling of photosynthesis in response to temperature, light intensity as well as CO_2 and O_2 concentration. At temperatures below the thermal optimum, O_2 insensitivity of photosynthesis and its saturation at relatively low CO_2 concentrations indicate a limitation due to regeneration of inorganic phosphate. From our fluorescence data and the O_2 sensitivity of photosynthesis we conclude that the RuBP regeneration capacity was limiting up to ca. 40°C, followed by a RuBP consumption limitation at higher temperatures. The temperature at which this transition occurred was dependent on CO_2 partial pressures. The modeled response generally agreed with the observed O_2 sensitivity of photosynthesis. In contrast, above the thermal optimum at 40°C and 20 mbar O_2 we observed a decrease in the initial slope of the photosynthetic CO_2 response relative to the values predicted by our theoretical model. Here, a high O_2 sensitivity of photosynthesis was apparent up to a C_i value of 1500ppm. Taken together, this may indicate that photosynthesis is limited by the carbamylation state of Rubisco, which could reflect heat lability of Rubisco activase."
(a) Department of Ecology and Evolutionary Biology, University of Toronto

P45019 *Miscanthus x giganteus*: The New Cool Kid On The Block
Spence, Ashley K-presenter spence4@uiuc.edu(a) Wang, Dafu (a) Long, Steve P (a)
"*Miscanthus x giganteus* can maintain photosynthetically active leaves at temperatures 6°C below the minimum for maize (*Zea mays*), allowing exceptional productivity in cool climates. Understanding the basis for this cold tolerance could indicate how maize a close relative, could be modified. When *M. x giganteus* and maize grown at 25°C were transferred to 14°C, light-saturated CO2 assimilation and quantum yield of photosystem II declined by 30% and 40%, respectively. The decline continued in maize but recovered in *M. x giganteus*. This recovery paralleled an accumulation of pyruvate phosphate dikinase (PPDK) protein, which declined in maize. PPDK and Rubisco are considered to share metabolic control of light-saturated C4 photosynthesis, but only PPDK increased. The increase in protein corresponded to an increase in extractable enzyme activity and a large increase in transcripts. PPDK is cold labile, below 12°C but by increasing concentrations of the recombinant protein *in vitro* we show decreased lability. The increases in PPDK *in vivo* may result either from increased PPDK RNA transcription and/or the stability of this RNA. To investigate the basis of this difference further, microarrays of 42,000 maize unigenes were used to determine what other genes expression changes when *M. x giganteus* leaves grown in 25°C are transferred to 14°C. Many of the observed changes were in putative heat shock proteins (HSPs). These include transcripts corresponding to the wheat small heat shock protein hsp23.6 precursor, a rice 101 kDa HSP, a rice RHSF13-like HSP precursor and a homologue to a rye HSP (p-value .01). These changes in proteins known to increase thermal stability may be critical to the unusual increase in PPDK protein at low temperature."
(a) University of Illinois

P45020 "STN8 kinase, a thylakoid protein, specific for photosystem II core protein phosphorylation, partially impaired the Photosystem II potential by suppression of D1 protein degradation in the mutant of *Oryza sativa* L."
Nath, Krishna-presenter krishnanath125@hotmail.com(a) Mishra, Sujata R (a) Zulfugarov, Ismayil S. (a) Lee, Choon-Hwan (a) An, Gynheung (b,b)
"STN8 kinase was previously identified in *Arabidopsis thaliana* as a protein involved in PS II core protein phosphorylation. In the present study, the effect of PSII core protein phosphorylation in wild type (WT) and STN8 kinase lacking mutant of *Oryza sativa* L. (*Osstn8*) on the functional activity of PS II under control and photoinhibitory conditions has been investigated. To elucidate the function of *Osstn8* kinase in photosynthetic process particularly in PS II inactivation and D1 protein degradation, the T-DNA inserted knock-out rice mutant line was selected from the pool of T-DNA insertion lines. Here, we investigated that the level of phosphorylation of PSII core proteins was significantly abolished in mutant as compared to WT whereas; LHC II phosphoproteins seemed to be phosphorylated to the WT level. The extent of PSII inactivation due to high light (HL) stress was more in mutant with less subsequent recovery than in WT however; it was decreased rapidly with similar rates in both genotypes without recovery in the presence of lincomycin suggesting that the repair of PSII is protected by STN8 kinase related PSII core phosphorylation. In addition, D1 protein was not degraded as fast as in the mutant as they were in WT support that PSII core protein did not get phosphorylation in former and thus, there is prevention of D1 degradation which is essential for PS II repair and which cause mutant plants become more susceptible to photoinhibition. "
(a) Department of Molecular Biology, Pusan National University, Busan, Korea (b) Division of Molecular and Life Sciences, Pohang, Korea

P45021 Mitochondrial proteome in young bamboo rhizome from *Bambusa oldhamii* and *Phyllostachys edulis*
Chien, Lee-Feng-presenter lfchien@dragon.nchu.edu.tw(a) Wu, Yuan-Ching (a) Ko, Guang-Chung (a)
"The young bamboo rhizome mitochondria were isolated from summer bamboo *B. oldhamii* and winter bamboo *P. edulis* during rapid shooting stage. The structural organization and the assembly of functional mitochondrial respiratory supercomplexes were studied using BN-PAGE and in-gel activity staining. In both species, almost 90% of total complex I was assembled into supercomplexes while P. edulis contained a greater amount of large complex-I-comprising supercomplexes than B. oldhamii. About 40% of complex III was assembled into supercomplexes in *B. oldhamii* whereas about 50% assembled into supercomplexes in *P. edulis*. In assembling complex V, both species performed in a similar manner, in that about 75% of complex V was assembled into supercomplexes. Three novel supercomplexes were recognized as $I+III_2+V_2$, $I+III_2+V$, and $I+F_1$: these were not found in other plants. *P. edulis* also contained a greater amount of complex-V-comprising supercomplexes than *B. oldhamii*. Subsequently, the mitochondrial individual protein components were analyzed by 2D BN/SDS-PAGE. Certain protein spots of *B. oldhamii* bamboo mitochondria were subjected to MS-based analysis in which six subunits of complex V were identified as α, β, γ, δ, b and d subunits, and two subunits of complex III as α and β subunits. Furthermore, mitochondrial proteins from *B. oldhamii* and *P. edulis* were separated and visualized on 2D IEF/SDS-PAGE. Some visualized spots of *P. edulis* mitochondrial protein on IEF/SDS-PAGE were identified by MALDI-TOF MS or LC-MS/MS. Generally these identified protein spots can be divided into four functional groups including electron transport chain, metabolism, stress protein, and transcription."
(a) Department of Life Sciences, National Chung Hsing Universtiy

P45022 Molecular marker development for the analysis of Crassulacean acid metabolism evolution in neotropical orchids (subtribe Oncidiinae).

Gulle, Bahay-presenter bahaygulle@yahoo.com(a) Albion, Becky L (a) Silvera, Katia (a) Cushman, John C (a)

"Crassulacean acid metabolism (CAM) is an important water-conserving photosynthetic pathway present in approximately 7% of vascular plant species from 33 families. Orchidaceae is the largest family of vascular plants with about 25,000 species, and approximately 50% of these species are likely to exhibit some degree of CAM. Oncidiinae, which is the second largest orchid subtribe, was selected for evolutionary analysis, because it has one of the most well established molecular phylogenies available. In order to text the hypothesis that CAM evolution is accompanied by gene duplication events and recruitment of discrete isogenes by enhanced mRNA expression, we have studied the structure of the carbonic anhydrase (CA) and glucose-6-phosphate/Pi translocator (GPT) gene families in Oncidiinae orchid species performing C3 photosynthesis, weak CAM, and strong CAM by cDNA sampling and mRNA expression studies. Our results indicate that the evolutionary progression towards the CAM state is accompanied by the selective recruitment of discrete isogenes that show increased mRNA expression and circadian clock regulation in order to fulfill the enhanced metabolic demands of CAM to perform nocturnal CO2 fixation."

(a) University of Nevada, Reno

P45023 Investigation of *Arabidopsis* leaf carotenoid turnover by ^{14}C pulse-chase labeling

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"Carotenoids are essential components of the pigment-protein complexes in the photosynthetic membranes (thylakoids). They not only harvest light for photosynthesis, but also protect thylakoid membranes against photooxidative damage by dissipating excess light energy and scavenging reactive oxygen species. In the past decades nearly all the enzymes involved in carotenoid biosynthesis have been identified for *Arabidopsis*, and some also investigated with respect to their catalytic activities. However, our current understanding about the regulation of carotenoid biosynthesis in photosynthetic tissues is largely restricted to the level of gene transcription or steady-state pigment concentrations that are determined by the rate of *de novo* synthesis and degradation under given conditions. In order to gain further insights into the regulation of leaf carotenoid biosynthesis in higher plants, it is necessary to study dynamic changes in the contribution of each process and turnover of different carotenoid species. In the present study, we investigated short-term responses of carotenoid biosynthesis in leaves of *Arabidopsis* under different light environments by ^{14}C pulse-chase labeling experiments. A $^{14}CO_2$ application system was developed for highly efficient labeling of single leaves, and a HPLC method using a C30 column was established for radio-HPLC analysis of photosynthetic pigments. The first results indicated rapid and high ^{14}C incorporation into carotene and chlorophyll *a*, but not into xanthophylls and chlorophyll *b*, regardless of the changes in the xanthophyll cycle pool size. Distinct turnover rates and precursor pools for carotene and xanthophylls will be discussed in the context of D1 protein turnover and localization of different carotenoid pigments in the thylakoids."

(a) Institut Phytosphaere ICG-3, Forschungszentrum Juelich, Germany (b) BIOSPEC, Forschungszentrum Juelich, Germany

P45024 Deep transcriptome analysis of C3 and C4 Flaveria Species using 454 sequencing - A new approach to understand C4 photosynthesis

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"C4 photosynthesis was developed several times independently during the evolution of higher plants. While the basic biochemistry of the C4 cycle is well understood, nearly nothing is known about the genes responsible for the altered leaf morphology typical for C4 plants. In addition only few transport proteins necessary for the increased intracellular flux of metabolites required for the C4 pathway are identified so far. To gain insight into the extent to which gene expression is altered in C4 compared to C3 leaves, and to provide a platform to identify candidate genes required for the establishment of C4 photosynthesis, we have carried out a comparative transcriptome analysis of leaves of the closely related C3 and C4 species Flaveria pringlei and Flaveria bidentis, using massively-parallel pyrosequencing. Such a digital gene expression analysis is based on the random generation of sequence tags that are proportional to the abundance of the corresponding transcripts in a particular sample. We used 454 sequencing to produce more than 470000 reads with an average length of 230 bp for each species. These reads were mapped to the cDNA sequences of the TAIR 8 release of the Arabidopsis genome in the 'protein space'. The TAIR 8 release of the Arabidopsis genome contains 33282 gene loci. Together, the reads from both Flaveria species detected expression of 17063 of orthologuos of these genes, with 15132 detected with F. bidentis and 15397 detected with F. pringlei."

(a) Heinrich-Heine University, Botany

P45025 "Induction of CAM in the slender ice plant, *Mesembryanthemum nodiflorum*, by salinity and low water treatments"

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"*Mesembryanthemum nodiflorum*, the slender ice plant, is a succulent annual that is native to southern Africa, but introduced to almost all continents of the world. In Western Australia it favours the saline and degraded soils of agricultural regions. The tolerance of this plant to harsh environments is hypothesised to be an ability to switch from C_3 photosynthesis to Crassulacean Acid Metabolism (CAM) during its growth. While CAM induction in the common ice plant (*Mesembryanthemum crystallinum*) has been well-studied, and is triggered by high salinity and water stress, little work has been done on CAM induction in the slender ice plant. In this study diurnal changes in tissue acidity were measured in samples taken from the slender ice plant grown in a glasshouse under three different treatments: control plants were well-watered, some plants received a low water treatment, whilst others were well-watered with 1 M NaCl solution. Within 12 days, a distinct C_3 to CAM switch was induced in glasshouse plants receiving saline solution and low water treatments, as indicated by 7- and 4-fold increases in nocturnal acid accumulation, respectively, whilst control plants showed no CAM induction."

(a) School of Biomedical, Biomolecular and Chemical Sciences, University of Western Australia (b) School of Plant Biology, University of Western Australia (c) Department of Agriculture and Food Western Australia, Centre for Ecohydrology, University of Western Australia

P45026 Identification of a novel gene required for normal pyrenoid formation in *Chlamydomonas reinhardtii*

Ma, Yunbing-presenter yma3@tigers.lsu.edu(a) Pollock, Steve V (a) Xiao, Ying (a) Cunnusamy, Khrishen (b) Moroney, James V (a)

"*Chlamydomonas reinhardtii* possesses a CO_2 concentrating mechanism (CCM) that allows the alga to grow at low CO_2 concentrations. One common feature in photosynthetic organisms possessing a CCM is that Rubisco is tightly packaged within the cell. In cyanobacteria, Rubisco is localized to carboxysomes. In many eukaryotic algae, Rubisco is localized to the pyrenoid, an electron dense structure within the chloroplast. In order to identify genes required for a functional CCM, insertional mutants were generated using a gene that confers resistance to Zeocin. Transformants were first identified by their resistance to Zeocin and then were screened for CCM deficient phenotype. One mutant identified by this screen was named *cia6*. Physiological studies established that *cia6* grows poorly on air levels of CO_2 and has an impaired ability to accumulate inorganic carbon. A gene encoding a protein that has sequence homology to SET domain is interrupted in *cia6*, and members of this protein family are often methyltransferases. Inspection of *cia6* cells using electron microscopy revealed that the pyrenoid in this mutant is highly disorganized even though

the amount of Rubisco is about the same as in wild type. The current hypothesis is that either Rubisco or some other proteins must be methylated for proper pyrenoid organization, and failure of pyrenoid formation leads to a defective CCM. Current experiments include testing whether CIA6 is a methyltransferase by first overxpressing CIA6 in *E.coli*. Components of the pyrenoids are also investigated by isolating this microcompartment from both mutant and wild type. In this presentation we will describe the molecular characterization of this gene and the physiology of *cia6*. This work is supported by the National Science Foundation. "
(a) Department of Biological Sciences, Louisiana State University (b) Division of Basic Sciences, UT Southwestern Medical Center

P45027 Investigation of putative bicarbonate transporters in *Chlamydomonas reinhardtii*.
Mukherjee, Bratati-presenter bratati23@gmail.com(a) Moroney, James V (a)
"The unicellular eukaryotic green alga *Chlamydomonas reinhardtii*, is able to acclimate successfully to low CO_2 conditions with the help of a Carbon Concentrating Mechanism [CCM].One of the key features of this mechanism is the uptake and transfer of charged inorganic carbon species across the plasma, chloroplast and thylakoid membranes. To date, the bicarbonate transport process in this alga has not been completely characterized, although a number of genes encoding putative bicarbonate transporters have been identified. This work deals with the investigation of the role of two such candidate bicarbonate transporter genes, *NAR1.2* and *LCI1*. *NAR1.2* is a member of a group of six genes belonging to the NAR gene family, showing sequence homology with the formate/nitrite transporter family. Recent electrophysiological studies have shown that *NAR1.2* can transport both nitrite and bicarbonate anions [Mariscal *et al*; Protist (2006) 157:421~433.]. In contrast, *LCI1* encodes a protein with no sequence homology with characterized proteins. In this work, we have shown that the transcription of these genes is highly upregulated under low CO_2 conditions and that both genes are under the control of the *CIA5* gene, which regulates the expression of many key CCM genes. In order to investigate the physiological role of these two proteins, attempts are being made to knock down their expression, either singularly or in conjunction, employing RNAi. The knockdown mutants, if successfully generated, will be inspected for any changes in the ability to take up bicarbonate. Also, since the location of these two proteins will throw a great deal of light on their possible function, antibodies raised against these two proteins will be used for the purpose of immunolocalization. Work supported by NSF."
(a) Department of Biological Sciences,Louisiana State University

P45028 Quality control of Photosystem II: localization and structure properties of the FtsH proteases involved in the degradation of photo- or heat-damaged D1 protein
Yoshioka, Miho-presenter dns19402@s.okayama-u.ac.jp(a) Inagawa, Kayo (a) Yamamoto, Yasusi (a)
"It has been suggested that FtsH proteases are abundant in the stroma thylakoids and the grana margin because these domains are the site of repair of the damaged D1 protein in the thylakoids. In the present study we assayed the relative amount of the protease in the thylakoids, grana, stroma thylakoids, PSII membranes and PSII core. The active FtsH protease forms hexameric ring structure, but it is not clear whether the hexameric proteases are present in every membrane domain (fraction). We analyzed the structure of the FtsH proteases under control dark conditions, as well as heat or light stress conditions, using clear native gel electrophoresis."
(a) Faculty of Science, Biology, Okayama University

P45029 Quality control of Photosystem II: thylakoid unstacking is necessary for efficient degradation of the D1 protein under light and heat stresses in spinach thylakoids
Khatoon, Mahbuba-presenter mkhatoon2002@hotmail.com(a) Inagawa, Kayo (a) Yoshioka, Miho (a) Noriko, Morita (a) Yamamoto, Yasusi (a)
"Light and heat stresses induce damage to Photosystem II (PSII) complex. A repair cycle operates to replace the damaged subunits within PSII, in particular, the D1 protein, by newly synthesized copies. In the present study, we investigated the relationship between the light- and heat- induced damage to the D1 protein and its repair, and unstacking of the thylakoids using spinach thylakoids. To enable efficient migration of the photo-damaged PS II complexes from the grana to the stroma thylakoids where the damaged PSII is repaired, the grana should become unstacked. We used digitonin fractionation method to estimate the membrane unstacking. During the photoinhibition of PSII, the thylakoids became markedly unstacked. Interestingly, the extent of D1 degradation seemed to be proportional to the rate of the thylakoid unstacking.These results suggest that the thylakoid unstacking is necessary for the efficient degradation of the photo- or heat-damaged D1 protein in PS II. "
(a) Faculty of Science, Biology, OKAYAMA UNIVERSITY

P45030 High throughput screening of T-DNA tagging rice lines for altered contents of total soluble sugars and absorbance spectra of photosynthetic pigments
Woo, Young-Min-presenter ymwoo@postech.ac.kr(a) Kim, Ji-Hyun (a) An, Kyungsook (a) Sung, Min-Jung (a) Moon, Sunok (a) An, Gynheung (a)
"T-DNA or transposon tagging mutants are invaluable for functional genomics. A great number of insertion mutants in rice are available worldwide for forward and reverse genetic approaches. Forward genetic approach initiates with specific phenotypes and eventually identifies the responsible genes. However, it is considered to be relatively inefficient due to multiple insertions in the genome of a single line and tissue culture-derived somatic variation and retrotransposon activity. Thus, we attempted to improve the efficiency of forward genetic approach by using homozygous T-DNA tagging lines. Ongoing genotyping of our T-DNA population has isolated about 1,000 homozygous lines in gene families including receptor-like kinases, transcription factors, and E3 ligases. To isolate rice genes playing significant roles in photosynthesis-related processes, we screened the homozygous lines for altered contents of total soluble sugars and absorbance spectra of photosynthetic pigments. Water-soluble sugars extracted from leaves were acid-hydrolyzed, and their contents were determined by dinitrosalicylic acid assay. For absorbance spectra of photosynthetic pigments, the pigments in leaves were extracted in 80% acetone, and absorbance was scanned from 350 to 750nm wavelength. A few lines with exceptionally high or low soluble sugar contents and altered absorbance spectral patterns were isolated. Confirmation and further analyses of the isolated lines are in progress. This research was supported in part by Biogreen21 Research Project, RDA, Korea (Grant No. 20080401034027) and the Korea Science and Engineering Foundation (KOSEF) through the National Research Laboratory Program funded by the Ministry of Science and Technology (Grant No. M10600000270 6J00007010)."
(a) National Research Laboratory, Biotechnology Research Center, Pohang University of Science and Technology, Pohang 790-784, Korea

P45031 Redox control of chlorophyll metabolism governs acclimative responses of the light harvesting anetnna
Schwarz, Eliezer M.-presenter eschwarz@life.uiuc.edu(a)
"In order to make use of the suns energy plants must be able to absorb enough light to photosynthesize efficiently while still avoiding oxidative damage. This balance is achieved by a variety of processes at different timescales. Long term acclimation of the photosynthetic apparatus to the light environment is achieved by adjusting the relative stoichiometry of the photosystems and the size of the light harvesting antenna pool, which occurs

both during initial greening of the plant in development as well as under changing conditions throughout the life of the plant. The coordination of such photosystem rearrangements requires regulation of cellular processes on many levels. Chlorophyll, as an indispensable cofactor for proteins of the photosystems, potentially plays a key regulatory role in photosystem protein dynamics. To investigate the role of chlorophyll metabolism in governing photosynthetic acclimation, incorporation radiolabeled chlorophyll into protein complexes was followed by native green gel electrophoresis. Results indicate that production of chlorophyll and its incorporation into photosynthetic proteins are repressed by normal growth light, and this repression is mediated by the redox state of the plastoquinone pool. "
(a) University of Illinois Urbana Champaign

P45032 Inorganic carbon acquisition by an acid-tolerant green alga
Colman, Brian-presenter colman@yorku.ca(a) Powe, Chris (,b)
"The acid-tolerant green alga *Stichococcus bacillaris* grows in media ranging from pH 3.0 to 9.5. The cells express an external carbonic anhydrase (CA) when grown in media above pH 5.0 and CA increases from pH 6.5 to 9.5. The capacity for HCO_3- uptake by cells treated with the impermeable CA inhibitor acetazolamide (AZA) was determined by comparing the calculated rate of CO_2 supply with the rate of photosynthetic oxygen evolution. Active bicarbonate transport was found in cells grown above pH 7.0. Monitoring CO_2 uptake and O2 evolution by mass spectrometry, indicated that air-grown, AZA-treated cells grown at pH 8.0 accumulated inorganic carbon and reduced the extracellular CO2 concentration to a CO_2 compensation concentration at pH 7.5 to 18.7 µ;M which is above the CO_2 equilibrium concentration at this pH suggesting that the cells do not exhibit active CO_2 uptake. This was confirmed by mass spectrometry when suspensions of AZA-treated cells were pulsed with CO_2-saturated water. However, O_2 evolution continued at CO_2 compensation point, confirming the capacity of these cells for active bicarbonate uptake. These data indicate that this alga has a CO_2 concentrating mechanism when grown at alkaline pH, but the δpH between the cell interior and the external medium is large enough to allow the accumulation of inorganic carbon by the diffusive uptake of CO_2 and the expression of external CA at neutral pHs would ensure an equilibrium CO_2 concentration at the cell surface to maintain the CO_2 supply. "
(a) York University, Department of Biology

P45033 Efficiency of the CO_2-Concentrating Mechanism in Single-Cell C_4 Metabolism
King, Jenny L.-presenter jennylking@wsu.edu(a) Edwards, Gerald E. (a) Cousins, Asaph B. (a)
"The availability of atmospheric CO_2 often limits rates of photosynthesis and leads to an increase in rates of photorespiration, particularly when stress induces stomatal closure. In response, some plants in the Chenopodiaceae family evolved a CO_2-concentrating mechanism termed C_4 photosynthesis, which concentrates CO_2 around Rubisco and reduces rates of photorespiration. It was previously thought that C_4 photosynthesis required a dual-cell anatomy to enable CO_2 to be concentrated around Rubisco and prevent diffusional CO_2 loss from a leaf. Recently, however, a C_4 photosynthetic pathway using dimorphic chloroplasts within a single cell to concentrate CO_2 around Rubisco was discovered. Combining the increased efficiency of C_4 photosynthesis in an anatomically simpler package has tremendous potential for engineering C_4 traits into C_3 crops. However, the photosynthetic efficiencies of such novel C_4 types are unknown and must be investigated before we can estimate their true potential as a strategy for enhancing photosynthesis in C_3 crops. The aim of this project is to characterize the efficiency of the CO_2-concentrating mechanism in two different subtypes of single-celled C_4 photosynthesis. One form has Bienertioid type anatomy, which uses central and peripheral cytoplasmic compartments to concentrate CO_2 around Rubisco (*Bienertia sinuspersici*), and the other, having Borszczowoid type anatomy, utilizes distal and proximal compartments (*Borszczowia aralocaspica*). We are using a coupled gas exchange and isotope-ratio mass spectroscopy system to measure real-time carbon isotope fractionation. This system will be used to determine the efficiency of the carbon-concentrating mechanism during single-cell C_4 photosynthesis in response to changing light and CO_2 availability."
(a) Washington State University

P45034 "Over expression of sedoheptulose 1,7, bisphosphatase (SBPase) significantly improves carbon assimilation at elevated CO_2"
Rosenthal, David M-presenter davidrosenthal2@gmail.com(a,c) Raines, Christine A (b) Ort, Donald R (a,c)
"Biochemical models of photosynthesis (A) show that it is most frequently limited by the slowest of two processes, maximum carboxylation capacity of the enzyme Rubisco ($V_{c,max}$) or the regeneration of RuBP via electron transport (J). At current CO_2 levels Rubisco is not saturated by its substrate, therefore elevating CO_2 increases the velocity of carboxylation. At light saturation the transition between Rubisco limited and RuBP limited photosynthesis occurs at current ambient CO_2. Therefore, in the future leaf A should be increasingly limited by RuBP regeneration, as CO_2 is predicted to increase by 50% by 2050 to ca. 550 ppm. If this is the case then plant engineered to have an increased capacity to regenerate RuBP should have a greater stimulation of photosynthesis and productivity at elevated CO_2 relative to wild type plants. We tested this hypothesis by growing three transgenic tobacco lines over expressing the enzyme SBPase and compared their response to wild type (WT) tobacco in greenhouse growth chambers in near ambient (400ppm) and at elevated (750 ppm) CO_2. Increased light saturated A of transgenic lines in elevated CO_2 was associated with a 2 to 4 fold greater increase in the quantum yield of electron flux through PSII (ΦPSII) than that of wild type plants. As a result, plants over expressing SBPase exhibited a 45 % increase of light saturated photosynthetic rates (A) whereas wild type plants only had a 35 % increase at elevated CO2. In vivo Rubisco capacity ($V_{c,max}$) was down regulated in both WT and transgenic plants. However, downregulation of carboxylation capacity in transgenic plants was 1/2 to 1/3 of that in wild type plants. Over expressing this enzyme alone could significantly increase carbon assimilation, growth, and crop productivity in the future."
(a) USDA-ARS-Photosynthesis Research Unit (b) University of Essex (c) University of Illinois, Institute For Genomic Biology

P45035 Dynamic photosynthetic discrimination at low light
Mirabal, Susan-presenter mirabals@unm.edu(a) Chuchra-Zbytniuk, Kathleen (a) Pater, Dianne (a) Hanson, David (a)
"Enzymatic fractionation caused by ribulose-1,5-bisphosphate carboxylase oxygenase and phospho*enol*pyruvate carboxylase, along with the effects of diffusion, respiration, and photorespiration drive the net discrimination against $^{13}CO_2$ in the leaf. However, there is considerable uncertainty in the relative importance of these factors under low light conditions. We employed a new tunable diode laser gas exchange system to analyze transient isotopic shifts in leaf CO_2 exchange associated with step changes in light intensity. We measured leaf-level isotopic exchange at a frequency of 10Hz and found that step changes from high to low light caused net leaf level discrimination to be much higher than predicted values based on the standard model. Discrimination at low light was dynamic, yielding very high values in the first two to four minutes after the initiation of low light conditions. This was followed by a longer period (approximately twenty minutes) of high discrimination before achieving steady-state. We interpret this response as evidence for decarboxylation of a metabolite pool (e.g. malate) generated by a metabolic imbalance in the leaf. This would be analogous to the isotopic signature associated with the up-regulation of respiration in the dark during the period of light-enhanced dark respiration. Furthermore, the steady-state levels achieved at low light are inversely related to the final light intensity in a manner not fully predicted by current models. We interpret this increase as evidence for the up-regulation of respiration under the low light conditions."
(a) University of New Mexico

P45036 Converting sunlight energy into biomass at almost 9% efficiency: There is a plant that does it repeatedly in the field under low-input conditions.
Dohleman, Frank G. (a) Arundale, Rebecca A.-presenter rarundal@illinois.edu(a,b) Long, Stephen P. (a,b)
http://www.miscanthus.illinois.edu
"Bioenergy crops are criticized as alternate energy sources because of their perceived low conversion efficiency of solar radiation into biomass energy. Crop productivity is determined as the product of total solar radiation incident on an area of land, and the efficiencies of interception, conversion and partitioning of that sunlight energy into plant biomass. In replicated side-by-side trials of mature stands of the perennial bioenergy feedstocks *Miscanthus* x *giganteus* and *Panicum virgatum* cv. Cave-in-Rock in the Midwestern USA, above and below-ground biomass accumulation was measured bi-monthly over the 2006, 2007 and 2008 growing seasons. Average peak shoot biomass for *M.* x *giganteus* was 46.5 t ha^{-1}, declining to 38.1 t ha^{-1} of dry biomass by December, significantly higher than the peak of 20.7 t ha^{-1} declining to 11.3 t ha^{-1} for *P. virgatum*. While rhizome biomass did not appear to change annually for either species, *M.* x *giganteus* rhizome biomass varied through the growing season, while *P. virgatum* rhizomes did not show a similar trend. Average annual conversion efficiency of photosynthetically active radiation (PAR) to harvestable end-of-season dry biomass of *M.* x *giganteus* was 0.031, nearly three times higher than the 0.011 conversion efficiency found in *P. virgatum*. Over the 2 month period of largest total biomass gain in both species, between June and August, PAR conversion efficiency was 0.087 for *M.* x *giganteus*, double the 0.044 of *P. virgatum* and nearly 75% of the theoretical maximum for C4 species of 0.12. This short-term conversion efficiency is among the highest reported and shows that if this window of high conversion efficiency could be extended for a longer period of the growing season, it could lead to significant increases in total biomass yield."
(a) University of Illinois - Urbana Champaign (b) Energy Biosciences Institute

P45037 Engineering the light-harvesting chlorophyll antenna size to improve solar energy conversion efficiency
Kirst, Henning-presenter hkirst@nature.berkeley.edu(a) Melis, Anastasios (a)
"Biofuels production by plants and algae requires a high solar-to-product conversion efficiency. Under direct and bright sunlight, when productivity of plants and algae ought to be maximal, photosynthesis becomes saturated and ~80% of the absorbed photons are wasted by non-photochemical quenching. Attenuation of light absorption by the photosynthetic apparatus can be achieved by substantial reduction in the size of the chlorophyll (Chl) antenna, resulting in higher productivity by up to 3-fold. This objective is pursued by mutagenesis through which to identify genes that determine the size of the Chl light-harvesting antenna. A new DNA insertional transformant of the model photosynthetic organism, Chlamydomonas reinhardtii, with a **t**runcated **l**ight harvesting Chl **a**ntenna size (tla2) was isolated and investigated. The work reports on measurements of the Chl antenna size in the tla2 mutant, found to be about 50% of that in the wild type. The tla2 mutation did not affect the photochemical charge separation efficiency of PSII, as evidenced by Fv/Fm measurements and electron-transport capacity, as measured by oxygen evolution. Under mass culture and high light intensity conditions, the truncated Chl antenna size of the tla2 strain helped increase photosynthetic productivity of the algae by 2-fold. Southern blot analyses revealed a single copy of the plasmid insert in the tla2 mutant. Flanking sequence analysis of the insertion site was achieved by TAIL-PCR, and further analysis showed that several nuclear genes have been deleted by the insertion. Genetic crosses of the tla2 mutant to a wild type strain showed co-segregation of the exogenous plasmid and the tla2 phenotype, suggesting linkage between insert and phenotype. The functional role of the TLA2 gene will be discussed."
(a) UC Berkeley

P45038 Varietal differences in growth and photosynthetic response to elevated CO_2 in wheat under low irradiance
Seneweera, Saman-presenter samans@unimelb.edu.au(a,a) Norton, Rob M (a,a) Rebetzke , Greg (b,b)
http://www.unimelb.edu.au/
"To investigate the underlying physiological mechanism of intraspecific variation in response to elevated CO_2, ten wheat (*Triticum aestivum* L.) cultivars reflecting different tillering capacity and contrasting genetic background were evaluated (cvv Janz, Yitpi, Halberd, Hartog, Batavia, Federation, Excalibur, Sunvale, Westonia and H45). These cultivars were grown in controlled environment conditions at ambient (390 µ;mol CO_2 mol) or elevated CO_2 (700 µ;mol CO_2 mol), with a mid-day maximum photon flux density of 700 µ;mol photons m^{-2} s^{-1}. Total plant dry mass at 40 days after sowing was increased an average 16% for plants grown under elevated CO_2. Among cultivars, Sunvale showed the largest relative increase in dry matter (+43%) while growth was unchanged for cv. Janz. Light saturated photosynthetic rates were increased by about 60% for all cultivars. However, photosynthetic acclimation to elevated CO_2 was not evident for any of the cultivars except Sunvale. Mechanistic analysis of gas exchange data showed large variation in maximum carboxylation capacity of Rubisco (V_{cmax}) and photosynthetic electron transport rate (J_{max}). In most cases, V_{cmax} was increased while J_{max} decreased in plants grown at elevated CO_2. This suggests that a reallocation of biochemical resources occurs in favour of Ribulose Bisphosphate Carboxylation over RuBP regeneration under elevated CO2. This response could be an adaptive strategy to respond to elevated CO_2 under the relatively low irradiance conditions. "
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SESSION P46 – PLANT BIOTECHNOLOGY & RISK ASSESSMENT

P46001 Glycosylation of biological active compound using plant cultured suspension cells
Hamada, Hiroki-presenter hamada@dls.ous.ac.jp(a) Ohiro, Azusa (a) Kimura, Eriko (a) Kondo, Mai (a) Sato, Daisuke (a) Nakajima, Nobuyoshi (b) Shimoda, Kei (c)
"Many kinds of secondary metabolites, such as saponins, are produced in the form of glycosides in plant cells. To produce the bioactive saponin many scientist synthesized the bioactive saponin and glycosylation of organic compound improves their bioavailability and pharmacological properties. We had investigated the biotransformation of foreign substrate using plant cultured suspension cells and clarified that the plant cultured suspension cells have the ability such as stereoselective reduction, enantioselective oxidation and regioselective hydroxylation. In this study we study the biotransformation of biological active compound using plant cultured suspension cells and we report that plant cultured suspension cells glycosylate regio- and stereoselectively the hydroxyl group of biological active phenolic compound in a good yield and the bioactivity: anti-oxidation, anti-obesity, anti-allergy and anti-cancer of saponin (biotransformation product)."
(a) Okayama Univ. of Sci., (b) Okayama Prefectural Univ. (c) Oita Univ.

P46002 Enhanced Expression of Hantaan Viral Antigens using GPOS™ Chloroplast Technology
Kode, Vasumathi-presenter vasumathi@kashawaii.com(a) Champagne, Michele (a) Stokes, Jill (a) Yang, Jie (a) khaiboullina, svetlana (c) St.Jeor, Steven (c) Kuehnle, Adelheid (a,b)
"Hantaviruses are RNA viruses that cause significant human mortality, are endemic worldwide and are transmitted to humans by aerosolized mouse urine or excreta. The global distribution of Hantaviruses makes them a worldwide public health concern. Old World strains found in Europe and Asia

cause hemorrhagic fever with renal syndrome; New World strains are responsible for hantaviral cardiopulmonary syndrome, and cause pulmonary edema with fatality rates approaching 36%. To manufacture a diagnostic test, a reliable source of recombinant nucleoprotein is critical. Hantavirus nucleoproteins expressed in E.coli, Vaccinia virus, yeast, tobacco, potato and mammalian cells demonstrate antigenicity, but yield has been poor (0.1% to 2% of protein). Thus, there is a need for a higher yielding production platform. Chloroplast genetic engineering has emerged as an attractive tool for hyper-expression of biopharmaceuticals in plants. We have designed and proven a novel method of chloroplast recombinant protein production termed G-POSTM (Gene Positioning System). Here we demonstrate a 3- to 4-fold elevated expression of Hantaan and Andes nucleoprotein antigens in plastids of tobacco to approximately 6% to 8% of total soluble protein without system optimization. G-POS-based antigens are sufficiently reactive with convalescent human antisera to function in an in vitro diagnostic assay, with reactivity similar, or superior, to that prepared in E. coli. G-POS TM can produce protein antigens at low cost and be applied to other species of chloroplast-based production platforms. This work is supported by the US Army Medical Research and Materiel Command under Contract W81XWH-06-C-0009 and NSF SBIR Award 0548640 to Kuehnle AgroSystems and Grant AI-65359 to S. St. Jeor. "
(a) Kuehnle Agrosystems, Inc (b) Department of Tropical Plant and Soil Sciences, 3190 Maile Way, University of Hawaii, Honolulu, HI 96822 U.S.A. (c) Department of Microbiology and Immunology, School of Medicine, University of Nevada, Reno 89557

P46003 Rapid high-level production of multiple-subunit protein therapeutics and vaccines in plants
Huafang , Lai (a) Waranyoo, Phoolcharoen (a) Chen, Qiang-presenter qiang.chen.4@asu.edu(a,b)
"Plant viral vectors have great potential in rapid production of important pharmaceutical proteins. However, due to the competing nature of viral vectors derived from the same virus, no efficient viral expression system is yet available for high-yield production of heterooligomeric pharmaceuticals which require the expression and assembly of two or more protein subunits. We report the employment of a geminivirus-derived expression system in high-yield rapid production of oligomeric protein complexes of pharmaceutical importance, including vaccines and monoclonal antibodies (mAbs) derivatives in plants. Our expression platform is a single-vector noncompeting high-yield transient expression system based on Gemini virus replicons. Using our vectors, we obtained high-level expression of several therapeutic mAbs within 4 to 7 days post infiltration (dpi) of Nicotiana benthamiana leaves. The correct assembly of the mAb into full-size functional oligomeric structures was demonstrated. Our system, therefore, represents the most advanced plant transient expression technology which eliminates the daunting needs for identifying non-competing virus and needs for co-infection of multiple expression modules for producing multiple-subunit protein complexes."
(a) THe Biodesign Institute, Arizona State Univeristy (b) Department of Biological Sciences, Arizona State University

P46004 Transient Expression of Ebola Immune Complex in *Nicotiana benthamiana*
phoolcharoen, waranyoo-presenter pang@asu.edu(a) Arntzen, Charles (a) Mason, Hugh (a) Qiang , Chen (a,b)
"Plants have been shown to be good bioreactors for producing recombinant proteins. Plants offer many advantages over other recombinant systems for protein production, including product safety, ease of production, product storage, and low production cost. A previous study showed that immune complex can be expressed in *Nicotiana tabacum*. The plant expressed immune complex was reported highly immunogenic in mice and appeared to act as a self adjuvant. In this work, we intend to express Ebola immune complexes in *Nicotiana benthamiana* and to study the effects of a variety of agonists in enhancing the immunogenicity of these Ebola vaccine candidates. To create Ebola immune complex, we used Ebola glycoprotein (GP1) and a humanized GP1 monoclonal antibody (mAb) (6D8) which specifically binds to the 6D8 epitope on GP1. The coding sequence of GP1 was fused to the 3' end of the 6D8 heavy chain gene. The 6D8 heavy chain-GP1 fusion was co-expressed with the light chain of 6D8 using a Geminiviral replicon system. The Ebola immune complex was extracted from leaf tissue of *N. benthamina*, and purified by ammonium sulfate precipitation and protein G affinity chromatography. The purified Ebola immune complex was subcutaneously injected into BALB/C mice. Our results showed that the humanized 6D8 mAb induced strong mouse anti-human antibody response. In contrast, antibody response to Ebola GP1 was relatively weak. However, the anti-GP1 response is enhanced when TLR7/8 and TLR3 agonists was injected with Ebola immune complex. In conclusion, plant expressed Ebola immune complex showed its immunogenicity in mice, which was enhanced by the inclusion of a combination of TLR agonists. "
(a) The Biodesign Institute and School of Life Sciences, Arizona State University, Tempe, Arizona 85287 (b) Department of Applied Biological Sciences, Arizona State University, Mesa, Arizona 85212

P46006 Fostering Plant Science Research at MU Plant Transformation Core Facility
Kennon, Angela A.-presenter kennona@missouri.edu(a) Zhou, Liwen (a) Yin, Xiaoyan (a) Yin, Jingwei (a) Sahoo, Diptimayee (a) Chen, Xinlu (a) Lee , Hyeyoung (a) Wan, Neng (a) Zhang, Zhanyuan (a)
http://www.plantsci.missouri.edu/muptcf
"Since its establishment in 2000, the University of Missouri (MU) Plant Transformation Core Facility has been providing state-of-the-art research support services in genetic engineering of maize (Zea mays) and soybean (Glycine max). The Facility is aiming at promoting gene discovery, crop improvement, and funding opportunities for the plant science research community. The facility service categories include both standard and customized transient as well as stable transformation for maize and soybean upon the users request. The transformation systems for both maize and soybean utilizes Agrobacterium-mediated approaches. We have recently established fast and reproducible sorghum and switchgrass transformation systems and transformation services for these two species will be open to the public soon. Current research activities are geared towards developing high-throughput transformation, improving the quality of transgene integration and sufficient gene regulation to meet the needs of crop improvement and functional genomics. Some of these studies are conducted as collaborations with on- and off-campus researchers. More details of these activities will be presented at the conference. "
(a) University of Missouri-Columbia

P46007 A biotech strategy for Garlic Mosaic Disease.
Kenel, Fernand O. (a) Eady, Colin C. (a) Brinch, Sheree A.-presenter BrinchS@crop.cri.nz(a)
"Garlic Mosaic Disease is caused by an insidious virus complex made up of *Potyviruses*, *Carlaviruses* and *Allexiviruses*. Once present in garlic it can cause a gradual decrease in vigour, resulting in yield reductions of up to 35%. The main causal agent in this complex is thought to be the potyviruses Onion Yellow Dwarf Virus (OYDV) and/or Leek Yellow Stripe Virus (LYSV). These suppress the plant defence system such that other viruses can proliferate, thus causing a reduction in vigour of the host. To combat this disease we have designed a OYDV/LYSV coat protein gene fusion construct based around sequences of high homology within the respective OYDV and LYSV strains. This has been cloned into pHannibal to create an RNAi gene-silencing cassette directed against both OYDV and LYSV. The cassette has been inserted into a pART27-based binary vector that also confers hygromycin resistance and GFP activity in plant hosts, and has been transformed into garlic (*Allium sativum*). GFP-positive plants have been recovered after selection on hygromycin, and molecular analysis reveals intact T-DNA in some transgenic lines. Progress towards the characterisation of the RNAi expression is also presented."
(a) The New Zealand Institute for Plant and Food Research

P46008 Bioassay system for polychlorinated biphenyls using polychlorinated biphenyls-response genes in the genome of *Arabidopsis thaliana*

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"The potential of halogenated aromatic hydrocarbons (HAH) to disrupt the endocrine system of animals has long been a cause of great concern. HAHs include numerous polychlorinated biphenyls (PCBs) which have been used for industrial and commercial applications since the 1960s. Among the 209 possible PCB congeners, the environmental toxicity of coplanar PCBs (Co-PCBs) is increasingly problematic, particularly in Japan. Although the usage of polychlorinated biphenyls (PCBs) was widely prohibited over thirty years ago owing to their high toxicity, a significant amount of them are still detected even now in almost all environments. The precise quantitative analysis of pollution levels of PCBs has been performed using a gas chromatograph, equipped with a high-resolution mass spectrometer; however, both the high cost and the high level of skill required to administer this technique are seen as disadvantages of this method. The present study reports on the existence of several genes in the genome of *Arabidopsis thaliana (A. thaliana)* that respond to the chemical stress of 3,3',4,4',5-pentachlorobiphenyl (PCB 126), one of the Co-PCBs. They were detected by cDNA microarray assay using probes prepared from PCB 126 and biphenyl exposed *A. thaliana*. Twenty nine and 36 genes were up-regulated and down-regulated respectively, by exposure to PCB 126 alone. The development of a bioassay system for PCBs using PCBs-response genes found in the genome of *A. thaliana* was proposed, with the aim of investigating its efficiency for risk assessment of PCBs contamination. This bioassay system is expected to be a good substitution for instrumental analysis, as a first step simple analysis method of PCBs in the environment."

(a) Azabu University, Department of Life and Environmental Science

P46009 Evaluation of the D-Serine Ammonia Lyase (dsdA) Gene for Use As A Selectable Marker in Maize Transformation

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"In this study, we evaluated the D-serine ammonia lyase (dsdA) gene from E. coli as a selectable marker for maize transformation. DSDA detoxifies D-ser via metabolism in a substrate-specific reaction to pyruvate, ammonia and water. D-ser inhibits germination of isolated immature maize embryos and growth of embryogenic callus from wild-type plants in a concentration range between 2 to 15 mM. Transgenic plants were recovered under the preferred selection conditions with dsdA as the selection marker at efficiencies comparable to using a mutated ahas selection marker gene as a control. D-serine provided tight selection without escapes. Molecular analysis confirmed the integration of the dsdA gene into the genome of the transgenic plants. No adverse phenotypes were observed in the greenhouse and expression of the dsdA marker had no affect on agronomic characteristics or grain yield in a multi-location field trial. Seed composition analysis results showed that there were no significant differences in the contents of seed protein, starch, fatty acids, fiber, phytic acid, and free amino acids between transgenic and non-transgenic control plants. These data indicate that the dsdA gene is properly expressed in maize and the DSD protein functions appropriately to metabolize D-serine during in vitro selection. Preliminary safety assessments indicated no adverse affects would be expected if humans were exposed to DSD protein in the diet from an allergenicity or toxicity perspective. The dsdA gene in combination with phytoinhibitory levels of D-serine represents a new and effective selectable marker system for maize transformation. "

(a) BASF Plant Science

P46010 Measuring the leakage of paternal ptDNA *via* pollen indicates that plastid transgenes are efficiently contained by plastid localization in tobacco plants

Maliga, Pal-presenter maliga@waksman.rutgers.edu(a) Svab , Zora (a,a)

"Plastid DNA in cultivated tobacco (*Nicotiana tabacum*), as in most crops, is transmitted to the progeny by the maternal parent only. We have shown by selection for a plastid-encoded spectinomycin resistance gene *in vitro* that the paternal ptDNA may be transmitted to the progeny in ~ 1 out of 10,000 seedlings (10^{-4}; PNAS 104: 7003, 2007). To estimate the likelihood of paternal ptDNA transmission under field conditions, we designed a novel marker system that facilitates scoring the presence of paternal plastids in three-week old greenhouse-grown seedlings. The screen is based on using transplastomic maternal plants with handicapped 'golden' plastids that delay plant development. The paternal plants carry normal green plastids. If rare paternal plastids are transmitted *via* pollen, we expect appearance of variegated or green seedlings with paternal plastids that grow faster than the seedlings carrying only the 'golden' maternal plastids. In a population of ~370,000 hybrid seedlings we found only one variegated progeny suggesting the presence of paternal ptDNA. This indicates that the frequency of paternal ptDNA transmission under field conditions is at least 30x lower, ~3 x 10^{-6}, than what is detected by direct selection for the paternal ptDNA marker *in vitro*. This suggests that most paternal ptDNA copies are in a cell type that will never contribute plastids to leaf cells or to the germline. "

(a) Rutgers University

P46011 Optimizing Chloroplast Transgenes for Production of Microbial Proteins

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"Transformation of plant chloroplast genomes with constructs carrying coding regions derived from various organisms has given variable results. Proteins encoded by some transgenes have been expressed at high levels, but others result in low or undetectable levels of proteins, even when the same promoter and terminator sequences are incorporated into the vector. We have investigated the role of the downstream box region in accumulation of microbial enzymes expressed from chloroplast transgenes. We placed coding regions for a *Thermobifida fusca* endoglucanase and β-glucosidase under the control of the phage T7 G10 5-prime untranslated region and a plastid terminator and introduced constructs into the tobacco *(Nicotiana tabacum)* chloroplast genome by particle bombardment. Transgenes were expressed by read-through from the native plastid 16S rRNA promoter. Sequences were fused at the 5-prime end of the coding regions to express proteins carrying different 14 amino acid N-terminal fusions. In homoplasmic transgenic plants, the most effective transgenes resulted in plants that accumulated the enzymes to as much as 10-12% of total leaf soluble protein (TSP), while less than 0.3% TSP was obtained from leaves of plants carrying the least effective transgenes. Enzyme accumulation was stable during plant development. Endoglucanase could be purified on cellulose affinity columns and enzyme activity correlated well with protein quantification by immunoblot. The chloroplast-expressed β-glucosidase was active against cellobiose. This study demonstrates the feasibility of high-level chloroplast-based synthesis of biomass-degrading enzymes. Supported by USDA grant NRI 2007-02133 to BAA and MRH and an NSF Fellowship to BNG."

(a) Molecular Biology & Genetics, Cornell University (b) Biological & Environmental Engineering, Cornell University

P46012 Overexpression of *Aspergillus oryzae leucine aminopeptidase* in tomato

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"An leucine aminopeptidase (LAP) liberates the hydrophobic amino acid residues in the N-terminus of the peptides of different origin. To study the regulation and function of LAP in plants, we developed the transgenic tomato (*Lycopersicon esculentum*) overexpressed *Aspergillus oryzae LAP* gene.

Comparisons of the amino acid sequences revealed that *Aspergillus oryzae* LAP protein shared rather low similarity to the two LAP isoforms of tomato: LAP-A (6.45%) and LAP-N (5.03%). Transgenic lines harboring *Aspergillus oryzae LAP* gene were screened by PCR using the primer pairs specific to *neomycin phosphotransferase II (NPTII)* gene. The expression of *Aspergillus oryzae LAP* gene in different transgenic lines was determined by RT-PCR analysis. Furthermore, the observation that overexpression of *Aspergillus oryzae LAP* contrarily inhibited the LAP activity in transgenic lines implicated an possible regulation between LAP activity and *LAP* gene expression in plant."
(a) Department of Molecular Biotechnology, Da Yeh University, Changhua, Taiwan (b) Department of Bioindustry Technology, Da Yeh University, Changhua, Taiwan (c) Department of Life Sciences, National Chung Hsing University, Taichung, Taiwan

P46013 The *Activator (Ac)* element from maize: transposition mechanism and advancement as a mutagenesis tool
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"The *Activator/Dissociation (Ac/Ds)* transposable elements from maize are used as mutagenesis and gene isolation tools in various plant species. However, the transposition frequencies vary widely and in some plant species they are too low for large scale gene tagging approaches. *Ac* transposition underlies complex post-transcriptional regulation mechanisms that are still not well understood. We established yeast (*S. cerevisiae*) as an experimental system to study the transposition mechanism and regulation and to test mutant *Ac* derivatives. To survey the activity of putative hyperactive *Ac* transposase mutants in transgenic *Arabidopsis* plants we developed a novel bifunctional reporter construct encoding a phosphinotricin acetyltransferase (PAT) fused to the enhanced green fluorescent protein (eGFP). There we combined the ability to phenotypically score somatic *Ds* excision with the possibility to select for rare germinal excision events."
(a) Free University Berlin, Institute of Biology/Applied Genetics

P46014 Monitoring the occurrence of genetically modified oilseed rape growing along a Japanese roadside: 3-year observations
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"Monitoring for escape of genetically modified (GM) oilseed rape (*Brassica napus*) during transport can be performed by means of roadside evaluations in areas where cultivation of this GM crop is not conducted, such as in Japan. We performed a survey of oilseed rape plants growing along a 20-km section of Japan's Route 51, one of the main land transportation routes in central Japan for imports of GM oilseed rape from the Port of Kashima into Keiyo District. Oilseed rape plants were found each year, but the number of plants varied substantially during the 3 years of our study: 2162 plants in 2005, 4066 in 2006, and only 278 in 2007. The low number in 2007 was probably caused by roadwork. Herbicide-resistant individuals were detected in the three consecutive years (26, 8, and 5 individuals with glyphosate resistance), but glufosinate-resistant plants (9 individuals) were detected only in 2005. The roadside plants occurred mainly along the inbound lane from Kashima to Narita. These plants are likely to have their origin in seeds spilled during transportation of cargo from the port because there are no potential natural seed source plants for *B. napus* near Route 51. This is the first detailed report on the transition and distribution of herbicide-resistant oilseed rape plants following loss and spillage along Japanese roads."
(a) National Institute for Environmental Studies

P46015 Development of effective species-specific DNA markers using DNA arrays
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"Species-specific DNA markers (SSMs) are genetic tools essential in investigation of biodiversity and biological interactions in fields. However, developing them is a time-consuming task. Here we aimed to establish a convenient method for preparation of SSMs that distinguish 3 *Brassica* sepecies (*B. rapa, B. napus, and B. juncea*) using cDNA micro arrays. Plants were grown for two weeks in a greenhouse. RNA was isolated from the 2nd leaves, then cDNA was synthesized from 10µ;g of total RNA. Complimentary DNAs were labeled with fluorescent dyes, then hybridized to *Arabidopsis* oligo cDNA array (Affimetrix Arabidopsis ATH1). We selected 192 genes exhibiting the most significant differences in the signal intensity among the 3 species. We amplified DNA fragments with primers for selected genes using genomic DNA isolated from the *Brassica* species as a template. The primers were designed according to the nucleotide sequences of *Arabidopsis* orthologs. We successfully amplified 53 different PCR products for all 3 species. Fifteen of them have been sequenced so far and all exhibited polymorphic alleles for the 3 species. These data suggested that development of SSMs using DNA arrays is a powerful strategy for construction of SSMs. "
(a) The National Institute for Environmental Studies

P46016 Fostering Plant Science Research at MU Plant Transformation Core Facility
Kennon, Angella M-presenter kennona@missouri.edu(a) Zhou, Liwen (a) Yin, Jingwei (a) Sahoo, Diptimayee (a) Yin, Xiaoyan (a) Karpova, Olga (a) Lee, Hyeyoung (a) Barampuram , Shyamkumar (a) Wan, Neng (a) Zhang, Zhanyuan (a)
http://www.plantsci.missouri.edu/muptcf
"The University of Missouri (MU) Plant Transformation Core Facility has been providing state-of-the-art research support services in genetic engineering of maize (Zea mays) and soybean (Glycine max). The Facility is aiming at promoting gene discovery, crop improvement, and funding opportunities for the plant science research community. Our staff is strongly dedicated and committed to providing various types of transformation support services and conducting research in transgenic technology development with a focus on maize and soybean. The facility service categories include both standard and customized transient as well as stable transformation for maize and soybean upon the users request. The transformation system for maize and soybean employ the Agrobacterium-mediated approach. We have recently established fast and reproducible sorghum (Sorghum bicolor) transformation system and now are ready for providing sorghum transformation services. Current research activities are geared towards developing high-throughput transformation, improving the quality of transgene integration and sufficient gene regulation to meet the needs of crop improvement and functional genomics. One of our most recent efforts is in developing efficient switchgrass transformation process to meet the need of biofuel crop engineering. Our specific interest in soybean genetic engineering is to regulate several economically important genes conditioning soybean seed polyunsaturated fatty acids and short chain sugars, secondary metabolites, abiotic stress, virus resistance, etc. Some of these studies are conducted as collaborations with on- and off-campus researchers. More details of these activities will be presented at the conference."
(a) University of Missouri

P46017 aFGF expressed in plants by transient expression system
Wang, Xingzhi-presenter mailwxz@yahoo.com.cn(a) Liu, Jingying (a) Yang, Liping (a) Fan, Yajun (a) Li, Liang (a) Wang, Hongwei (a) Li, Wei (a) Wang , Lei (a) Luan, Ming (a) Zhu, Xiaojuan (a)
"Acidic fibroblast growth factor (aFGF) plays an important role in morphogenesis, angiogenesis and wound healing and is therefore of potential

medical interest. Three technologies have been applied in transient expression to express aFGF in our group. 1. aFGF expressed by leaf injection Human aFGF has been transiently expressed in Nicotiana benthamiana by leaf injection. Approx. 1 week after agroinfection, the recombinant haFGF accumulated in the agroinfected plants reached up to 1% of the total soluble protein. The growth of NIH 3T3 cells was stimulated by the purified haFGF derived from plants. 2. aFGF expressed by root absorption We have developed a simple system named i°root absorptioni± to express foreign proteins in plants successfully. Various factors influencing the gene expression were studied including Agrobacterium cell density, seedling age, plant materials and inoculation conditions. 3. aFGF expressed in germinated pea seeds The aFGF gene was introduced into pea plants by vacuum infiltration of germinated seeds. After germination the seeds with 2-3 cm roots were vacuum infiltrated with bacterial suspensions (OD600 =1.0-1.5) at 0.08 MPa for 1 min. aFGF was expressed in pea plants by the optimized conditions. The advantages of the vacuum infiltration of germinated pea seeds are facility, a short production cycle, efficiency and inexpensiveness. 4. The expression of scfv antibody fragment The scFv of aFGF antibody has been expressed in tobacco plants by leaf injection, vacuum infiltration and root absorption methods. The expression of scFv has been confirmed by Western hybridization and RT-PCR, which showed the normal function compared to the whole antibody. "
(a) Northeast Normal University

P46018 Beneficial mutants of *Brassica napus* produced by UV irradiation of in vitro microspores
shi, Shuwen -presenter shishuwen@hotmail.com(a) Wu, Jiangsheng (a) Sun, Xiuli (a) Wang, Pin (a)
" Since the single cell character of microspores,the mutants generated from microspore in vitro mutagenesis are pure homozygote whereas those derived from tissues and organs are generally chimera.Thus,the technique of microspore in vitro mutagenesis by physical or chemical mutagens has been applied in *B.napus* to create new germplasms and improve varieties.We report here got *B.napus* mutants regenerated from UV irradiation of microspores. The microspores of cultivar Zhongyou 821 and 3 lines (2004-2159,2001-3014,2001-7212) grown in the field were isolated by B5 medium (Gamborg et al. 1968)with 13% sucrose.Purified microspores were pipetted into 7.5cm dishes with Lichter medium (1981) added 70mg/L colchicine and irradiated for 95s by 253.7nm wave ultraviolet light.Using the method of chromosome doubling of Shi et al (2001), irradiated microspores were then incubated at 32.5 °C for 48h followed by removing to same medium without colchicine and cultured at 25 °C to induce embryogenesis. The plantlets formed were transplanted to the field and the self-seeds was analysed by NIRS.Zhongyou 821 DH population occurred one genic male sterile plant with temperature sensitive trait and 2 mutants either having 44.57% oil(donor 40.15%) or low glucosinolates of 19.59µ;M/g seed (donor 93.85µ;M),respectively.One mutant with purple young leaveas at seeding stage and the other apetalous mutant appeared in a DH population of line 2004-2159. 2 plants with 51.66% oil (donor 47.92%) or 0.01% erucic acid (donor 24.18%) was detected respectively,in line 2001-3014. 2 DH plants from line 2001-7212 either contains 47.23% oil (donor 42.79%) or 28.32µ;M/g seed glucosinolate(donor 69.85µ;M),respectively.These mutants would be of greater value for use in rapeseed breeding programs. "
(a) Plant Science & Technology College, Huazhong Agricultural University

P46019 Development of banana bunchy top virus-resistant transgenic banana plants
Borth, Wayne (a) Perez, Eden (a) Cheah, Kheng (a,b) Chen, Yan (a) Xie, Wenshuang (a) Gaskill, Doug (a) Khalil, Said (a) Hu, John-presenter johnhu@hawaii.edu(a)
"Embryogenic cell suspensions (ECS) initiated from immature male flowers of banana cultivar Dwarf Brazilian were transformed using Agrobacterium tumefaciens containing one of four constructs derived from the replicase-associated protein (Rep) gene of of Banana bunchy top virus (BBTV). Each construct was engineered under control of the CaMV 35S promoter in the binary plasmid pBI121. Plantlets that survived antibiotic selection were challenged with viruliferous banana aphids (Pentalonia nigronervosa). Ten adult or late instar aphids were allowed to feed for 2-4 weeks on test plants. All test plants were kept in the greenhouse and monitored for symptom expression for a period of six months. Control plants transformed with empty vector pBI121 only were included in all tests. A total of 270 test plants and 63 control plants were screened for BBTV resistance using this approach. One of 32 test plants transformed with the M1 (mutant Rep gene) construct, 4 of 74 test plants transformed with the AS (antisense Rep gene) construct, 5 of 38 test plants transformed with the ?2/5 (partial Rep gene) construct, and 10 of 126 test plants transformed with the R?2/5 (inverted repeat of partial Rep gene) construct were found to be resistant to BBTV challenge and showed no bunchy-top symptoms. All of the control plants became infected with BBTV under these experimental conditions. Plants that survived BBTV challenge were analyzed by quantitative PCR (qPCR) and Southern hybridizations to determine the number of transgenes that were present in their genomes. Results from these analyses indicated that the resistant plants contained from 2 to more than 9 copies of the NPTII (kanamycin resistance) transgene carried on the pBI121 plasmid. "
(a) Plant & Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, HI, USA (b) Tropical Plant and Soils Sciences, University of Hawaii at Manoa, Honolulu, HI, USA.

P46021 Commercialization of Potato Tuber Moth Resistant Potatoes in South Africa
Zarka, Kelly A-presenter zarka@msu.edu(a) Douches, David (a) Brink, Johan (a) Quemada, Hector (a) Pett, Walter (a) Koch, Muffy (c) Visser, Diedrich (b) Maredia, Karim (a) Boone, Anne (a)
"The potato tuber moth (PTM), Phthorimaea operculella (Zeller) is a major pest problem facing potato farmers in developing countries. Currently, the primary means to control the PTM and avoid major crop losses is the use of chemical pesticides. Michigan State University, funded by the U.S. Agency for International Development, through its Agricultural Biotechnology Support Project, initiated biotechnology research on the development of PTM resistant varieties in 1992. A Bacillus thuringiensis Berliner (Bt)-cry1Ia1 gene, was obtained from ICI Seeds (now Syngenta seed company) and successfully introduced into several potato varieties, including Spunta. Transgenic lines were shown to have complete efficacy against PTM. A regulatory dossier has been submitted to the South African government for release approval. If approved, this Bt-potato will be one of the first public sector developed products to reach farmers in developing countries and will serve as a model for the public sector deployment of insect resistant transgenic crops. The commercialization project includes six components: 1) Product development, 2) Regulatory file development, 3) Obtaining freedom to operate on intellectual property/proprietary technologies and establishing licensing relationships, 4) Marketing and technology delivery, 5) Documentation of socio-economic benefits, and 6) Public communication. The expected benefits of this Bt-potato to farmers and end-users will be increased marketable yield, improved quality, reduced storage losses, reduced post-harvest losses and reduced human exposure to pesticides."
(a) Michigan State University (b) Agricultural Research Council-Vegetable and Ornamental Plant Insitute South Africa (c) Independent Consultant

P46022 Genetic engineering of Leucaena leucocephala for reduction of mimosine content
Jube, Sandro LR-presenter sandro@hawaii.edu(a) Borthakur, Dulal (a)
http://www.sandrojube.blogspot.com
"*Leucaena leucocephala* (leucaena) is an important leguminous tree in many tropical and subtropical countries. Its foliage makes an excellent animal fodder due to its palatability and high protein content. The use of leucaena as an animal fodder is however restricted due to the presence of a toxic compound, mimosine. We have identified and sequenced a set of genes that are involved in mimosine degradation from the leucaena symbiont *Rhizobium* sp. TAL1145. The catabolism of mimosine by TAL1145 involves two major steps: the *mid* genes encode enzymes that break down

mimosine to 3-hydroxy-4-pyridone (HP), which is further degraded by a dioxygenase and a hydrolase, encoded by the *pydA* and *pydB* genes, respectively. We hypothesize that the expression of the bacterial HP-degrading genes *pydA* and *pydB* in leucaena will disrupt the biosynthesis of mimosine, generating transgenic leucaena plants with reduced mimosine content. We have previously reported the development of an in vitro propagation and *Agrobacterium*-mediated transformation protocol for leucaena. After developing synthetic *pydA* and *pydB* genes that were codon-optimized for preferred codon usage of leucaena, we constructed a translational fusion between *pydA* and *pydB* and cloned the single genes and the fusion gene (g3) in the vector pCAMBIA3201 to obtain four different binary plasmids: pCAM-pydA, pCAM-pydB, pCAM-g3, and pCAM-pydA+pydB. A total of 15 independently transformed lines of leucaena were obtained, and the presence, stable integration and expression of the transgenes in leucaena were confirmed through PCR, Southern blot and reverse transcriptase PCR. These transgenic plants are now being micropropagated for the determination of mimosine content and assessment of other physiological and biochemical properties."
(a) University of Hawaii at Manoa, MBBE

P46023 Transgenic maize expressing a balsam pear class I chitinase gene (Mcchit1) has enhanced resistance against Exserohilum turcicum
Zhao, Degang-presenter dgzhao@gzu.edu.cn(a) Zhu, Youyin (a)
"Northern corn leaf blight (NCLB), caused by the fungus *Exserohilum turcicum*, can cause yield losses in humid areas where maize is grown. The genetic engineering may provide an approach to enhance NCLB resistance. The objectives of this research were to develop transgenic maize expressing a chitinase gene and to test the transgenic lines against *E. turcicum* infection under greenhouse. A balsam pear class I chitinase gene (*Mcchit 1*) driven by the maize ubiquitin-1 (ubi1) promoter-intron was introduced into the maize inbred line, *B73*, by using *Agrobacterium*-mediated transformation. Nine transgenic lines were identified that expressed the chitinase transgene and exhibited enhanced resistance in greenhouse conditions. An excised leaf challenge assay was used to assess the functionality of the Mcchit1 protein in the transgenic plants. Necrotic lesions of untransformed control plant leaves appeared on day 3 after inoculation with *E. turcicum*, while transgenic maize plant expressing the *Mcchit 1* gene were visible only on day 5 after inoculation. The number of lesions was significantly fewer, and the size of lesions was significantly smaller compared to the controls. These results suggested that transgenic maize expressing a balsam pear class I chitinase gene exhibited enhanced resistance against *E. turcicum*. "
(a) Guizhou Key Laboratory of Agricultural Bioengineering, Guizhou University

P46024 Production of transgenic plants in *Okitsu wase* Satsuma mandarin (*Citrus unshiu* Marc.) transformed with *hptII* and *bar genes*
Kim, Jae-Hoon-presenter kimjh@cheju.ac.kr(a,b) Jin, Seong-Boem (b) Park, Jeong-Won (c) Boo, Kyung-Hwan (d) Bachchu, Md Adnan (a,b) Yun, Jeong-Hun (a,b) Lee, Hyo Yeon (a,c) Riu, key Zung (a,c)
"Satsuma mandarin (*Citrus unshiu* Marc.) is most commercially important and widely cultivated in Jeju, Korea. An efficient and stable transformation system is necessary for its molecular breeding, however this citrus species is known to be difficult to produce transgenic plants due to low efficiencies in selection of transgenic lines and regeneration of normal plants. We focused on the development of a screening system for transgenic lines of this citrus species using two different selectable marker genes. Hygromycin resistant gene was used for primary screening and bialaphos resistant gene for secondary screening. The hygromycin- and bialaphos-resistances were introduced into callus cells of *Okitsu wase* Satsuma mandarin (Citrus unshiu Marc.) with Agrobacteerium tumefaciences strain EHE101 containing the binary vector pGTV-HPT harboring hptII and bar genes. The transformed cells were successfully regenerated into plants via somatic embryogenesis onto MT medium containing 1.0 mg/L of GA3,. 20 ml/L of Coconut water, 10 ml/L of coumarin stock (1.46 mg/L) and 1.0 mg/L of NAA. The Integration of the genes into the citrus genome was confirmed by polymerase chain reaction (PCR) analysis with *hptII* and *bar*-pecific primers. "
(a) Faculty of Biotechnology College of Applied Science, Jeju National University (b) Gene & Material Bank for Citrus Breeding, Jeju National University (c) Research Institute for Subtropical Horticulture, Jeju National University (d) Department of Plant Science, University of Califonia, Davis

P46025 "A rice cleistogamous mutant, *superwoman1-cleistogamy*, is a useful tool for gene containment in GM rice."
Ohmori, Shinnosuke-presenter sohmori@affrc.go.jp(a) Itoh, Jun-Ichi (b) Yoshida, Hitoshi (a) Horigome, Ayako (b) Uchida, Eiji (a) Kimizu, Mayumi (a) Matsumura, Yoko (a) Kusaba, Makoto (b) Satoh, Hikaru (c) Nagato, Yasuo (b)
"Cleistogamy (non flower-opening pollination) is an efficient strategy for preventing gene flow from genetically modified (GM) crops. We identified a cleistogamous mutant of rice harbouring a missense mutation (the 45th residue isoleucine to threonine; I45T) in the class-B MADS-box gene *SUPERWOMAN1* (*SPW1*), which specifies the identities of stamens and lodicules that are equivalent to petals and have important function for rice flower-opening. In the mutant, designated *spw1-cls*, the stamens are normal, but the lodicules are transformed homeotically to lodicule-glume mosaic organs, thereby engendering cleistogamy. Since this mutation does not affect other agronomic traits (yield, fertility, grain quality and so on), it can be used in crosses to produce transgenic lines that do not cause environmental perturbation. Molecular analysis revealed that the reduced heterodimerization ability of $SPW1^{I45T}$ with its counterpart class-B proteins OsMADS2 and OsMADS4 caused altered lodicule identity. *spw1-cls* is the first useful mutant for practical gene containment in GM rice. Because grass flowers have a conserved structure, cleistogamy is possible in many cereals by engineering class-B floral homeotic genes and thereby inducing lodicule identity changes. Because of its nature of missense mutation, *spw1-cls* is predicted to show temperature-sensitive phenotype. Evaluation of the temperature-sensitivity will also be presented. "
(a) Hokuriku Research Center, National Agricultural Research Center (b) Graduate School of Agricultural and Life Sciences, University of Tokyo (c) Institute of Genetic Resources, Faculty of Agriculture, Kyushu University

P46026 Improving Soybean Oil for Food and Industrial Uses
Ulmasov, Tim-presenter tnoulm@monsanto.com(a) Voelker, Toni (a) Klopf, Gary (a) Heise, Jerry (a) Vallabh, Makadia (a) Ahmed, Gulam (a) Jenkinson, Jonathan (a) Jenkins, Dan (a) Crawford, Aaron (a) Mroczka, Andrew (a) Roberts, Peter (a) Schreckengost, Bill (a) Wagner, Nick (a) Hoffer, Paul (a) Keithly, Greg (a) Hess, Steve (a) Wilkes, Rick (a)
"Worldwide, soybean oil is one of the largest sources of vegetable oil in the human diet; in US it comprises two thirds of all edible oil consumption. However, the composition of soybean oil today is far from optimal from both functionality and nutritional value standpoints. Oxidative instability due to high levels of linoleic and linolenic acids prevents unmodified soybean oil from being used in many food applications. As a result, soybean oil used to be hydrogenated to increase its shelf life and stability in applications such as frying. Most industrialized countries have recognized the risks of trans-fat created during hydrogenation and have adopted policies strictly regulating its presence in food supply. Today, food companies scrambling to reformulate their products away from trans-fat are limited in their options to either more expensive/less abundant oils or oils rich in saturated fat. Using a combination of biotechnology (RNAi) and breeding, Monsanto was able to develop high-yielding soybean varieties containing increased (>70%) oleic acid, <3% of linolenic acid and significantly reduced (6-7% vs. 15% in the normal soybean) saturated fat. This profile results in significant functional improvement over low linolenic oils introduced in 2005, with a direct benefit to consumer in the form of lower saturated fat. This profile is ideal for heavy-duty applications such as commercial frying, providing an abundant supply of inexpensive and stable oil with saturated fat

content lower than in most vegetable oils, rivaled only by canola. This well-rounded oil will also find its use in industrial applications requiring improved physicochemical properties (enhanced stability, lubricity, cold flow), making it a preferred feedstock for biodiesel, lubricants and hydraulic fluids."
(a) Monsanto

P46027 "Antimicrobial activity on human pathogens of a protegrin 1 peptide (PG1), produced in tobacco chloroplast"
Alpuche-Solis, Angel G.-presenter alpuche@ipicyt.edu.mx(a) Patino-Rodriguez, Omar (a) Herrera-Diaz, Areli (b) Montes de Oca-Luna, Roberto (c) Castillo-Collazo, Rosalba (a) Korban, Schuyler S (d)
http://sipicyt.ipicyt.edu.mx/web/curriculaInvestigador_new2.Portada?p_cvePersonal=18
"The indiscriminate use of antibiotics has led to the selection of pathogenic microorganisms resistant to a wide range of antibiotics. In this context, antimicrobial peptides are an effective alternative for combating a large number of pathogenic microorganisms. The protegrins are antimicrobial peptides obtained from pig neutrophils, with antibacterial, antifungal and antiviral activities. The use of transgenic plants to produce proteins of pharmaceutical importance at low cost is an excellent alternative and recently it has been possible to introduce genes into the chloroplast genome increasing the production levels of recombinant proteins. We expressed the protegrin-1 (PG1) of porcine leukocytes encoded by a synthetic gene optimized for tobacco chloroplast expression and tested the antibacterial activity against *Klebsiella pneumoniae* and *Staphylococcus aureus*. A modified pKCZ vector with sites for homologous recombination was used for tobacco chloroplast transformation. The vector has the *Prrn* promoter and 5'UTR region of the protein 10 from phage T7 as well as the Protegrin-1 (PG1) gene fused to ubiquitin. Additionally, the selection gene spectinomycin (*aadA*) was included generating a bicistronic vector. Particle bombardment was used in tobacco young leaves and after two rounds of selection and 16-18 weeks, transplastomics shoots were obtained, which were transferred to a rooting media. Putative transplastomic lines were evaluated by RT-PCR to confirm the transgene transcription. The presence of ubiquitin-protegrin protein production was confirmed by Western-blot assay. The preliminary results suggest that the protegrin 1 produced in tobacco chloroplast is processed and functionally active against *K. pneumoniae*, and *S. aureus* as is shown in the *in vitro* assays. "
(a) IPICYT, Plant Biology Division (b) Munich University, Institute of Botany (c) UANL, Medicine Faculty (d) University of Illinois, Department of Natural Resources and Environmental Sciences

P46028 Improving *Agrobacterium tumefaciens*-mediated Gene Delivery to Understand and Improve Sorghum
Lemaux, Peggy G.-presenter lemauxpg@nature.berkeley.edu(a) Gurel, Songul (a) Gurel, Ekrem (a) Kaur, Rajvinder (a) Wong, Joshua (a) Miller, Tamara (a) Lee, Cindy (a) Linden, Katrina (a) Wu, Stephanie (a) Buchanan, Bob (a)
"Grain sorghum (*Sorghum bicolor* L. Moench), the fifth most important cereal worldwide, possesses multiple agronomic traits that make it a desirable future crop - the ability to survive abiotic stresses, like drought, flood, and high temperatures, while using far fewer inputs than other crops like maize. Sorghum grain is an important food source in semi-arid regions, and its grain, stems and sugars, in the case of sweet sorghum, can be used for biofuel production. Recently the entire sorghum genome was sequenced, opening the door to understanding gene function and to using that information to improve sorghum. Certain of our efforts have focused on developing efficient, reproducible transformation systems for sorghum. Multiple treatments were used to induce a stress response before *Agrobacterium* inoculation, which led to higher embryo survival rates, increased callus initiation and an ~8% transformation frequency of one sorghum variety, P898012. To reduce the time to generate and characterize transgenics, most recently we have used a short-season sorghum variety, N247, and achieved an 85% transient transformation efficiency; regeneration of these tissues is currently underway. Other efforts have focused on biochemical analyses to understand protein and starch digestibility of sorghum using *in vitro* pepsin, α-amylase and pancreatin digestion, Dumas combustion and gel-based and western blot protein analyses. Developing an understanding of starch and protein digestion will provide insights into digestibility, revealing features that can be used to improve sorghum for food, feed and biofuel use. "
(a) University Of California

P46029 *Helleborus* Micropropagation
Dan, Yinghui-presenter yinghui.dan@ialr.org(a,b) Pantazis, Christopher (a)
"Hellebores, the Genus *Helleborus* in the Family Ranunculaceae, are perennial flowering plants and increasing in commercial importance because of their evergreen nature, perennial winter and early spring blooming and use as a cut flower. It is also valued for their environmental adaptation such as frost, acid soil and deer-resistance. Hellebores have an annual market value of approximately one million dollars, with a potential market value of three million dollars in Virginia. However, the difficulty in sexual and asexual propagation of *Helleborus* species has highly restricted their production worldwide. Due to the problem, Virginia can only produce approximately 30% of the plants required for the one million dollar market. We have developed an efficient micropropagation system, which is composed of two major elements of 1) decontamination methods for the explant materials from field, greenhouse and growth room and 2) micropropagation protocols for *Helleborus* species. This system can potentially enable a massive and rapid production of hellebores to meet the current and future market demands in Virginia. "
(a) Institute for Advanced Learning & Research (b) Departments of Horticulture and Forestry, Virginia Polytechnic Institute And State University

SESSION P47 – PLANT HERBIVORE INTERACTIONS

P47001 Differential gene expression in buffalograss cultivars infested with chinch bugs
Twigg, Paul G-presenter twiggp@unk.edu(a) Langenfeld, Katie A (a) Barber, Anna K (a) Nuxoll, Austin S (a) Heng-Moss, Tiffany M (b) Shearman, Robert C (b)
"Buffalograss (*Buchloe dactyloides*) is a warm season grass native to the central U.S. It is low growing and quite drought resistant. These characteristics make buffalograss appealing for home use. It however has problems, which make wide adoption difficult. The color and texture of buffalograss are not quite the same as bluegrass, which most homeowners prefer. The other more significant problem is that most varieties are susceptible to chinch bug infestation. Susceptible cultivars will quickly turn brown when infested. Breeding efforts have largely focused on the appearance of the grass without directly considering insect susceptibility. Our efforts have been focused on helping to combat this problem using subtractive hybridization to identify candidate genes involved in tolerance of some buffalograss cultivars to chinch bugs (Prestige) and susceptibility in others (378). Once identified, we have measured the levels of some transcripts using qRT-PCR. We will present and analyze our findings for these cultivars at various damage levels. This project was supported by NIH grant P20 RR016469 from the BRIN program of the National Center for Research Resources, a grant from the United States Golf Association, and a grant from the Nebraska Research Initiative."
(a) University Of Nebraska-Kearney (b) University Of Nebraska - Lincoln

P47002 Soybean responses to soybean aphids assessed using subtractive hybridization
Barber, Anna K-presenter barberak@unk.edu(a) Twigg, Paul G (a) Heng-Moss, Tiffany M (b) Reese, John C (c)
"Aphids are perhaps the most damaging group of agricultural pests worldwide. They transmit diseases, withdraw phloem sap, and can elicit drastic responses in the plant. Recently, the soybean aphid (*Aphis glycines*) has been introduced the to Midwest, and represents a growing risk to soybean production. The development of insecticide resistance is common, and plant resistance based on antibiotic factors is often short-lived. In our study, we attempt to address this problem by examining soybeans tolerant to aphid infestation. Tolerance has a much broader genetic basis than resistance and is therefore more durable. We infested soybean plants tolerant to soybean aphid and in parallel another variety that was susceptible. For each, total RNA and mRNA were extracted to perform subtractive hybridization. From the resulting subtracted libraries, we sequenced 100 clones each to get an overview of the differences in the reactions of the two genotypes at the transcript level. The sequences were submitted to GenBank through a BLAST search to find putative identities and functions. These were further classified into functional groups. We will present and discuss the results of this analysis. This project was supported by NIH grant P20 RR016469 from the BRIN program of the National Center for Research Resources and a grant from North Central Soybean Research Project."
(a) University Of Nebraska-Kearney (b) University Of Nebraska - Lincoln (c) Kansas State University

P47003 Activity profiling of green leafy volatiles
Engelberth, Juergen-presenter jurgen.engelberth@utsa.edu(a)
"Green leafy volatiles (GLVs) which are generally emitted by plants in response to mechanical damage and insect herbivory have been found in recent years to play an important role in inter- and intra-plant signaling. Plants receiving these volatile signals generally appear to prime their defenses resulting in a stronger and faster response when under actual attack. To further study the effects of GLVs on plants we employed analytical and molecular techniques to gain further insights into the regulatory networks activated by these compounds. A structure/function analysis of various GLVs and related compounds in corn revealed the structural requirements for GLV activity measured as jasmonic acid accumulation. In contrast we also found that small α, β-unsaturated carbonyls like acrolein do significantly inhibit JA biosynthesis induced by mechanical wounding, insect elicitor treatment, and GLV exposure. A comparative analysis of GLV responses in different plant species on a metabolic level showed that a variety of plants respond to this treatment by rapid changes is the free fatty acid composition. Additionally, we studied early responses to GLVs on a genomic level by microarray analysis. While the results confirmed previous findings with regard to GLV-induced defense gene expression, several new groups of genes affected by this treatment were detected comprising a distinct set of transcription factors, genes involved in lipid- and fatty acid signaling, as well as genes involved in direct and indirect defense responses. The results will provide new insights into GLV-induced signaling processes and will be discussed in the context of plant defense signaling, gene networks, and the consequences for defense priming.αβ"
(a) UTSA, Dept. Biology

P47004 How does the aphid *Myzus persicae* respond to feeding on droughted *Brassica nigra*: a transcriptomics analysis
Vickers, Laura H-presenter lhv448@bham.ac.uk(a) Bale, Jeff S (a) Pritchard, Jeremy (a)
"Aphids are major global pests; the damage they cause can be direct via their specialist phloem feeding mechanism or indirect in their ability to transmit plant viruses. The response of the plant-aphid interaction to increased drought expected under climate change is unknown. Some predictions suggest that aphids will perform better due to the increased concentration of amino acids within the phloem. Others predict that the aphids will perform worse as they are faced with increased need for osmoregulation as phloem solutes increase. To understand the aphid response to droughted plants at the genetic level, the gene expression of *Myzus persicae* exposed to two different drought regimes was analysed on the host plant *Brassica nigra* using microarrays. The physiological state of the plants was quantified as fresh to dry weight ratios, water potential and amino acid concentrations. The feeding behaviour and honeydew production of the aphids was recorded. Changes in aphid gene expression are interpreted within the context of the changes in the physiological status of the plant. This information will help to identify aphid's mechanisms of feeding under drought."
(a) University of Birmingham

P47005 Interactions between phytochrome and jasmonate signaling in Arabidopsis. Shedding light on the dilemma of plants.
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http://www.ifeva.edu.ar/en/staff/ballare.htm
"Previous studies have shown that plants down-regulate the expression of costly chemical defenses against herbivore insects when their phytochrome system signals an increased risk of competition with neighboring plants. We demonstrated that this effect is not a simple by-product of increased allocation to shade-avoidance responses, since the auxin-deficient *sav3* mutant, which fails to induce morphological responses to phytochrome inactivation, also responded to far-red radiation (FR) with an attenuated defense phenotype (Moreno et al. 2009, PNAS). In those experiments, down-regulation of defense by FR correlated with a reduced sensitivity to methyl jasmonate. In the experiments reported here we found that FR did not affect the expression of *JAR1*, which encodes an enzyme that generates the jasmonyl-isoleucine conjugate, suggesting that FR does not act by modulating the formation of bioactive jasmonates. FR down-regulated several genes involved in the jasmonate response, including genes coding for transcription factors such as ERF1 and targets such as HEL. Interestingly, however, FR increased the expression of *JAZ10.4*, a splice variant of JAZ10 that recent work has identified as being resistant to jasmonate-induced degradation (Chung & Howe 2009, Plant Cell). Because JAZ proteins are repressors of the jasmonate response pathway, we postulate that up-regulation of *JAZ10.4* by FR provides a plausible molecular explanation for the observed attenuation of jasmonate sensitivity. Modulation of jasmonate sensitivity by phytochrome-perceived light signals appears to be a critical element in the mechanisms that allow plants to generate growth and defense strategies that optimize resource utilization in natural environments."
(a) CONICET and University of Buenos Aires (b) Michigan State University

P47006 Elevated carbon dioxide alters phytohormone signaling in *Glycine max*
Casteel, Clare L.-presenter ccastee2@igb.uiuc.edu(a,b) Berenbaum, May R. (b,c) DeLucia, Evan H. (a,b)
"Plants encounter countless challenges in the environment including insect and pathogen attack. Perception of attack and elicitation of defenses is mediated largely by phytohormones. Human activities are increasing the concentration of CO_2, potentially altering the relationship between plants and herbivores. Based on previous studies in soybeans, elevated CO_2 is hypothesized to modulate plant resistance through alterations in hormone signaling and defenses against herbivorous pests. To determine the impact of elevated CO_2 exposure on plant-insect interactions in soybeans, the magnitude and timing of three major hormone signaling pathways (jasmonic acid [JA], salicylic acid [SA], Ethylene [ET]) and related defenses were examined in open field environments under elevated CO_2 after a feeding episode by Japanese Beetle [JB]. Elevated CO_2 decreased the induction of JA and ET related transcripts (*lox7*, *aos*, *hpl* and *acc*). Accumulation of defense related transcripts and metabolites (polyphenol oxidase; PPO, protease inhibitors; PIs) initially increased to a higher level after Japanese beetle attack in elevated CO_2 compared to ambient grown plants, but over

time decreased to levels lower then ambient grown plants. In addition, elevated CO_2 increased the accumulation of SA in soybeans. SA and JA are known to have an antagonistic relationship in other plants, and may explain the reduction in JA related transcripts. The modulation of JA and ET signaling transcripts and metabolites resulted in lowered plant chemical defense over time and can explain increases in insect damage observed in previous soybean studies."
(a) Dept. of Plant Biology, University Of Illinois - Champaign Urbana (b) Institute of Genomic Biology, University Of Illinois - Champaign Urbana (c) Dept.of Entomology, University Of Illinois - Champaign Urbana

P47007 Aphid-induced defense responses in *Arabidopsis thaliana*
Martin, De Vos (a) Georg, Jander-presenter gj32@cornell.edu(a)
"Unlike Lepidoptera and other chewing insects, phloem-feeding aphids cause relatively little visible damage to the plant tissue on which they are feeding. Nevertheless, plants are able to recognize aphid infestation and mount appropriate defense responses. In the model plant *Arabidopsis thaliana* and other crucifers, glucosinolates, a diverse class of defensive secondary metabolites, provide a chemical protection against *Myzus persicae* (green peach aphid) and other herbivores. Bioassays with *A. thaliana* mutants show that elevated levels of tryptophan-derived indole glucosinolates in the phloem reduce *M. persicae* fecundity. Indole glucosinolate breakdown within the aphids produces a variety of products, some of which are strongly aphid-deterrent. In response to aphid feeding on both *A. thaliana* and *Brassica oleracea* (cabbage), there is specific accumulation of 4-methoxyindol-3-ylmethylglucosinolate, which is also the most aphid-deterrent glucosinolate in artificial diet experiments. DNA microarray experiments show that *M. persicae* feeding and infiltration of aphid saliva samples into *A. thaliana* leaves induce similar gene expression changes, which include upregulation of known indole glucosinolate biosynthesis genes. Further bioassays with fractionated aphid saliva samples indicate that a small protein or peptide is recognized by *A. thaliana* to mount a defense response. Other *M. persicae* salivary components appear to counteract these plant resistance mechanisms."
(a) Boyce Thompson Institute

P47008 Diatraea saccharalis herbivory on sugarcane plants induces a pathogen-inducible Barwin gene
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"In sugarcane fields, colonization of the stalk by opportunistic fungi usually occurs after the caterpillar Diatraea saccharalis attacks sugarcane. Plants respond to insect attack by inducing and accumulating a large set of defense proteins. In a search for defense-related proteins in sugarcane, two homologues of a barley wound-inducible protein (barwin) were identified by in silico analysis, and were designated sugarwin1 and 2 (sugarcane wound-inducible proteins). Using quantitative real-time polymerase chain reaction for monitoring of transcripts, we showed that the induction of sugarwin transcripts is late induced, restricted to the site of damage and occurs in response to mechanical wounding, D. saccharalis, and methyl jasmonate treatment. Subcellular localization using green fluorescent fusion protein indicates that SUGARWINS are secreted proteins. Recombinant SUGARWIN1 protein incorporated into D. saccharalis diet, showed no effect on insect development. BARWIN proteins are wound- and pathogen-inducible proteins that possess in vitro antipathogenic activities against fungi. Multiple sequence alignment of BARWIN proteins from sugarcane and from other mono and dicotiledoneous species reveals high similarity, suggesting that their function is conserved among species. This is the first report of a BARWIN-like protein inducible by herbivory. Our results show that SUGARWIN protein has no activity against D. saccharalis. We hypothesized that sugarwin gene induction by herbivory is part of a concerted strategy against opportunistic pathogens that are commonly found in the site of caterpillars' attack. "
(a) University of Sao Paulo

P47009 Arabidopsis CPKs are key mediators of gene regulation in defense responses to insect herbivory
Kanchiswamy, Chidananda N-presenter chidubt@me.com(a,b) Takahashi, Hirotaka (e) Maffei, Massimo E (d) Bossi, Simone (d) Boland, Wilhelm (c) Takabayashi, Juni (b) Sawasaki, Tatsuya (e) Arimura, Gen-ichiro (a,b)
"Plant Ca2+ signals are involved in a wide array of intracellular signalling pathways after pest invasion. Ca2+-binding sensory proteins such as Ca2+-dependent protein kinases (CPKs) have been predicted to mediate the signaling following Ca2+ influx after insect herbivory. However, until now this prediction was not testable. To investigate the roles CPKs play in a herbivore response-signaling pathway, we screened the characteristics of Arabidopsis CPK mutants damaged by a feeding generalist herbivore, Spodoptera littoralis. Following insect attack, the cpk3 and cpk13 mutants showed lower transcript levels of plant defensin gene PDF1.2 compared to wild-type plants. The CPK cascade was not directly linked to the herbivory-induced signaling pathways that were mediated by defense-related phytohormones such as jasmonic acid. CPK3 was also suggested to be involved in a negative feedback regulation of the cytosolic Ca2+ levels after herbivory and wounding damage. In vitro kinase assays of the nucleic/cytosolic CPK3 proteins with a suite of substrates demonstrated that the protein phosphorylates five transcription factors (including ERF1, WRKY and HSF22 [HSFB2a]) and ATL2 that functions as an E3 ubiquitin ligase (a ubiquitination enzyme) in posttranslational modulations. Other nucleic/cytosolic CPK13 proteins phosphorylated only one of them (HSF22 [HSFB2a]). These results reveal a novel, intricate array of signal transduction networks with ecological significance for plant-insect interactions."
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P47010 Elucidation of the LAP-A-dependent regulatory network involved in the late branch of wounding in tomato.
Smith, Melissa A-presenter msmit024@student.ucr.edu(a) Walling, Linda L (a) Fowler, Jonathan H (a) Girke, Thomas (a)
"Recent studies have shown that leucine aminopeptidase A (LAP-A) plays a key role in insect deterrence in tomato. Previous transgenic studies have shown that LapA-overexpressing (*LapA-OX*) plants are more resistant to insect feeding while LapA-silenced (*LapA-SI*) plants are more susceptible to insect feeding compared to wild type (WT). These studies also revealed that LAP-A has a regulatory role in the late branch of wounding, which is involved in direct insect deterrence. However, the mechanism by which LAP-A functions in the pathway is currently unknown. In order to further characterize LAP-A's role during wounding, cDNA microarray analysis was performed using TIGR 10K (version 3) potato cDNA microarrays. Transcript levels were determined from WT and *LapA-SI* lines 0, 1, and 8 hours after wounding. A subset of genes appears to be differentially regulated in *LapA-SI* versus WT plants prior to wounding. These genes are being investigated as molecular markers for LAP-A's regulatory role in tomato. After wounding, *LapA-SI* and WT lines have similar temporal and spatial changes in gene expression during these early wounding timepoints. Results from the microarray analysis have provided insight into specific metabolic pathways regulated in WT tomato leaves in response to wounding. "
(a) Botany and Plant Sciences, University Of California, Riverside

P47011 Analyses of plant resistance to thrips attack using Arabidopsis and Chinese cabbage

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"Plants are exposed to many types of abiotic or biotic stresses. Many analyses have performed to explain the mechanism of plant response to these stresses. However, the mechanism of plant response to feeding damage has not been well understood. We analyzed the interaction between Arabidopsis/Brassica crop, Chinese cabbage (*Brassica rapa*) and western flower thrips (*Frankliniella occidentalis*), which is one of the most serious insect pests. Thrips is cell content feeding insect that penetrate single cells with stylet to suck out the contents. In addition, thrips transmit the virus from plant to plant. We focused on the function of the immunity-related plant hormones jasmonate (JA), ethylene (ET), and salicylic acid (SA) on plant resistance to thrips feeding. We also present the development of B. rapa full-length cDNA and EST clones. We obtained about 5,000 independent clones at this time. Most of these clones have higher sequence homology to Arabidopsis genes. We analyzed biotic stress response in *B. rapa* using these clones."

(a) RIKEN BioResource Center (b) Research Institute for Biological Sciences (c) National Agricultural Research Center (d) National Institute of Vegetable and Tea Science (e) National Institute for Agro-Environmental Sciences (f) National Institute of Agrobiological Sciences

P47012 QTLs FOR COMPENSATORY RESPONSES FOLLOWING MAMMALIAN HERBIVORY

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"Plants have evolved numerous traits that mitigate damage caused by pests and diseases. Although insect pests cause damage (usually on the order of 10% tissue loss), the damage caused by a single bout of mammalian herbivory is comparatively devastating, as a plant can lose ≥90% of its aboveground biomass. Nonetheless, many plants can tolerate or even take advantage of such high levels of herbivory (e.g., the classic example of overcompensation, increased fitness, following ungulate herbivory in Ipomopsis aggregata). This study aims to identify the QTL regions that are correlated for compensatory responses following herbivory. One hundred F8 derived recombinant inbred lines (RILs) developed from a cross of Columbia x Landsberg erecta were used for analysis. The RILs were grown in the greenhouse during Spring 2007 and Fall 2008. The plants were clipped at ground level at bolting stage to simulate mammalian herbivory. Numbers of siliques as a measure of fitness were recorded. Using composite and multiple interval QTL mapping strategies, three QTLs located on chromosome 1, 4 and 5 were found (experiment-wise threshold α=0.05) responsible for compensatory responses to mammalian herbivory. In a separate microarray experiment a total of 107 genes were differentially expressed between plants, simulated with or without apical damage. Combining QTL and microarray data we found at least one potential candidate gene (At5g35790, Glucose 6 phosphate dehydrogenase 1) that could play an important role in response to herbivore damage. Based on initial mutant line analyses, other candidate genes eg., cytoplasmic invertase (At1g35580.1) may also contribute to compensatory responses following herbivory."

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P47013 How do endophytic fungi affect leafcutter ant attack?

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"Leafcutter ants are among the dominant herbivores in Neotropical forest and grassland systems. They sit within a complex web of mutualistic and antagonistic interactions, chief of which is the mutualism with the fungi which make up their fungus gardens. The plants which they attack also have mutualistic and antagonistic interactions, notably with endophytic fungi. In fragments of Atlantic Forest in southeastern Brazil, we are exploring the interactions between leafcutter ants and their symbionts, on the one hand, and plants and their endophytic symbionts on the other. We have identified endophytic fungi which could interact in an antagonistic fashion with the ants' symbiont fungus, specifically in cut leaf fragments being transported to the nest. We raise the possibility that this is a form of defence against ant herbivory on the part of the plant and propose mechanisms by which the ants could overcome this defence, or otherwise respond. "

(a) Federal University of Vicosa

P47014 Botanical characteristics of the rosette gall in *Aster scaber*

Lee, Doseung (a,c) Ha, Chan Man (e) Boo, Kyung Hwan (a,c) Ko, Seung Hee (a) Jeon, Gyeong Lyong (a) Kim, Jae Hoon (c,d) Lim, Pyung Ok (b,c) Lee, Hyo Yeon (c,d) Riu, Key Zung-presenter kzriu@cheju.ac.kr(c,d)

"The plant gall occurring in *A. scaber* is interesting because they grew to a miniature plant showing a unique well-organized rosette-like morphology (rosette gall) although it shows extreme dwarfism. The normal sizes of mature rosette galls were 0.3 - 2.0 cm in diameter and 0.3 - 1.0 cm in height, respectively. One of the most distinctive feature of these secondary ectopic mini-plants was to develope even a floral organ unlike other ordinary plant galls forming only an amorphous gall tissue. The typical rosette galls of *A. scaber* did not develope petiole, internodal stem, inflorescence or root. However, some of the rosette galls developed petioles and/or floral axes. Furthermore, the rosette galls showed sometimes *de novo* development of roots even though this was rarely observed under the open field condition. The rosette galls were usually formed on a leaf surface of the host plant. In this case, the rosette galls are developed from the non-meristemic cells between leaf veins unlike ordinary plant organs which differentiated mostly from the meristemic cells near the veins. In addition, the rosette galls could be developed from any organ of the host plant including leaf (adaxial and/or abaxial surface), petiole, stem, node, shoot apex and even root under the natural environment. The developments of rosette galls from the non-merstemic cells of various differentiated organs of *A. scaber* provided direct evidences for the intrinsic totipotency of the host plant cells regardless of their origin and degree of differentiation. "

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P47015 The insect inducing the rosette gall in *Aster scaber*

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"The rosette gall of *A. scaber* appeared to be induced by an insect which was identified as *Dasineura asteriae* Shinji (Diptera Cecidomyiidae). Under the natural condition of Jeju, Korea the adult insects mated and layed eggs from April to May when the radical leaves of *A. scaber* emerged from the soil surface. The female layed eggs on the surface of young leaves of the host plant. The egg hatched in 2-4 days, and the larva moved around and settled down at a certain position on the surface of plant organs where the rosette gall was later developed. Thereafter the larva made a chamber within the gall tissue of the rosette gall and lived in the chamber. The larval chamber became surrounded by ectopic leaflets with growth of the rosette gall, and later the chamber developed an additional organ resembling floral bud inside of the rosette gall leaflets. Two or more larvae were

often observed in a single rosette gall, and in this case each larva occupied a floral bud. The larva resided within the rosette gall throughout its larval stages. After the rosette gall aged to senescence in the late of autumn (November), the larva came out of the rosette gall and entered into the underground soil. The larva developed to a pupa and hibernated during winter time in the soil until next spring. "
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P47016 Process of the rosette gall development in *Aster scaber*
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"The mature rosette gall of *A. scaber* divided into three basic structures which consisted of ectopic leaves, floral organs and hemispherical gall at the basement. The rosette gall initiated with forming a tiny gall tissue on the surface of any organ in *A. scaber*. In the second phase, the ectopic leaves were differentiated from the gall tissue. In the third phase, the ectopic leaves grew and were organized into a rosette shape miniature plant. At last a floral organ was developed from the center of the rosette gall. The overall process of the rosette gall development included the dedifferentiation of a gall from the differentiated organs of the mother plant, the redifferentiation of ectopic leaves from the dedifferentiated gall, the growth and organization of the ectopic leaves to form a rosette shape, and the floral development from the mini-plants. The development of rosette galls appeared to be precisely regulated by the insect of *D. asteriae*. At the beginning of the rosette gall development, the initial gall was formed from the point of plant surface where insect larva settled down, not from the point of oviposition. Therefore the gall initiation appeared to be induced by the insect larva, not by egg or adult female. After the gall induction the larva stayed inside the rosette gall throughout the development of ectopic leaves and floral organs. The extreme dwarfism in the typical rosette galls implied that the larva inhibited the developments of stems and petioles as well as auxiliary buds and roots."
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P47017 Genes differentially expressed in the rosette gall in *Aster scaber*
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"To understand rosette gall development in *A. scaber* in relation with hormonal controls, the genes involved in the metabolism of phytohormones were cloned and their expressions were examined. Three genes of *NIT*, *IPT* and *GA3ox* encoding key step enzymes in the synthesis of auxin, cytokinin and gibberellin, respectively, were cloned. *NIT* was highly expressed in the inner and outer leaves of the rosette gall and its expression in the gall tissue was similar to mother leaf. In case of *IPT*, it was highly expressed not only in the inner and outer leaves of the rosette gall but also in the gall tissue by the similar level. In contrast to *NIT* and *IPT*, *GA3ox* was suppressed in the inner leaf of rosette gall while its expression in outer leaf and gall tissue were similar to mother leaf.On the other hand, an unknown gene denoted as '*GAS*' was highly expressed in the inner leaf of rosette gall whereas the expressions in outer leaf and gall tissue were similar to mother leaf. The length of this gene was relatively short, 450bp (150 amino acid), and had no introns. Therefore, *GAS* was supposed to play as a regulatory element in the rosette gall development, probably *via* the down-regulation of *GA3ox* because the expression of *GA3ox* was coincidently suppressed with the expression of *GAS*, and *vice versa*."
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P47018 A determinant role for the N-terminal trunk of plant cystatins interacting with herbivorous pest cysteine proteases
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"Cystatins regulate cysteine proteases in various physiological processes, including storage protein turnover, organogenesis, programmed cell death and defense against herbivorous pests. At the structural level, recent evolutionary data suggested a significant role for the N-terminal trunk of plant cystatins during the protease inhibitory process. Here we engineered N-terminal truncated variants of plant cystatins from different evolutionary clades to assess the impact of the N-terminal trunk on the inhibition of extracellular cysteine proteases from herbivorous arthropods and parasitic nematodes. In agreement with current structural models for mammalian cystatins, truncated forms of the cystatins lacking 15-20 amino acids at the N terminus systematically exhibited low inhibitory activity against the proteases tested, as deduced from $K_{i(app)}$ values for the different protease:inhibitor complexes increased by two or three orders of magnitude compared to wild-type cystatins. As inferred *in silico* from 3-D structural models, deletion of the N-terminal trunk had little impact on both the protein's central core and spatial orientation of the two inhibitory loops, which suggests instead a specific interaction with the target protease as documented earlier for mammalian cystatins. These observations confirm, overall, a central role for the N-terminal trunk of plant cystatins interacting with herbivorous pest cysteine proteases. They also point to the potential of this structural element as a useful target for the engineering of improved cystatin variants, in line with the previously reported occurrence of hypervariable amino acid sites in the N-terminal region strongly impacting cysteine protease inhibition."
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SESSION P48 – PLANT PATHOGEN INTERACTIONS

P48001 "NIK-mediated antiviral signaling, a novel layer of innate plant defenses suppressed by the geminivirus nuclear shuttle protein"
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"The NSP-interacting kinase (NIK) receptor-mediated antiviral signaling has been identified recently as a virulence target of the geminivirus nuclear shuttle protein (NSP). However, the NIK-NSP interaction does not fit into the elicitor-receptor model of resistance and hence the molecular mechanism that links this antiviral response to receptor activation remains obscure. Here we identified a ribosomal protein, rpL10, as a specific partner and substrate of NIK that functions as the immediate downstream effector of NIK-mediated signaling. Phosphorylation of cytosolic rpL10 by NIK redirects the protein to the nucleus where it may act to modulate viral infection. While ectopic expression of normal NIK or a hyperactive NIK mutant increases the accumulation of phosphorylated rpL10 within the nuclei, an inactive NIK mutant fails to redirect the protein to the nuclei of co-transfected cells. Likewise a mutant rpL10 defective for NIK phosphorylation is not redirected to the nucleus. Furthermore, loss of rpL10 function enhances susceptibility to geminivirus infection, resembling the phenotype of nik null alleles. We also provide evidence that geminivirus infection directly interferes with NIK-mediated nuclear relocalization of rpL10 as a counterdefensive measure. However, the NIK-mediated defense signaling

neither activates RNA silencing nor promotes a hypersensitive response but inhibits plant growth and development. Therefore, the NIK antiviral signaling may represent a novel layer of the innate plant defenses that has the potential to affect basic compatibility functions. Although geminivirus NSP overcomes this layer of defense in Arabidopsis, the NIK1-mediated signaling response may be involved in restricting the host range of other viruses."
(a) Universidade Federal de Vicosa

P48002 "A putative repressor interacts with ORA59, a transcription factor integrating JA and ethylene signaling"
Korbes, Ana Paula-presenter a.p.korbes@biology.leidenuniv.nl(a) Memelink, Johan (a)
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"Plant defense against pathogens and herbivores depends on the action of several endogenously produced hormones, including jasmonic acid (JA) and ethylene. In certain defense responses, JA and ethylene signaling pathways synergize to activate a specific set of defense genes, including PDF1.2 and HEL. The AP2-domain transcription factor ORA59 acts as the integrator of the JA and ethylene signalling pathways (Pre et al. 2008). Experimental results suggest that JA and ethylene affect the activity of ORA59 at the protein level via mechanisms that are unknown. To understand ORA59 regulation, we set out to identify and functional characterize ORA59-interacting proteins. ZFAR1 was identified as a putative interacting protein from a yeast two-hybrid screening. Expression assays in Arabidopsis cell suspension protoplasts suggested that ZFAR1 acts as a repressor of ORA59 activity. Plants overexpressing ZFAR1 did not show significant differences in the expression of PDF1.2. Double knockout zfar1 zfar2 plants also did not show significant difference in the expression of PDF1.2 but differences in the expression pattern of HEL were observed. Future studies will focus on determining whether and how ZFAR1 affects ORA59 activity in planta. Pre et al. (2008) Plant Physiology 147:1347-1357 "
(a) Leiden University

P48003 Functional studies of the bipartite begomovirus *CP* promoter
Sunter, Garry-presenter garry.sunter@utsa.edu(a) Lacatus, Gabriela (b)
"The AL2 protein of bipartite geminiviruses of the genus *Begomovirus* activates transcription of late viral genes through two viral sequences. One sequence is located 60-125bp upstream of the transcription start site for the coat protein (*CP*) gene and is required for AL2-mediated activation in mesophyll cells. A second element is located between 1.2-1.5kbp upstream of the *CP* gene and is required for AL2-mediated derepression in phloem. Binding assays indicate that sequences mediating repression and activation of the *Tomato golden mosaic virus* (TGMV) and *Cabbage leaf curl virus* (CaLCuV) *CP* promoter bind different nuclear factors common to tobacco, spinach and tomato. However, chromatin immunoprecipitation demonstrates that TGMV AL2 can interact with both sequences independently, but AL2 does not specifically bind either sequence directly. Thus, AL2 is likely targeted to responsive promoters via interaction with a host factor(s). A plant specific DNA binding protein, PEAPOD2 (PPD2), was cloned from *Arabidopsis* using a yeast one hybrid screen. PPD2 specifically binds to sequences known to mediate activation of the *CP* promoter of TGMV and CaLCuV in mesophyll cells. In vivo fluorescence microscopy demonstrates that PPD2 is associated with the nucleus, as expected for a transcriptional regulator, but is not capable of activating transcription directly. Thus, geminivirus AL2 protein and PPD2 likely form a complex at the *CP* promoter to activate *CP* gene expression. However, PPD2 does not bind sequences required for AL2-mediated derepression in phloem tissue. This is consistent with a model in which AL2 interacts with different components of the cellular transcription machinery that bind viral sequences important for repression and activation of begomovirus *CP* promoters."
(a) University of Texas at San Antonio, Department of Biology (b) Greheey Children's Cancer Research Institute, UTHSCSA

P48004 The role of protease inhibitors in the hypersensitive response
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"Pathogens that escape the plant's initial response to pathogen associated molecular patterns deploy effectors that contribute to pathogen virulence. Effector-triggered immunity is classically encoded by R proteins whose activation results in the hypersensitive response (HR). RPM1 is a typical NBS-LRR R protein that recognises the bacterial effectors AvrRpm1 and AvrB. Here we describe characterisation of RIN12, a protease inhibitor that interact with RPM1 in yeast 2-hybrid and whose mis-expression modifies the HR. RIN12 overexpression causes delayed HR and enhanced bacterial growth; reduction in RIN12 leads to faster HR upon elicitation with AvrRpm1-expressing bacteria. The RIN12 protein shows structural homology to serine protease inhibitors, although the combining loop unusually contains a proline. *In vitro* expressed RIN12 inhibits subtilisin-type serine proteases Mutation of the combining loop abolishes both subtilisin activity and RPM1 interaction in yeast. These data are consistent with a 'guard hypothesis' model in which bacterial effector activity relieves RIN12 association with RPM1 enabling activation of the RPM1 signalling network. Paradoxically, RIN12 also exhibits bacterial defensin activity, which appears specific to bacteria that employ a type III secretion system. This RIN12 defensin function is independent from the protease inhibitory activity. *RIN12* transcripts are induced by PAMPs. These data suggest RIN12 may have evolved as part of a secondary host pathogen-triggered immune response to overcome effector-triggered susceptibility. The effector-triggered immunity role of RIN12 possibly evolved as a secondary function in R protein signalling. "
(a) University of Exeter

P48005 Artificial MicroRNAs Highly Accessible to Targets Confer Efficient Virus Resistance in Plants
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"Short-hairpin RNAs based on microRNA (miRNA) precursors to express the artificial miRNAs (amiRNAs) can specifically induce gene silencing and confer virus resistance in plants. The efficacy of RNA silencing depends not only on the nature of amiRNAs but also on the local structures of the target mRNAs. However, the lack of tools to accurately and reliably predict secondary structures within long RNAs makes it very hard to predict the secondary structures of a viral genome RNA in the natural infection conditions in vivo. In this study, we used an experimental approach to dissect how the endogenous silencing machinery acts on the 3'untranslated region (UTR) of the Cucumber mosaic virus (CMV) genome. Transiently expressed 3'UTR RNAs were degraded by site-specific cleavage. By comparing the natural cleavage hotspots within the 3'UTR of the CMV-infected wild-type Arabidopsis to those of the triple dcl2/3/4 mutant, we acquired true small RNA programmed RNA-induced silencing complex (siRISC)-mediated cleavage sites to design valid amiRNAs. We showed that the tRNA-like structure within the 3'UTR impeded target site access and restricted amiRNARISC-mediated cleavage of the target viral RNA. Moreover, target recognition in the less-structured area also influenced siRISC catalysis, thereby conferring different degrees of resistance to CMV infection. Transgenic plants expressing the designed amiRNAs that target the putative RISC accessible target sites conferred high resistance to the CMV challenge from both CMV subgroup strains. Our work suggests that the experimental approach is credible for studying the course of RISC target recognition to engineer effective gene silencing and virus resistance in plants by amiRNAs."
(a) Institute of Microbiology, Chinese Academy of Sciences

P48006 Molecular-genetic characterization of the Arabidopsis *SFD1*-encoded dihydroxyacetone phosphate reductase's role in plant lipid metabolism and systemic acquired resistance

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"Diseases result in crop losses that exceed $10 billion per year in the US. Furthermore, our health and environment are endangered due to the increasing dependence on toxic chemicals for protecting plants. Novel targets and alternative strategies that exploit the inherent ability of plants to control diseases are needed for controlling plant damage. Systemic acquired resistance (SAR) is a defense mechanism that confers resistance against a broad spectrum of pathogens. The activation of SAR requires prior localized infection with a pathogen and the translocation of a factor from the pathogen-inoculated organ to the other organs where SAR is activated. The *Arabidopsis thaliana SFD1* (*suppressor of fatty acid desaturase deficiency1*)gene, which encodes a dihydroxyacetone phosphate (DHAP) reductase is required for the translocation of the SAR signal and for glycerolipid synthesis in the plastids. The SFD1 protein catalyzes the interconversion of DHAP and glycerol-3-phosphate. Since composition of plastid, but not the ER synthesized lipids is affected in the *sfd1* mutant, SFD1 protein may most likely function in the plastids. The focus of this study is to determine (i) if SFD1 is a chloroplast targeted protein, and (ii) if SFD1's DHAP reductase activity is required for SFD1's involvement in lipid metabolism and SAR. Recombinant versions of the *SFD1* gene carrying mis-sense mutations in key nucleotides have been constructed and their impact on the DHAP reductase activity, Arabidopsis lipid metabolism and SAR are being investigated. Progress on these experiments will be presented."

(a) University of North Texas

P48007 Conserved threonines residues within the A-loop of the receptor NIK differentially regulate the kinase function required for antiviral signaling

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"NSP-interacting kinase (NIK1) is a receptor-like kinase identified as a virulence target of the begomovirus nuclear shuttle protein (NSP). We found that NIK1 exhibits a stepwise pattern of phosphorylation within the A-loop with distinct roles for different threonine residues. Mutations at Thr-474 or Thr-468 impaired autophosphorylation and were defective for kinase activation. In contrast, a mutation at Thr-469 did not impact autophosphorylation and increased substrate phosphorylation, suggesting an inhibitory role for Thr-469 in kinase function. To dissect the functional significance of these results, we used NSP-expressing virus infection as a mechanism to interfere with wild type and mutant NIK1 action in plants. The NIK1 knockout mutant shows enhanced susceptibility to virus infections, a phenotype that could be complemented with ectopic expression of a 35S-NIK1 or 35S-T469A transgenes. However, ectopic expression of an inactive kinase or the 35S-T474A mutant did not reverse the enhanced susceptibility phenotype of knockout lines, demonstrating that Thr-474 autophosphorylation was needed to transduce a defense response to geminiviruses. Likewise, mutations at Thr-474 and Thr-469 residues antagonistically affected NIK-mediated nuclear relocation of the downstream effector rpL10. These results establish that NIK1 functions as an authentic defense receptor as it requires activation to elicit a defense response. Our data also suggest a model whereby phosphorylation-dependent activation of a plant receptor-like kinase enables the A-loop to control differentially auto- and substrate phosphorylation. "

(a) Universidade Federal de Vicosa

P48008 *Candidatus* Phytoplasma solani induces significant reprogramming of the leaf transcriptome in the field grown grapevine

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"Phytoplasmas are intracellular plant pathogens from class *Mollicutes*, causing diseases in hundreds of economically important plants, but the mechanisms of their interactions with hosts are largely unknown. We present the first global transcriptional profiling in grapevine (*Vitis vinifera*) responses to phytoplasmas. The gene expression patterns were followed in leaf midribs of grapevine cv. Chardonnay naturally infected with *Candidatus* Phytopalsma solani, which is associated with the grapevine yellows disease Bois noir. We established an expression system in a productive vineyard that allowed both a molecular analysis and tracking of plant natural histories. The two-year-long experiment revealed that genes involved in primary and secondary metabolic pathways were changed. A hypothesis that phytoplasmas interact with the plant carbohydrate metabolism was proven and some possibilities for how the products of this pathway might be utilized by phytoplasmas were shown. In addition, several photosynthetic genes were largely down-regulated in infected plants, whereas defense genes from the metabolic pathway leading to formation of condensed tannins and PR-5 proteins were significantly induced. Genes involved in defense-signaling were differentially expressed in healthy and infected plants. A set of ten selected genes from several differentially expressed pathways was confirmed to be suitable for a reliable classification of infected plants. Collectively, our results indicate that gene expression changes in response to infection by phytoplasmas may support their nutrition by promoting alterations in the host's metabolism. In addition, this study provide novel markers for the characterization of defence pathways and susceptibility features under field infection condition. "

(a) National Institute of Biology (b) University of Ljubljana, Biotechnical Faculty, Department of Biology (c) UMR SPO, Campus Agro-M/INRA

P48009 Gene expression pattern of *Phakopsora pachyrhizi*-*Glycine max* interaction in susceptible plant

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"Soybean is one of the top five agricultural products in the U.S. Its protection from all pathogens is very important for soybean production. Soybean rust, caused by the obligate fungus Phakopsora pachyrhizi Sydow, is responsible of large yield losses. From this perspective, we need to understand the molecular biology under this plant-pathogen interaction. On the pathogen side, we identified genes from P. pachyrhizi that might be involved in infection and reproduction. Thus, we constructed and analyzed cDNA library from RNA extracted from P. pachyrhizi uredinia isolated by laser capture microdissection (LCM) 10 days after inoculation (dai). This library was sequenced, contigs were formed, and blast searches were conducted. Forty unisequences have been found with similarities to sequences deposited in the NCBI protein database including proteins involved in energy production, cellular communication/signal transduction, and transcription. On the plant side, we identified genes from susceptible soybean cultivar Williams 82 expressed during P. pachyrhizi infection using microarrays and deep sequencing. Palisade layer cells adjacent to an uredinium or showing a brownish coloration were isolated by LCM 10dai. RNA from these samples and from none-inoculated plants was used in microarray experiment. Analysis indicates that 164 genes were up-regulated and 777 genes were down-regulated in inoculated plants. Most of the up-regulated genes seem to be associated with disease and plant defense. The down-regulated genes were mostly associated with metabolism, energy production, and protein synthesis. Deep sequencing results are currently being analyzed. In the future, target pathogen and plant genes will be studied to determine if they can be used to control P. pachyrhizi in soybean."

(a) USDA-ARS-Plant Sciences Institute-Soybean Genomics and Improvement Laboratory (b) USDA-ARS-Crop Genetics and Production Research (c) Towson University (d) USDA-ARS-MSA Genomics Laboratory

P48010 Bacterial Effectors Target A Common Signaling Partner To Impede Host Immunity and Development
He, Ping-presenter pinghe@tamu.edu(a,c) Shan, Libo (b,c) Sheen, Jen (c)
"Successful pathogens have involved diverse elegant virulence strategies to infect hosts. Many pathogenic bacteria deploy the type III secretion system to deliver virulence effectors into host cells to promote pathogenicity. The molecular actions of these effectors remain largely elusive. We performed a molecular cellular screen and identified two sequence-distinct effector proteins, AvrPto and AvrPtoB from a ubiquitous plant pathogen Pseudomonas syringae, as potent suppressors of host immune responses triggered by multiple microbe-associated molecular patterns (MAMPs). AvrPto and AvrPtoB target a receptor-like kinase BAK1, a shared signaling partner of diverse MAMP receptors and the plant hormone brassinosteroid receptor BRI1 in Arabidopsis. This targeting interferes with the ligand-dependent association of MAMP receptors with BAK1 during infection, and impedes plant immunity to nonpathogenic bacteria, and brassinosteroid-mediated plant development. The identification of BAK1 as a host target of AvrPto and AvrPtoB virulence uncovers a novel action of bacterial effectors in blocking signaling initiation from multiple receptor complexes. From an evolutionary point of view, it is parsimonious for pathogen effectors to target a common component involved in multiple plant signaling pathways. "
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P48011 Tomato defense responses to *Botrytis cinerea* and interaction with other response pathways
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"Plants deploy diverse molecular and cellular mechanisms to survive in stressful conditions. *Botrytis cinerea* is a typical necrotrophic fungus that causes the gray mold disease in many crops resulting in significant yield losses. We identified two regulatory components, tomato protein kinase 1b (TPK1b) and abscisic acid-induced myb1 (SlAIM1), that are required for tomato resistance to *Botrytis*. In addition to defense against *Botrytis*, *TPK1b* also plays a key role in resistance to insect herbivory in tomato. Reducing *TPK1b* gene expression through RNA interference (RNAi) increases tomato susceptibility to both *Botrytis* and tobacco hornworm (*Manduca sexta*) feeding larvae. The susceptibility to *Botrytis* and insect feeding is correlated with reduced expression of the *proteinase inhibitor II* (*PI-II*) gene in response to *Botrytis* and 1-aminocyclopropane-1-carboxylic acid (ACC). Second, the *SlAIM1* transcription factor is a key regulator of tomato responses to pathogens and some abiotic stress factors. The *SlAIM1* RNAi plants show an increased susceptibility to *Botrytis*, sensitivity to salt stress, but a reduced sensitivity to ABA, suggesting that *SlAIM1*-mediated ABA responses are required for tomato responses to biotic and abiotic stresses. Interestingly, elemental profiling of leaf tissues reveals that *SlAIM1* RNAi plants exposed to high salinity levels accumulate more Na+, thus suggesting that SlAIM1 regulates ion fluxes. Altogether, plant responses to *Botrytis*, insect herbivory and abiotic stress share common regulatory mechanisms mediated by different plant hormones."
(a) UAE University (b) Purdue University

P48012 An intact cuticle in distal tissues is essential for the induction of systemic acquired resistance in plants
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"Systemic acquired resistance (SAR) is initiated upon recognition of specific microbial effectors by cognate plant resistance proteins and immunizes distal tissues of plants against secondary infections. SAR involves the generation of a mobile signal at the site of primary infection, which then translocates to and activates defense responses in the distal tissues via unknown mechanism(s). We have recently shown that an acyl carrier protein, ACP4, is required for the processing of the mobile SAR signal in distal tissues of Arabidopsis. Although acp4 plants generated the mobile signal, they were unable to respond to this signal to induce systemic immunity. The defective SAR in acp4 plants was not due to impairment in salicylic acid (SA)-, methyl SA-, or jasmonic acid-mediated pathways but was associated with the impaired cuticle of acp4 leaves. Other genetic mutations impairing the cuticle or physical removal of the cuticle from wild-type plants also compromised SAR. This cuticular requirement was only relevant during the time of mobile signal generation and translocation to the distal tissues. Together, these results demonstrate a novel role for the plant cuticle as the site for SAR-related molecular signaling. Xia et al., 2009, Cell Host & Microbe 5: 151-165."
(a) Department of Plant Pathology, University of Kentucky, Lexington, KY (b) US Department of Agriculture, Agricultural Research Service, Prosser, WA (c) Department of Plant Science, University of Kentucky, Lexington, KY

P48013 Broadening resistance of soybean to the soybean cyst nematode using biotechnology
Matthews, Benjamin-presenter ben.matthews@ars.usda.gov(a) Klink, Vince (b) Kim, Kyung-Hwan (c) Lee, Seong-Kon (c) Park, Soo_Chul (c) Alkharouf, Nadim (d)
"Soybean cyst nematode (SCN; *Heterodera glycines*) is the major pathogen of soybean (*Glycine max*) and causes an estimated $0.8 billion in losses per annum in the U.S. We identified genes expressed in soybean roots and in SCN before and during infection using microarrays containing 37,500 soybean and 7,500 SCN gene probes. We used laser capture microdissection (LCM) to isolate syncytia from roots to study gene expression specifically at the feeding site. This provided a group of genes that may increase soybean resistance to SCN. A subgroup of SCN genes were selected by comparing the SCN EST (expressed sequence tag) database with genes from *Caenorhabditis elegans*. Genes were identified that would cause *C. elegans* death if mutated or silenced. We developed a system to rapidly transform soybean roots and screen DNA constructs to determine their effect on SCN survival. Transformation vectors were constructed for gene over-expression, gene silencing (RNAi), and promoter analysis. The vectors use Gateway (Invitrogen) technology to rapidly clone DNA. They contain the tetracycline resistance gene for easy selection of transformed *Agrobacterium rhizogenes* K599 and the gene encoding enhanced green fluorescent protein for easy selection of transformed roots. We transformed soybean roots with a series of vector constructs. The transformed soybean roots were challenged with SCN and analyzed to determine if there was a change in resistance compared to control roots. Several constructs may reduce the number of females achieving maturity at 30 days as indicated by Female Index comparisons. "
(a) USDA-ARS, Beltsville, MD (b) Mississippi State University, MI (c) Rural Development Administration, South Korea (d) Towson University, MD

P48014 Turnip crinkle virus coat protein mutants that fail to bind the NAC transcription factor TIP display slower accumulation in susceptible *Arabidopsis thaliana*
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"The coat protein (CP) of turnip crinkle virus (TCV) has previously been shown to interact with a member of the NAC family of transcription factors called TCV-interacting protein (TIP). The fully replication competent mutant virus (R6A) was made with a single amino acid replacement in the N-terminal region of the CP that failed to interact with TIP. R6A was observed to cause more severe symptoms in the susceptible Col-0 ecotype, and to break resistance in the resistant Dijon-17 ecotype. We hypothesized, based on these observations, that the interaction of TCV CP with TIP evolved to reduce the host innate immune response and thus favor more rapid systemic invasion. To test this we compared the rate of accumulation wt TCV

and mutant R6A in both inoculated and systemic leaves over the course of a virus infection for up to 8dpi. Susceptible Col-0 and mutant *Arabidopsis* lines compromised in their ability to mount a fully functional innate immune defense response were inoculated in parallel experiments with either TCV or the R6A mutant. Viral RNA accumulation was monitored by Northern blot analysis. Expression levels of select host defense-related genes were monitored using Northern analysis and semi-quantitative PCR. The data showed that accumulation of the R6A mutant relative to wt virus was compromised in wt *Arabidopsis* but not in mutant plants that lacked a fully functional SA defense pathway. This phenotype was also displayed in infections of plant lines with altered TIP expression levels. These data support the conclusion that the TIP-CP interaction is important in modulating the innate immune response to virus infection in susceptible hosts. "
(a) University of Nebraska - Lincoln (b) Doane College (c) University of Nebraska - Kearney (d) Ohio State University

P48015 Elimination of *Sorghum mosaic virus* from sugarcane (*Saccharum* sp.)
Cheong, Eun Ju-presenter Eunju.Cheong@ars.usda.gov(a) Mock, Raymond (a) Ruhui , Li (a)
"*Sorghum mosaic virus* (SrMV), which causes mosaic disease of sugarcane, has been eliminated from mature axillary buds through *in vitro* culture of meristem tips. Mature stem nodes containing an axillary bud from infected sugarcane plants were surface sterilized and cultivated in liquid medium with or without antiviral agents, oxytetracycline or thiouracil, at 26C. An RT-PCR assay detected SrMV in leaves of all *in vitro* shoots cultivated at 26C prior to harvest of the meristem. Meristem tissues (1-2 mm) excised from the axillary shoots grown in liquid culture were cultivated in agar-solid medium and then transplanted to soil in the greenhouse. RT-PCR results showed that all regenerated shoots were free of SrMV as *in vitro* plantlets and later as potted plants in the greenhouse for 8 months. Single node setts were cut from the mature tissue culture plants grown in the greenhouse and placed in soil in the greenhouse for an additional generation of growth. These replanted seed canes have tested free of SrMV for 3 months after planting. Prior research on in vitro therapy of viruses in sugarcane has mostly been confined to apical meristems, limiting the amount of material available for therapy protocols. However, this study demonstrates that *in vitro* techniques using mature axillary buds in liquid culture can be used to produce virus-free plants from SrMV-infected sugarcane. Harvesting 1-2 mm meristematic tips from *in vitro* grown plantlets at 26C, was successful in production of mature greenhouse grown virus-free plants. This method greatly expands the number of candidate therapy plants, and therefore increases the probability of successfully eliminating SrMV from infected sugarcane."
(a) USDA/ARS/NGRL Plant Disease Research Unit

P48016 Specific targeting of RPW8 to the interfacial membrane encasing the fungal haustorium renders cost-effective broad-spectrum resistance
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http://shadygrove.umbi.umd.edu/xiaogroup/
"Powdery mildew fungal pathogens penetrate the plant cell wall and develop a feeding structure named the haustorium to steal photosynthetate from the host cell. The haustorium is encased by an enigmatic extra-haustorial membrane (EHM) believed to be derived from the host cell plasma membrane. This interfacial membrane is of critical importance to both the host and the pathogen for defense and pathogenesis respectively. However, to date, not a single EHM-resident protein has been identified from either the host or the pathogen. Here, we report that the Arabidopsis resistance protein RPW8.2 is induced and specifically targeted to the EHM during haustorium biogenesis. There, RWP8.2 activates a defense strategy that concomitantly enhances the encasement of the haustorial complex and onsite accumulation of H2O2 to constrain the haustorium while reducing oxidative damage to the host cell. Salicylic acid signaling is required for the defense function of RPW8.2, but is dispensable for its EHM-targeting. The interception of haustoria pinpoints the nature of RPW8-mediated broad-spectrum mildew resistance."
(a) Center for Biosystems Research, University of Maryland Biotechnology Institute

P48017 Stomatal Immune Response to Bacterial Pathogens
Zeng, Weiqing-presenter zengweiq@msu.edu(a) Gao, Xiaoli (b,c) Jones, Daniel (b,d) He, Sheng Yang (a,e)
"Stomata closure is an early plant innate immune response against bacterial infection on the leaf surface to reduce the entry of bacteria into plant interior tissues. This response can be induced by either bacteria or pathogen-associated molecular patterns (PAMPs) and is suppressed by bacterial virulence factors such as coronatine. We report here that FLS2, the innate immune receptor for bacterial flagellin, has an essential role in mediating stomata-based resistance against the coronatine-deficient *Pseudomonas syringae* bacteria in Arabidoposis. To identify new components involved in bacterium-triggered stomatal closure, we conducted a random genetic screening for mutant Arabidopsis plants that have lost their stomata closure response by exploiting the predicted enhanced susceptibility to coronatine-deficient *P. syringae* bacteria. Five *stomatal closure-defective (scd)* mutants were identified in this screen. Further characterization showed that *scd7* is involved in abscisic acid (ABA) signaling, and *scd13* is an allele of *eds5*, which is required for salicylic acid (SA) biosynthesis. This random genetic screen is consistent with the emerging evidence that not only ABA, but also SA, play a key role in regulating bacterium-triggered stomatal immune response. We further showed that both the heterotrimeric G protein alpha (GPA1; involved in ABA signaling), and NPR1 (required for SA signaling) are necessary for stomatal immune response. These results provide molecular insights into the poorly understood innate immune function of plant stomata."
(a) DOE-Plant Research Laboratory, Michigan State University (b) Department of Biochemistry and Molecular Biology, Michigan State University (c) Genetics Program, Michigan State University (d) Department of Chemistry, Michigan State University (e) Department of Plant Biology, Michigan State University

P48018 Expression of PFLP (plant ferredoxin-like protein) targeting to extra-chloroplast in *Arabidopsis thaliana* is required in resistance against bacterial disease
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"PFLP, a photosynthetic type ferredoxin isolated from sweet pepper, contains a signal peptide at its N-terminal region for targeting towards chloroplasts. The transgenic tobacco plants containing this protein in both extra- and intra-chloroplast could be converted to be resistant against bacterial pathogens. We attempted to clarify the contribution of PFLP localization on disease resistance. The responses of transgenic Arabidopsis plants with different version of PFLP against four strains of bacterial wilt pathogen, *Ralstonia solanacearum*, were different. The transgenic plants containing PFLP with targeting sequence deleted version, or replaced version by having an extracellular secreted signal peptide, conferred resistance against all of the pathogenic strains. However, the resistance did not appear in the transgenic plants containing PFLP replaced with a chloroplast targeting sequence. The disease resistance raised by transferred PFLP in transgenic plants is also correlated with the harpin-mediated hypersensitive response (HR) which is a common plant defense response. We concluded that PFLP can mediate enhanced disease resistance when PFLP is outside the chloroplast. In addition, we further demonstrated that the casein kinas II (CK2) phosphorylation site at C-terminal of PFLP is also required for intensifying the harpin-mediated HR and disease resistance. Moreover, the CK2 phosphorylation site is commonly exist at C-terminal region of all ferredoxin isoproteins. Our findings provide a potential strategy for protecting economical crops against pathogen infection with ferredoxin."
(a) Institute of Plant and Microbial Biology, Academia Sinica (b) Department of Life Science, National Taitung University

P48019 Priming of Arabidopsis PAMP-triggered immunity by specific inhibition of the jasmonate response induced by coronatine
Tsai, Chia-Hong (a) Chen, Ching-Wei (a) Boachon, Benoit (b) Thomas, Jerome (c) Weber, Hans (c) Mauch-Mani, Brigitte (b) Zimmerli, Laurent-presenter lauzim2@ntu.edu.tw(a)
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"The chemical beta-aminobutyric acid (BABA) enhances Arabidopsis resistance to the virulent bacterial pathogen Pseudomonas syringae pv tomato DC3000 (Pst DC3000) through priming of salicylic acid (SA)-dependent defenses. The molecular mechanisms underlying this phenomenon remain elusive. Here we show that BABA potentiates PR1 mRNA up-regulation during infection with the type-III secretion system deficient mutant Pst DC3000 hrcC. This suggests that BABA primes for PAMP-triggered immunity (PTI)-mediated SA defenses. In addition, we demonstrate that BABA inhibits the Arabidopsis response to the bacterial effector coronatine (COR). COR is known to promote bacterial virulence by inducing the jasmonate (JA) response to antagonize SA signaling activation. BABA suppressed the COR response, without affecting other Arabidopsis responses to JA. Inhibition was observed after Pst DC3000 inoculation or purified COR treatment. BABA inhibition was SA-independent indicating that BABA suppression of the COR response is not the result of negative cross-talks between SA and JA. BABA-induced resistance and priming were strongly reduced in Arabidopsis inoculated with a strain of Pst DC3000 that does not produce COR or in the COR-insensitive mutant coi1-16. Together, these data suggest that BABA primes SA signaling through inhibition of the COR response. We propose that BABA confers resistance to Pst DC3000 by interfering with the bacterial suppression of PTI. In addition, these data point to the existence of a signaling node that distinguishes COR from other JA responses."
(a) National Taiwan University, Institute of Plant Biology (b) University of Neuchatel, Laboratory of Molecular and Cellular Biology (c) University of Lausanne, Center for Integrative Genomics

P48020 Analysis of Phytoalexins by High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) following Induction in *Salvadora persica*
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"Many plants defend themselves by accumulating newly synthesized antifungal compounds called phytoalexins. while screening Omani plants for phytoalexin induction, we looked at phytoalexin induction in *Salvadora persica*, of the family Salvadoraceae. *S. persica* leaves play a role as antibacterial. *Salvadora persica* (Arabic; Miswak), is used as chewing stick in the Middle East and Africa as an oral hygiene device for cleaning teeth and gum. The use of these sticks (Miswak) as a tooth brush is known to be effective in lowering condidiasis. So far no studies have been reported on phytoalexins production in *S. persica* or in the Salvadoraceae family. Chemical induction of phytoalexins by the leaves of *S. persica* was achieved using $CuCl_2$ solution, and UV induction with wavelength 254 nm. Extracted phytoalexins were detected using (TLC)-*Cladosporium bioassay* followed by large scale preparative TLC. Eluted antifungal compounds were purified by TLC and identified by HPLC-MS. *S. persica* leaves were induced with 10 mM $CuCl_2$ solution or exposure to U.V. for 20 minutes as abiotic elicitor. *Botrytis cinerea* was also used as biotic agent. Results of TLC bioassay of control and treated samples showed the presence of a number of phytoalexins such as Psorospermin [*Botrytis cinerea*], Gratiogenin and 1-Caffeoyl-β-D-glucoside [U.V.] and Diferulic acid [$CuCl_2$] produced by the leaves of S. persica. These compounds were analyzed and identified by High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS). Other antifungal compounds were also detected and identified e.g., lupeolic acid, however, these are considered as phytoanticipins since they were detected in control as well as treated samples. Some aspects of the biological activities of these phytoalexins have also been studied. "
(a) Sultan Qaboos University

P48021 Structure-Function Analysis of Coronatine-Mediated Formation of the COI1:JAZ Receptor Complexes and their Contribution to the Pathogenicity of *Pseudomonas syringae*
Withers, John C-presenter withersj@msu.edu(a,b) Mecey, Christy (a,b) Melotto, Maeli (c,d) He, Sheng Yang (a,b)
"The bacterial phytotoxin coronatine, produced by several pathovars of the foliar pathogen *Pseudomonas syringae*, contributes to suppression of plant immune responses both locally and systemically. Coronatine is a structural mimic of the plant hormone jasmonoyl-isoleucine and modulates host transcriptional responses to infection by binding to the SCF^{COI1} ubiquitin ligase and promoting the degradation of jasmonate ZIM-domain (JAZ) transcriptional repressors. Degradation of JAZ proteins leads to the induction of the jasmonate (JA)-response pathway, which regulates many aspects of plant growth, development, and response to pathogen attack or wounding. Receptor complex formation in the presence of coronatine occurs between the conserved C-terminal Jas domain of JAZ repressors and the leucine-rich repeat (LRR) domain of COI1. We have shown that coronatine, like JA-Ile, is capable of promoting interaction between multiple JAZ proteins and COI1 and that conserved residues within the C-terminal Jas domain of JAZ1 and JAZ9 are crucial for ligand-dependent formation of JAZ-COI1 complexes. To further investigate the importance of specific amino acids, site-directed mutagenesis was used to generate a comprehensive series of point mutations in the Jas domain of JAZ9 and the LRR domain of COI1. Additional JAZ9 mutants that disrupt the interaction with wild-type COI1 were identified, and wild-type JAZ proteins were screened against a suite of COI1 mutants. Here, we identify specific amino acids within both the C-terminal Jas domain of JAZ9 and the LRR domain of COI1 that are necessary for coronatine-mediated complex formation, providing molecular insights into ligand-dependent formation of a major plant hormone receptor complex."
(a) Michigan State University (b) DOE-Plant Research Laboratory (c) University of Texas - Arlington (d) Department of Biology

P48022 The strawberry cystatin gene family and in planta evaluation of anti-nematode ativity
Wang, Heidi H.Y.-presenter heidiwang@ufl.edu(a) Luc, John E. (b) Crow, William T. (b) Folta, Kevin M. (a)
"Phytocystatins are cysteine protease inhibitors implicated in a variety of plant processes, including plant defense. A number of prominent studies have shown that transgenic fortification of plants with phytocystatins can limit damage by pests and pathogens. The current study assesses the effect of strawberry phytocystatins as anti-nematode agents, targeting the ectoparasitic sting nematode *Belonolaimus longicaudatus*. Using the previously described *CYF1* gene as a starting point, a series of gene/allele variants were identified in cDNA libraries. Variant-specific expression was detected. One new variant was assessed for cysteine protease activity against commercial reagents and nematode extracts, demonstrating its effect. The same variant was introduced into diploid strawberry plants (*Fragaria vesca*) and evaluated for effects on plant morphology and nematode colonization. The results show evidence of decreased colonization under certain nematode population densities, but also show effects on plant growth that are likely not beneficial. The study demonstrates that use of such tools in strawberry are likely to be effective against sting nematode, but will require other modes of tissue-specific expression to ensure a desired effect on nematodes without affecting plant vigor."
(a) Department of Horticultural Sciences, University of Florida (b) Entomology and Nematology Department, University of Florida

P48023 Understanding the molecular mechanisms underlying the harpin-induced resistance to *Magnaporthe grisea* and other fungal diseases in rice
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"*Magnaporthe grisea* is a pathogenic fungal disease in rice that severely affects rice production, thus making engineering durable resistance to *M. grisea* one of the ultimate goals of rice breeding. We previously showed that the over-expression of a harpin-encoding gene (*HRF1*), derived from *Xanthomonas oryzae pv. oryzae* confers nonspecific and heritable rice resistance to all races of blast fungus *M. grisea*. However, nothing is known on the molecular mechanisms of such fungal resistance induced by *HRF1* over-expression. As the first attempt to address this question, we performed genome-wide microarray experiments using the Affymetrix 51K Rice Genome GeneChipTM to compare the transcriptome of *HRF1* transgenic rice plants to that of wild type. Our results showed that around 140 genes were up-regulated more than 2 fold and over 80 genes were down-regulated more than 2 fold by *HRF1* over-expression in comparison to their transcript levels in wild type rice plants. Several lines of evidence indicate the biological significance of our microarray results. First, RT-PCRs conducted on representative genes agreed with the microarray data. Second, genes known to be involved in disease resistance, such as NB-ARC domain-containing proteins, wall-associated protein kinase (WAK), WRKY transcription factors, were up-regulated in *HRF1*-transgenic rice. Third, a number of genes whose expression levels were altered by *HRF1* over-expression are also transcriptionally regulated by *M. grisea*. A portion of genes with altered expression levels by *HRF1* over-expression encode unknown proteins with novel domain organizations. Functional studies on these genes will undoubtedly shed lights on the molecular mechanisms underlying *HRF1*-induced rice resistance to blast fungus *M. grisea*. "
(a) Department of Plant Pathology, Nanjing Agricultural University, China (b) Plant Gene Expression Center, University of California, Berkeley

P48024 Salicylic acid stimulates secretion of the typically cytosolic metabolic enzyme mannitol dehydrogenase.
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"The sugar alcohol mannitol has well-documented roles in both metabolism and osmoprotection in plants and fungi. In addition to these traditional roles, mannitol is also reported to be an antioxidant, and as such, may play a role in host-pathogen interactions. Current research suggests that at least some pathogenic fungi secrete mannitol to suppress reactive oxygen-mediated host defenses. Electron microscopic, immunological and biochemical data from tobacco cells expressing the mannitol catabolic enzyme, mannitol dehydrogenase, from celery (AgMTD) show that this normally cytoplasmic enzyme is secreted into the apoplast in response to salicylic acid (SA) while retaining enzymatic activity. In contrast, the enzyme hexokinase, a cytoplasmic control, remained in the cytoplasm after SA-treatment. More recently, a quantitative, data-independent mass spectrometry acquisition strategy (LC/MS^E) was used to verify a 19-fold specific induction of MTD secretion in celery culture cells in response to SA treatment. Given that SA is an endogenous inducer of plant defense responses and that MTD catabolizes mannitol, induced MTD secretion may be a component of plant defense against fungal pathogens. Finally, MTD was not detected by immunolocalization in the Golgi apparatus of AgMTD expressing tobacco following SA treatment. In addition, SA-induced MTD secretion was resistant to brefeldin A, an inhibitor of Golgi-mediated protein transport. Given the absence of a known extracellular targeting sequence in the MTD protein, these data suggest that plant responses to pathogen challenge may include secretion of selected defensive proteins by as yet unknown mechanisms."
(a) Department of Horticultural Science, North Carolina State University (b) The Robert H. Smith Institute, The Hebrew University of Jerusalem (c) Department of Molecular and Structural Biochemistry, North Carolina State University

P48025 Influence of host chloroplast proteins on *Tobacco mosaic virus* accumulation and cell-to-cell movement
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http://www.noble.org
"*Tobacco mosaic virus* (TMV) form cytoplasmic inclusion bodies referred to as virus replication complexes (VRCs) upon infection. The composition and functions of these VRCs is not fully understood. To determine the content of the TMV replication complex, we isolated and purified a replicase-enhanced fraction from TMV-infected *Nicotiana tabacum* cv. Xanthi plants. The purified fractions were profiled using SDS-PAGE and compared with the fractions from healthy controls processed in parallel. We observed several host proteins at higher levels in the extracts from TMV-inoculated leaves. Two host proteins, ATP-synthase gamma subunit (AtpC) and rubisco activase (RA), identified by MALDI-TOF-MS were further characterized. TMV infection of N. tabacum cv. Xanthi plants resulted in a 50% reduction of AtpC and RA mRNA levels. To investigate the possible role of these host proteins in TMV accumulation and plant defense responses, we used a *Tobacco rattle virus* (TRV) vector to silence these genes in *Nicotiana benthamiana* plants prior to challenge with TMV expressing GFP. Silencing RA significantly enhanced TMV accumulation and pathogenicity compared with plants that were not silenced for RA (infiltrated with TRV not expressing an RA fragment), suggesting a role of this protein in the host defense response to TMV infection. TMV-induced green fluorescent lesions on AtpC-silenced *N. benthamiana* leaves were significantly larger and more intensively fluorescent than were lesions on AtpC-expressing leaves. Interestingly, silencing RA and AtpC in *N. benthamiana* did not influence the spread of *Tomato bushy stunt virus* (TBSV) and *Potato virus X* (PVX). The possible roles of RA and AtpC in TMV accumulation and plant defense responses will be presented"
(a) Samuel Roberts Noble Foundation (b) University of Florida (c) Dakota Wesleyan University

P48026 Two-component elements mediate susceptibility of Arabidopsis to the oomycete *Hyaloperonospora parasitica*
Argueso, Cristiana T.-presenter ca@email.unc.edu(a) Ferreira, Fernando J. (a) Schaller, G. Eric (b) Kieber, Joseph J. (a)
"Plant hormones play an important role in various developmental processes, as well as in responses to the constantly changing environment, including responses to pathogens. Cytokinins are involved in the regulation of many aspects of plant development and physiology, such as the regulation of cell division, meristem maintenance, chloroplast development, leaf senescence, vascular development, as well as regulation of source-sink relationships within the plant. In Arabidopsis, cytokinin signaling involves a phosphorelay pathway similar to two-component response systems, which are used by bacteria to sense and respond to a diverse array of environmental stimuli. Here we report that Arabidopsis plants altered in the cytokinin signaling pathway show altered responses to compatible isolates of the biotrophic plant pathogen *Hyaloperonospora parasitica* . Molecular characterization of these responses revealed that a subset of pathogen-responsive genes is differentially regulated in these genotypes, suggesting a role for cytokinin signaling in defense responses to pathogens. Interestingly, the altered responses were specific to *H. parasitica* and *Botrytis cinerea*, and not extended to *Pseudomonas syringae* pv. *tomato* DC3000, suggesting that the pathogenicity strategy of some pathogens, but not others, may involve alterations of the cytokinin signaling pathway."
(a) University of North Carolina at Chapel Hill, NC (b) Dartmouth College, NH

P48027 Dissecting the role of actin and myosin in the cell-to-cell movement of diverse RNA viruses
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"The actin microfilament network has been implicated in the movement of both plant and animal viruses. Here we survey a number of plant RNA viruses for the effect of pharmacological disruption of the actin cytoskeleton on cell-to-cell spread. Utilizing the actin inhibitor, Latrunculin B, we demonstrate that viruses from different genera rely upon the actin cytoskeleton for sustained movement. Surprisingly, the closely related

Tobamoviruses *Tobacco mosaic virus* (TMV) and *Turnip vein clearing virus* (TVCV) differ in their ability to move cell-to-cell in the absence of intact microfilaments. TMV depends upon microfilaments for normal cell-to-cell spread, while TVCV does not. We use viral proteins fused to the fluorescent reporter GFP to dissect the molecular basis for the observed difference in cytoskeletal movement requirements between these two viruses. We show that while the TMV 126-kDa protein forms cytoplasmic inclusions that traffic along microfilaments, the TVCV 125-kDa homolog does not. This finding correlates with the differing requirements for actin-dependent movement observed for these viruses. The dependence of sustained TMV intercellular movement on microfilaments led us to investigate the role of myosin motor proteins in this process. We show that virus-induced gene silencing of a specific *N. benthamiana* myosin XI gene has an inhibitory effect on the cell-to-cell movement of TMV. This effect is specific to TMV since other viruses that are also dependent upon actin for their sustained movement were not inhibited by silencing this myosin. These results demonstrate that distantly and even closely related viruses utilize unique movement strategies which may have been selected during co-evolution in specific hosts."
(a) Samuel Roberts Noble Foundation, Inc. (b) Texas AgriLife Research (c) Tokyo University of Agriculture and Technology (d) John Innes Centre

P48028 The bacterial blilght pathogen *Xanthomonas oryzae* adapts to resistant host genotypes by targeting alternative host susceptibility genes

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"Bacterial blight of rice represents a robust system for understanding the interaction and co-adaptation process of a bacterial pathogen and the host. *Xanthomonas oryzae* pv. oryzae (Xoo) like many members of the proteobacteria, depend on a type III secretion system for effective invasion and colonization of the host. The pathogen is noteworthy for the dependence on a family of type III effector genes , which consists of 19 members are named the transcription activator-like (TAL) effectors. We have identified four major TAL effectors based on their contribution to the virulence. One of the effector, PthXo1, targets the host gene *Os8N3* and causes a thirty-fold increase in mRNA levels. Some plants harbor the recessive xa13 allele of *Os8N3* and are resistant to bacteria that rely on PthXo1 for virulence. Strains of the pathogen that overcome *xa13*-mediated resistance target other members of the N3 gene family. Addition rice cultivars also have alternative recessive alleles for N3 genes, indicating that rice has evolved variants that avoid Pth-mediated expression. Xoo targets additional host genes using other members of the 19 member TAL gene, including genes for a bZIP transcription factor, a core transcription factor subunit, and miRNA regulation. The results indicate a complete adaptive history between Xoo and the host plant rice."
(a) Kansas State University (b) Iowa State University

P48029 Role of ribosomal proteins *NbRPL12* and *NbRPL19* in nonhost disease resistance in *Nicotiana benthamiana*.

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"Nonhost disease resistance is crucial to plants as a defense mechanism against a plethora of plant pathogens. Nonhost disease resistance response in plants is manifested either as hypersensitive response (Type II) or no visual symptoms (Type I). Understanding the molecular mechanisms underlying these responses is critical to elucidate the mode of action of nonhost resistance that will be beneficial to enhance disease resistance in plants. Virus-induced gene silencing (VIGS), a powerful tool, has been utilized in this study to identify genes that play a role in nonhost disease resistance. Several genes were identified using VIGS based fast-forward genetics approach. Of the many genes identified, ribosomal proteins L12 and L19 were selected for further characterization. Ribosomal proteins have been implied to play a role in disease resistance. Our study, for the first time, show that these proteins play a role in nonhost resistance but not in resistance against host pathogens in *N. benthamiana*. Nonhost bacterial growth was monitored in plants that were silenced for *NbRPL12* and *NbRPL19* genes and non-silenced plants. The silenced plants significantly support more growth of nonhost bacteria when compared to non-silenced plants. Characterization of the role of these proteins in nonhost disease resistance and model to propose the role for ribosomal proteins in plant defense will be presented."
(a) Samuel Roberts Noble Foundation (b) Bentham Science

P48030 A novel role of the phosphate transporter gene *SUP3* in plant innate immunity

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"Pathogen infection activates expression reprogramming of thousands of genes in plants. However, it remains challenging to identify which ones regulate plant disease resistance and elucidate how these genes function. Taking advantage of defense-dependent size change in the Arabidopsis mutant *acd6-1*, we developed a genetic screen to identify *acd6-1 suppressor (sup)* mutants, which potentially harbor mutations in novel defense genes. Among the genes identified was *SUP3* , encoding a phosphate transporter. The *sup3-1* mutant was found to suppress constitutive defense, high levels of salicylic acid (SA), and small size in *acd6-1*. In addition, *sup3-1* was compromised in basal defense against virulent but not avirulent *Pseudomonas syringae* strains. We also found that *sup3-1*-conferred susceptibility could be rescued by exogenous SA treatment, suggesting that SUP3 acts upstream in SA signaling. Consistent with the role of SUP3 in regulating SA-mediated defense, genetic analysis indicated that *sup3-1* acted additively with several known SA regulators, ALD1, EDS5, and SID2, to affect *acd6-1*-conferred phenotypes. The *sup3-1* mutant is disrupted in the fifth exon of the *SUP3* gene, leading to the accumulation of a shorter transcript. The *sup3-1* mutant is dominant, possibly due to the action of the truncated protein. Transgenic expression of the DNA fragment containing the *SUP3-1* region in the wild type recapitulated *sup3-1*-conferred susceptibility to *Pseudomonas* infection. In addition, introducing extra copies of the full length SUP3 genomic fragment into the wild type also resulted in enhanced disease susceptibility. Taken together, our data suggest that SUP3 is a novel negative regulator of basal defense, acting independently of ALD1, EDS5, and SID2 in the SA-mediated defense pathway."
(a) University of Maryland Baltimore County

P48031 Whole genome wide expression profiles of *vitis amurensis* grape responding to downy mildew infection by using solexa sequencing technology

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"Downy mildew, caused by fungal pathogen *Plasmopara viticola* (Berk.et Curtis) Berl et de Toni, is the single most damaging disease of grapes (*Vitis* L.) world wide. Solexa, a new generation of sequencing technology, was used for 'deep' sequencing a near complete set of transcripts derived from downy mildew infected leaves of *Vitis amurensis* Rupr. cv. Zuoshan-1. About 8.5 million 21-nt cDNA tags were sequenced in the cDNA library derived from pathogen-infected leaves, and about 7.5 millions were sequenced from the cDNA library constructed from the controlled leaves. Comparative analysis between these two cDNA libraries revealed a large number of pathogen-induced up-regulated (about 0.9% of the sequenced tags) and down-regulated (about 0.6% of the sequenced tags) genes, while 98.5% of the tags were found no prominent difference (within 5 fold) between the two samples. As a result, a total of 36,979 tags or 6,295 genes were found to be up-regulated, and 26,261 tags or 4,326 genes were found to be down-regulated at significant level of 0.01. Further analysis revealed that 123 tags were up-regulated more than 50 folds. Of which at least 32 tags with their corresponding genes have previously been described as disease resistant genes. While annotated with the 30,434 reference genes derived from the grape genome project, a total of 27,073 (88.96%) putative genes were constructed among the Solexa sequencing tags. Results from this

study indicated that the tag-based mRNA profiling technology is a powerful tool to identify transcripts regulated (up- and down-) by pathogen infection. The Solexa sequencing technology enables us to rapidly identify, quantify, and functionally annotate a large scale of plant host genes involved in plant-pathogen interaction."
(a) Florida A&M University (b) China Agricultural University (c) University of Florida

P48032 Quantitative changes in the Arabidopsis defense proteome and phospho-proteome
van Schie, Chris C.N. (a) Shen, Zhouxin (a) Mason, Amanda G. (a) Chung, Eui Hwan (b) Dangl, Jeff L. (b) Briggs, Steve P.-presenter sbriggs@ucsd.edu(a)
http://www-biology.ucsd.edu/faculty/briggs.html
"The Arabidopsis-Pseudomonas syringae pv tomato (Pto) interaction is an intensively studied model system for plant immunity. Mutant hunts and gene expression profiling have driven the field to a new plateau of understanding. However, many gaps remain in our understanding of how resistance (R)-proteins work. Signaling partners of R-proteins that are lethal if mutated or are genetically redundant will not be recovered in mutant screens. Microarray studies cannot detect the primary signaling events that ultimately give rise to transcriptional changes. To complement these approaches, we use sensitive proteomics methods using LC-MS/MS to identify proteins whose properties are regulated by immune signaling. We make use of the conditional avrRpm1 effector expression system to induce immune signaling through the R-protein RPM1 in adult plants. After the first (phospho-)proteome analysis from 3 replicates and 2 time-points, we identified approximately 4600 proteins and 1900 phospho-peptides (corresponding to 900 modified proteins), of which 330 and 220, respectively, were reproducibly changed in level. Our study confirms several changes observed at transcript level, and identifies regulation of proteins known to be involved in immune signaling like TRX5, CML24, PEN1 and PEN3. Novel exciting findings include changes in (phosphorylation-) levels of proteasome-related proteins, Calcium/Calmodulin binding proteins, kinases, phosphatases and transcription factors. Besides functional analysis of these proteins, we continue proteomics based analysis of the Arabidopsis immune system by parsing signaling pathways using mutant backgrounds, and we are building an interactome by identification of protein complexes after immuno-precipitations with various proteins around the RPM1-module."
(a) University of California, San Diego (b) University of North Carolina, Chappel Hill

P48033 Characterisation of the Role of Tobacco Methyl Salycylate Esterase in the SAR induced by chemical elicitors
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"Plant diseases caused by microbial pathogens result in devastating losses in productivity of food and fiber crops. To fight with these microbial pathogens, plants have evolved various pathways. One such pathway is mediated by a plant hormone salicylic acid (SA). SA is required both local resistance and development of a broad spectrum resistance, systemic acquired resistance (SAR) in the uninfected parts of the plants. Conversion of methyl salicylate (MeSA) synthesized upon pathogen infection into SA by a methyl salicylate esterase (SABP2) is critical. MeSA is also the phloem mobile signal for SAR. Plants which do not express SABP2 are unable to convert MeSA into SA and fail to develop effective SAR. Various functional analogs of SA have been developed and used to activate plants own natural defenses against microbial pathogens. ASM (Acibenzolar- S-Methyl) is one such functional analog which induces similar responses as pathogens in monocot and dicot plants belonging to various families. It has also been shown to enhance the nutritional quality of the grapes by increased biosynthesis of resveratrol and anthocyanin. Resveratrol has been thought to have an array of biological activities in medicine and nutrition. How ASM functions in the plant is mostly unclear? We have investigated the role of SABP2 in systemic acquired resistance induced by ASM. Our results show that SABP2 is required for the conversion of ASM into its active form which is essential for the expression of defense proteins and effective mounting of systemic acquired resistance in plants. Results provide a mechanism of action for acibenzolar-S-methyl."
(a) Department of Biological Sciences, East Tennessee State University (b) Department of Chemistry, East Tennessee State University (c) The Talent Expansion in Quantitative Biology Program, East Tennessee State University

P48034 The Yellow Leaf disease of sugarcane caused by a phloem-located luteovirus
Komor, Ewald-presenter ewald.komor@uni-bayreuth.de(a) Lehrer, Axel T (a)
"Axel T. Lehrer1,2, Shih-Long Yan1, Ewald Komor1* 1Pflanzenphysiologie, University Bayreuth, D-95440 Bayreuth 2Hawaii Agriculture Research Center, Experiment Station, Aiea, HI 96701, USA *ewald.komor@uni-bayreuth.de Ten years ago a new sugarcane disease was detected and a new luteovirus (Sugarcane Yellow Leaf Virus) was identified as the causal agent. A specific test revealed that the pathogen is distributed worldwide and has infected virtually all plants of the susceptible cultivars, but stayed mostly unrecognized because obvious and specific symptoms are often not developed. The carbohydrate physiology of non-symptomatic plants was studied and compared with plants of the same cultivar which were made virus-free by meristem tissue culture. The infected plants showed a small but significant inhibition of carbohydrate export. This was obvious as a higher level of assimilation starch which remained in the source leaves after the night. The starch accumulated not only in bundle sheath cells but also in mesophyll cells of infected plants, not so or less in virus-free plants. As a consequence, several further changes took place, such as a higher ATP/ADP ratio, a lower chlorophyll a/b ratio (not the total chlorophyll content), higher starch levels in bundle sheath and mesophyll, and a change of thylakoid packing in the chloroplasts. The small sugar export inhibition resulted finally in a growth inhibition of the infected plants especially in phases of bud germination, leading to a yield decline of ca. 30 %. "
(a) university Bayreuth

P48035 Testing the resistance of Anthuriums expressing antibacterial proteins against *Xanthomonas campestris* pv *dieffenbachiae.*
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"Anthurium is prone to bacterial blight disease caused by *Xanthomonas campestris* pv *dieffenbachiae* . Breeders have developed cultivars with favourable colour and floral characteristics but there is little blight disease resistant germplasm available for improvement through breeding. A transgenic approach based on genes encoding antibacterial proteins has been adopted in an attempt to improve blight disease resistance. An *Agrobacterium* -mediated transformation system was used with the commercial cultivars Marian Seefurth and Midori. Genes used for transformation included a lysozyme from bacteriophage T4, and attacin and cecropin from the giant silk moth, *Hyalophora cecropia* . We confirmed the antibacterial activity of the proteins expressed by these genes against *Xanthomonas campestris* pv *dieffenbachiae* . Genes encoding the antibacterial proteins were subcloned into the pBI121 binary vector with expression controlled by a constitutive 35S promoter and the NPTII gene as a selectable marker. Integration and expression of the transgenes in plants surviving on growth media containing G418 was confirmed by molecular analyses. Currently, we are conducting a laboratory assay to evaluate levels of resistance to the bacterium and to detect any differences in the bacterial infection process among the tested genes. Preliminary results showed that expression of antibacterial proteins in anthurium affords some protection against bacterial blight. Plants are also being tested in a field trial. "

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P48036 Understanding the mechanisms of basal and resistance gene-mediated immunity in soybean

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http://www.ca.uky.edu/agcollege/plantpathology/AKachroo/Index.html

"Soybean is an important source of protein for human nutrition and animal feed worldwide. Each year, soybean losses due to microbial diseases greatly impact agricultural economy. With the aim of improving our understanding of soybean defense mechanisms, we have been characterizing the defense-related functions of soybean sequences. We have used a virus-based vector to silence endogenous soybean sequences and studied their roles in defense against viral, bacterial and oomycete pathogens. We have shown that altering the levels of the monounsaturated fatty acid, oleic acid, induces a novel resistance-inducing pathway in soybean and that this pathway is conserved between diverse plants. We also showed that, although soybean defense signaling pathways recruit structurally conserved components, they have distinct requirements for specific proteins. For example, RAR1, SGT1 and HSP90 are conserved components of resistance (*R*) gene-mediated defense signaling, where RAR1 and SGT1 are thought to serve as co-chaperones of HSP90. R proteins recruiting RAR1 and/or SGT1 usually also require HSP90 for defense signaling. In soybean, however, Rsv1-mediated extreme resistance to Soybean mosaic virus (SMV), and Rpg-1b-mediated resistance to Pseudomonas syringae require GmRAR1 and GmSGT1, but not GmHSP90. GmRAR1 and GmSGT1-2 also contribute to basal and systemic immunity in soybean. We conclude that RAR1 and SGT1 serve as a point of convergence for basal, *R* gene-mediated and systemic resistance in plants, since Arabidopsis *rar1* mutant was also found to be defective in SAR. The functional characterization of these components will aid the isolation of additional factors essential for soybean defense against specific pathogens. "

(a) University Of Kentucky

P48037 "Collection and pathogenicity tests of Fusarium oxysporum, the causal agent of koa "

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"Koa (Acacia koa Gray) is a Hawaiian endemic tree and both ecologically and economically important to Hawaii. It provides habitats for endemic species such as the endangered birds, snails and many other plants that grow in its understory. However, in natural koa forests and in commercial plantations, an increasing number of trees are infected with disease and show symptoms such as chlorosis and wilting of crown and dieback. One of the pathogens is Fusarium oxysporum f. sp. koae, which was first isolated from young seedlings at the Hawaii Volcanoes National Park by Gardner in 1980. The long-term objectives of this study are to identify disease-free fields for new koa plantings and to develop a rapid molecular method to confirm the presence of pathogenic F. oxysporum. To achieve these goals, understanding of the genetic diversity within the population are essential and many cultures of F. oxysporum are required. In October 2008, the required collection of hundreds of F. oxysporum cultures was begun from six mature diseased koa trees in the College of Tropical Agriculture and Human Resources, Hamakua Research Station on the Island of Hawaii. A single hyphal tip was used to establish pure cultures of fungi that emerged from diseased specimens plated on agar. These tips were placed on nutrient agar, allowed to grow, and identified using morphological characteristics. Over 147 cultures were collected including 78 Fusarium containing 30 F. oxysporum. These Fusarium cultures were isolated from diseased branches, bark, trunk and roots. Pathogenicity tests were conducted on koa seedlings and 69 percent of the isolates were pathogenic. These isolates and future collections will be used for genetic analysis to develop molecular methods to identify pathogenic isolates. "

(a) Department of Plant and Environmental Protection Sciences. University of Hawaii

P48038 Myo-Inositol Oxygenase Genes are Involved in the Development of Syncytia Induced by *Heterodera schachtii* in Arabidopsis Roots

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"In plants, UDP-glucuronic acid is synthesized by the oxidation of UDP-glucose by UDP-glucose dehydrogenase. However, a second pathway has been described and involves the oxygenation of free myo-inositol by myo-inositol oxygenase (MIOX). In Arabidopsis, myo-inositol oxygenase is encoded by four genes *(MIOX1, MIOX2, MIOX4, MIOX5)*, *MIOX3* being a pseudogene. Transcriptome analysis of syncytia induced by the cyst nematode *Heterodera schachtii* in Arabidopsis roots revealed that two *MIOX* genes are among the most strongly upregulated genes in these specialized nematode feeding cells (*MIOX4* and *MIOX5*). The other two genes are expressed in both control roots and syncytia. These results have been confirmed by GUS analysis, in situ RT-PCR and real-time RT-PCR. The functional role of the MIOX genes in syncytia was also evaluated in a nematode infection assay using T-DNA insertion lines. Results of the infection assay with double mutants in two combinations (Δmiox1+2, Δmiox4+5) showed a significant reduction in the number of females per plant when compared with the wild-type. As functional syncytia are essential for female nematode development, the data indicate an important role of these genes for syncytium development. "

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P48039 How and where do plants make salicylic acid?

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"Salicylic acid (SA) is important for many responses in plants, including pathogen defence, during senescence and responses to heat, UV and salinity. Despite this importance, the synthesis of SA is not fully understood in plants. SA may be synthesised by hydroxylation of benzoic acid, which is derived from the phenylpropanoid (PP) pathway. However, there is increasing evidence for a PP-independent pathway whereby SA is synthesised directly from chorismate in a way analogous to certain bacteria. This alternative pathway requires two enzyme activities: an isochorismate synthase (ICS) and an isochorismate pyruvate lyase. In *Arabidopsis*, there are two ICS genes, which encode enzymes that convert chorismate to isochorismate. *Ics1* was identified from the *sid2* mutant, which is severely compromised in SA biosynthesis, and subsequently demonstrated to be an ICS. *Ics2* may be involved in the synthesis of phylloquinone, a photosynthetic electron acceptor. This project is aimed at characterising the roles of these two plastidial ICS proteins in *Arabidopsis*. The requirement for two separate proteins is unclear: one possibility is that the enzymes might have different kinetic characteristics, so recombinant ICS1 and ICS2 are being produced to address this. An alternative explanation is that the genes are expressed under different conditions. To test this, the regulation of *Ics1* and *Ics2* gene expression is being studied using reporter gene constructs and real-time RT-PCR under different biotic and abiotic stimuli. *Ics1* is known to be induced by different pathogens, but little is known about *Ics2*, which is likely to be constitutively expressed. This work will therefore provide further evidence for the role of isochorismate synthesis in pathogen

defence and other plant responses."
(a) Department of Plant Sciences, University of Cambridge

P48040 "Tzs, a host range factor, is involved in *Agrobacterium* virulence and growth"
Hwang, Hau-Hsuan-presenter hauhsuan@dragon.nchu.edu.tw(a,b) Wang, Ming-Hsuan (b) Lee, Ying-Ling (a) Li, Yi-Ho (a) Chen, Chun-Wei (a) Tsai, Yun-Long (b) Lai, Erh-Min (b)
"*Agrobacterium tumefaciens* is an organism capable of trans-kingdom DNA transfer, transforming mainly plants but also other eukaryotic species. Genetic transformation by *A. tumefaciens*, which in plants causes neoplastic growths called crown gall, results from the transfer and integration of a specific DNA fragment from the bacterium into the plant genome. Here, we characterized a Ti-plasmid encoded gene, *tzs* (*trans*-zeatin synthesizing), that is responsible for the synthesis of a plant hormone cytokinin in *A. tumefaciens* . To determine the role(s) of *tzs* in *A. tumefaciens* virulence, *tzs* deletion mutants and frame-shift mutants were generated and characterized. Quantitative tumor assays demonstrated that *tzs* mutants decreased their ability to cause tumors on *Arabidopsis* roots. Additionally, *tzs* mutants reduce transient transformation efficiency in *Arabidopsis* roots, suggesting that Tzs is likely involved in step(s) prior to T-DNA integrations. The exogenous applications of cytokinin during infections also restored the transient transformation efficiencies in the *tzs* mutants, suggesting that the cytokinin is responsible for the efficient transformation on *Arabidopsis* roots. Tumor assays on various plant species were also tested to determine if Tzs is a host range factor. The *tzs* mutants were able to enhance transformation efficiency on green pepper and cowpea, reduce transformation efficiency on white radish and other plant species. Interestingly, the *tzs* mutants are impaired in cell viability and/or growth in both AS-induced and infection conditions. These data suggest that Tzs, likely via synthesizing *trans*-zeatin at early stage(s) of infection process, is involved in the transformation efficiency of *A. tumefaciens* and may play different roles in different host plants. "
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P48041 Isolation and analysis of defense-related genes in infected sorghum seedlings
Li, Lei-presenter kellylei@hkusua.hku.hk(a) Chu, Apple H (a) Liu, Hong jia (b) Lo, Clive (a)
"To understand the key processes governing defense mechanisms in Sorghum bicolor upon infection by fungus *Colletotrichum sublineolum*, the causal agent of sorghum anthracnose, a cDNA library was constructed using the suppression subtractive hybridization (SSH) method. SSH was performed between cDNAs prepared from a resistant sorghum cultivar (DK18) inoculated with *C. sublineolum* and uninoculated control samples. A total of 41 unique cDNA clones were found differentially expressed in inoculated seedlings. They can be classified into 7 categories according to characterized or putative functions of their homologous sequences. These genes are potentially involved in plant defense, signal transduction, abiotic stress, secondary metabolism, protein synthesis and degradation. Gene expression patterns were compared in sorghum cultivars that are resistant or susceptible to the anthracnose pathogen following inoculation. In addition, expression of the pathogen-inducible genes was investigated in sorghum seedlings after challenges by non-host pathogens including *Cochliobolus heterotrophus* and *Magnaporthe grisea*. Selected sorghum genes were over-expressed in transgenic tobacco plants which were demonstrated to show enhanced resistance against *Pseudomonas syringae pv. tabaci*. Elevated expression levels of PR genes were recorded in the transgenic plants. Further characterization and functional analysis of these genes may allow us to identify sorghum genes that can be utilized for board-spectrum disease resistance in different plant species."
(a) School of Biological Sciences,The University of Hong Kong, Hong Kong (b) Zhejiang Academy of Agriculture Sciences

P48042 Characterization of *Arabidopsis* Receptor-like Kinases Involved in Fungal Innate Immunity
Yang, Xin-presenter xyang15@bama.ua.edu(a) Ramonell, Katrina M. (a)
"The ability to recognize general elicitors and rapidly initiate defense responses is integral to the basal resistance of plants to most potential pathogens. These responses can be activated upon recognition of elicitors such as bacterial flagellin, plant cell wall fragments released by pathogen damage and chitin fragments generated from the cell walls of pathogenic fungi. It has been shown that the recognition of elicitors involves receptor-like kinases (RLKs). In Arabidopsis, RLKs are a large family of genes including more than 610 putative members. Recently several RLKs have been shown to play a critical role in pathogen recognition and plant innate immunity. We compared publically available microarray data sets of Arabidopsis plants treated with Chitin, Flg22, and oligogalacturonides(OG). Our analysis showed that 141 genes were specifically induced and 279 genes were specifically repressed by chitin treatment. From these data we identified 26 LRR-RLK genes that were up-regulated by chitin and obtained Salk T-DNA insertional lines in these genes. Homozygous T-DNA insertional lines were tested for resistance to the powdery mildew pathogen, *Golovinomycetes cichoracearum*. Insertions in three of the RLK lines were more susceptible to powdery mildew than Columbia wild-type. Experimental Data on the characterization of these genes will be presented."
(a) The University of Alabama, Biological Sciences

P48043 Elucidating the function of ascorbic acid in response to virulent *Pseudomonas syringae*
Larrimore, Katherine E.-presenter klarrimo@mix.wvu.edu(a) Mukherjee, Madhumati (a) Ahmed, Naushin (a) Barghouthi, Nadia (a) Bedick, Tyler (a) Traw, Brian (b) Barth, Carina (a)
"The ascorbic acid (AA)-deficient *Arabidopsis thaliana vtc1* mutant exhibits increased resistance to the virulent pathogen *Pseudomonas syringae*, involving salicylic acid (SA)-dependent NPR1 signaling. To understand the mechanism of this enhanced disease resistance, we studied the Arabidopsis *vtc1*, *vtc2*, *vtc3*, and *vtc4* mutants and (i) tested if constitutively elevated SA and pathogenesis-related (*PR*) mRNA levels are a general phenomenon of AA deficiency, (ii) attempted to elucidate the signal that stimulates SA synthesis in *vtc* mutants, and (iii) identified the biosynthesis pathway through which *vtc* mutants accumulate SA. All *vtc* mutants, except for *vtc4*, contain four to six times more SA than the wild type and have higher *PR* mRNA levels under normal growth conditions. All mutants are more resistant to *P. syringae*. The *vtc1*, *vtc2*, and *vtc3* mutants contain 30-40% and *vtc4* contains 50% AA of the wild type. All mutants have slightly elevated levels of H_2O_2, which is known to stimulate SA biosynthesis. Analysis of double mutants with a defect in *VTC1* and the SA signaling pathway genes *PAD4*, *EDS5*, and *NPR1*, respectively, revealed that these double mutants exhibited decreased SA levels and enhanced susceptibility to *P. syringae*. This suggests that the *vtc* mutants synthesize SA through the isochorismate synthase pathway that requires the action of PAD4, EDS5 and NPR1. Collectively, our study provides novel insights on the role of AA in affecting SA biosynthesis, which partially occurs through the action of H_2O_2. We suggest that SA biosynthesis is constitutively upregulated in AA-deficient plants to compensate for the decreased antioxidant capacity, thereby priming plants for future pathogen attacks. "
(a) West Vriginia University (b) Univeristy of Pittsburgh

P48044 Characterization of an *Arabidopsis* E3 ligase involved in plant defense
Perricone, Adam J.-presenter ajperricone@bama.ua.edu(a) Ramonell, Katrina M. (a)
"Innate immunity is an ancient and conserved form of defense shared by plants, insects, and vertebrates. These defense responses can be activated

upon recognition of an array of general elicitors. One of these elicitors, the oligosaccharide chitin, elicits a strong defense response in Arabidopsis and other plants. We identified a gene, rapid response to elicitor 1 (rre1), which responds strongly to chitin. T-DNA insertional mutants of *rre1* showed an increased susceptibility to the powdery mildew pathogen, *Golovinomycetes cichoracearum*. RRE1 is a RING zinc-finger type E3 ubiquitin ligase belonging to the ATL family of stress response genes. To further characterize the role of RRE1 in innate immunity, we created an over-expression line of rre1 under the control of the CaMV 35S promoter, a GFP:rre1 fusion, and a GUS:rre1 fusion line. These lines will be tested for resistance to the powdery mildew pathogen and to determine the tissue-specific and cellular localization of RRE1 during infection. Additionally the over-expression line will be infected with powdery mildew and the growth of fungal colonies quantified. Data from these characterization studies will be presented."
(a) University Of Alabama

P48045 "The Pepper Pathogen-Inducible, Receptor-Like Cytoplasmic Protein Kinase CaPIK1 Gene Is Involved In Plant Signaling for Defense and Cell Death Responses to Microbial Pathogens "
Kim, Nak Hyun-presenter nakol@korea.ac.kr (a) Kim, Dae Sung (a) Choi, Hyong Woo (a) Hwang, In Sun (a) Choi, Du Seok (a) Lee, Dong Hyuk (a) Hwang, Byung Kook (a)
http://biotech.korea.ac.kr/
"Protein kinases are crucial for plant cell signaling in defense response to pathogen attack. In this study, we have identified and functionally characterized the *Capsicum annuum* pathogen-inducible kinase (*CaPIK1*) gene that is transcriptionally regulated by *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) infection and abiotic stresses. Phylogenetic analyses revealed that CaPIK1 is grouped into receptor-like cytoplasmic protein kinase (RLCK) VII subfamily. The functional serine/threonine (Ser/Thr) protein kinase CaPIK1 not only autophosphorylates itself, but also phosphorylates myelin basic protein (MBP), an artificial Ser/Thr kinase substrate. CaPIK1 is localized to the plasma membrane of onion cells, indicating its possible involvement at early events of the signal transduction pathway during infection. Silencing of *CaPIK1* in pepper plants confers enhanced susceptibility to virulent and avirulent *Xcv* strains. Salicylic acid (SA)-dependant defense responses are attenuated in the *CaPIK1*-silenced plants, including the expression of the SA-dependent marker genes such as *CaBPR1* and *CaSAR82A*, but not of the jasmonic acid-regulated pepper defensin (*CaDEF1*) gene. *Agrobacterium*-mediated transient expression of *CaPIK1* in pepper leaves induces defense-responsive phenotypes such as fluorescent phenolics accumulation, reactive oxygen species and nitric oxide generation, which ultimately leads to the HR-like cell death. Overexpression of *CaPIK1* in non-host Arabidopsis elevates basal resistance to virulent *Pseudomonas syringae* pv. *tomato* DC3000 and *Hyaloperonospora parasitica* isolate Noco2 infection. Together, these results suggest that CaPIK1 modulates the signaling required for defense response to pathogen infection in a SA-dependent manner."
(a) Korea University, School of Life Sciences and Biotechnology, Anam-dong, Sungbuk-ku, Seoul 136-713, Republic of Korea

P48046 Characterization of the role of chitin-responsive protein 1 in plant innate immunity
Antico, Christopher J.-presenter jayantico@gmail.com(a) Ramonell, Katrina M. (a)
"Plants are under constant threat from disease-causing organisms in the environment including bacteria, fungi, and insects. To defend against these pathogens, plants have evolved defense mechanisms that respond to specific disease agents and recognize pathogen-associated elicitors. One important elicitor is the oligosaccharide chitin that is a major component of fungal cell walls and insect exoskeletons. We have identified a gene in Arabidopsis, chitin response protein 1 (crp1) that is strongly induced upon exposure to chitin. CRP1 is a receptor-like protein that is predicted to be localized to the endomembrane system and contains several leucine-rich repeat (LRR) domains. T-DNA insertional mutants in crp1 showed an increased susceptibility to the powdery mildew pathogen, Golovinomycetes cichoracearum as compared to Columbia wildtype suggesting that CRP1 plays a role in chitin defense signaling. To further characterize crp1, over-expression lines have been generated and tested for disease resistance. Additionally, GFP and GUS constructs have been made to provide information on cellular and tissue-specific localization during infection. Since LRR domains are a hallmark of protein-protein interactions, yeast two-hybrid constructs have been generated to identify interacting protein(s) partners that will induce the chitin-signaling pathway. These studies will provide further information on chitin signaling in innate immunity leading to a better understanding of interactions between plants and pathogens."
(a) University Of Alabama

P48047 Molecular interactions between *Macrophomina phaseolina* and its plant host
Shuai, Bin-presenter bin.shuai@wichita.edu(a) Gaige, Andres Reyes (a)
"Charcoal rot is a plant disease caused by soil fungus *Macrophomina phaseolina*. This fungus has a wide range of plant hosts, and it can readily invade over 500 different species of plants including many important crops. Unlike most of fungal pathogens, *Macrophomina* prefers hot and dry conditions. That is why charcoal rot disease was used to be seen mostly in southern states in the US. However, the disease has migrated to northern states in recent years due to climate change resulted from global warming. Although charcoal rot disease is one of the leading causes of reduced crop yield in the US and around the world, we know very little about the pathogen and the mechanisms of host-pathogen interactions. Using *Medicago truncatula* as a model, we established a genetic screen to identify genes that play roles in disease development. We also investigated the expression of disease resistant genes in response to *Macrophomina* infection to gain better understanding on the disease at the molecular level. Our data showed that the plant has delayed response in several disease defense pathways, suggesting the possible mechanism for susceptibility to the fungal pathogen. We hope that the information gained from our study will become valuable for crop improvement in the future. "
(a) Wichita State University

P48048 A cassava geminivirus AC4 membrane protein is required for virus replication and pathogenecity in plant
Chen, Kegui-presenter kchen@desu.edu(a) Fondong, Vincent (a)
"Geminiviruses constitute a large group of plant viruses with circular single-stranded DNA genomes that replicate via double-stranded DNA intermediates following the rolling-circle replication mechanism, similar to bacteriophages. Replication of these viruses has been shown to be initiated by the virus-encoded AC1. Interestingly, geminiviruses encode another gene *AC4*, which is wholly contained within the *AC1* open reading frame but in a different reading frame. While investigating a role for AC4 in geminivirus pathogenicity, we mutated the translation start codon from ATG to ACG with no change in the AC1 amino acid sequence in *East African cassava mosaic Cameroon virus*. The resulting mutant virus completely lost the ability to infect *Nicotiana benthiamiana*. A Southern blot analysis of inoculated leaf tissues showed no evidence of virus replication. Given that there is another downstream methionine, six amino acids away from N-terminus of AC4, this result implies that either the encoded ATG can not function as initiation codon for the gene translation or loss of the motif with six amino acids results in malfunctioned protein and failure of virus DNA replication. We further made mutations within the 6 amino acids. The mutant of both Gly-2 and Cys-3, potential myristoylation site, caused a significant delay in virus symptom development in the infected plants. Taken together, these results suggest that the AC4 membrane protein is involved in cassava geminivirus replication and pathogenesis. "
(a) Delaware State University, Department of Biological Sciences

P48049 Exogenous NO induced resistance to *Botrytis cinerea* in *Arabidopsis*
Yang, Hong Yu-presenter Yanghongyukm@126.com(a) Zhao, Xiao Dan (b) Wu , Jia (b) Li, Xiang (b)
"Hong-Yu Yang[1ab], Xiao-Dan Zhao[2a], Jia Wu[2], Xiang Li[2] [1]College of Life Sciences and Technology, Kunming University, Kunming 650018, China [2]College of Life Sciences, Yunnan Normal University, Kunming 650092, China It has been assumed that NO stimulates the defensive responses of plants to a necrotrophic fungal pathogen *Botrytis cinerea* infection. However, how NO relates to the biochemical mechanisms associated with the resistance to disease is unclear. In this study, we found that *Arabidopsis* leaves pre-treated with NO donor sodium nitroprusside (SNP) suppressed the development of *B. cineres*. Additionally, the dosage levels of SNP applied to the leaves had no direct, toxic impact on the development of the pathogen. This suggests that NO induces intrinsic resistance of *Arabidopsis* to the pathogen. We detected H_2O_2 and $O_2^-$of leaves. We observed a positive correlation between the generation and accumulation of H_2O_2 in the host and the symptoms of the development of disease. The results also showed that NO restrained the generation and accumulation of ROS, especially H_2O_2, as the pathogen interacted with the plant. This suspension of reactive oxidant burst restricted the development of the symptoms of disease. The effect of NO on the antioxidant enzymes was investigated. The activities of CAT and POD were elevated in different degrees in SNP treated leaves, SNP pretreated and pathogen-inoculated leaves. However, the activity of superoxide dismutase was unchanged in the leaves studied and the decrease in H_2O_2 content probably was resulted from the increase in activities of POD and CAT. [a]These authors contributed equally to this work. [b]Corresponding author E-mail: Yanghongyukm@126.com "
(a) Kunming University (b) Yunnan Normal University

P48050 Regulation of *JAZ* gene expression during *Pseudomonas syringae* pathogenesis
Demianski, Agnes J-presenter ajdemian@artsci.wustl.edu(a) Kunkel, Barbara N (a)
"*Arabidopsis thaliana* uses the endogenous hormone jasmonic acid (JA) to regulate development, growth, and responses to abiotic and biotic stresses. JASMONATE ZIM DOMAIN (JAZ) proteins act as negative regulators of JA signaling. In the presence of JA-isoleucine (JA-Ile), an active derivative of JA, JAZ proteins interact with the F-box protein CORONATINE INSENSITIVE 1 (COI1) and are degraded. This activates transcription of JA responsive genes, including the *JAZ* genes themselves. The bHLH transcription factor JASMONATE INSENSITIVE 1(JIN1) has been implicated in the regulation of *JAZ* gene transcription. In this study we examine *JAZ* gene expression in *A. thaliana* during infection with the plant pathogen Pseudomonas syringae pv. tomato DC3000. This pathogen manipulates the JA signaling pathway by producing a virulence factor, coronatine (COR), which mimics JA-Ile. We show that most, but not all, *JAZ* genes are induced during infection in a COR-dependent manner. We also show that the induction of most *JAZ* genes during infection is only partially dependent on *JIN1*, indicating that other unknown transcription factors also regulate *JAZ* genes. Overall, our expression studies reveal that not all *JAZ* genes are regulated in the same way, suggesting that JAZ proteins may have specific functions. Additionally, we demonstrated that *JAZ10* is the mostly strongly induced *JAZ* gene during infection. We identified a *jaz10* mutant and examined the disease susceptibility of the mutant to DC3000 infection. The *jaz10* mutant is more susceptible to DC3000 infection, suggesting that *JAZ10* is a negative regulator of the branch of the JA signaling pathway required for DC3000 susceptibility. "
(a) Washington University in Saint Louis

P48051 RNA-binding proteins confer plant defense against pathogen infection by functioning as an RNA chaperone
HwaJung, Lee-presenter smile5154@hanmail.net(a) Hyun Ju , Jung (a) Hunseung, Kang (a)
"Although a number of recent studies have demonstrated that RNA-binding proteins (RBPs) play important roles in the posttranscriptional regulation of plant defense response against pathogen infection, the cellular function and mode of action of most RBPs in defense response is largely unknown. Here, the biological function of RBPs and their modes of action in defense response were determined in *Arabidopsis thaliana* and tobacco. Glycine-rich RNA-binding protein 7 (GRP7), the RBP of which is ADP-ribosylated by HopU1 and quells the host immunity, conferred defense against fungi and viruses as well as bacteria. To understand the action mechanism of GRP7 in defense, the effect of ADP-ribosylation on the nucleic acid-binding and RNA chaperone activity of GRP7 were investigated. Results showed that ADP-ribosylation of GRP7 by HopU1 abolished RNA chaperone activity of GRP7. Analysis of loss-of-function mutants of pathogen-regulated RBPs demonstrated that a specific type of RBP confers defense against diverse pathogens including bacteria, fungi, and viruses. Collectively, the results show that RBPs play a role as a posttranscriptional regulator of defense response in plants, and particularly perform a function as an RNA chaperone during pathogen infection."
(a) Chonnam National University

P48052 DNA damage and priming for resistance against pathogens
Cahill, David M-presenter david.cahill@deakin.edu.au(a) Kay, Pippa (a) Schenk, Peer M (b) Mintoff, Sharl (a)
"Plant disease is caused by complex biotic and abiotic interactions and is responsible for significant losses to agriculture and the environment. A major challenge is to cut through this complexity and define the basic principles that can be used for disease control. Ultraviolet (UV) light is a ubiquitous component of the environment to which all plants are exposed. UV light damages cellular components, proteins and DNA but plants have shielding and repair mechanisms that enable them to tolerate UV light. UV-C treatment of *Arabidopsis thaliana* induced resistance to virulent isolates of the biotrophic pathogen *Hyaloperonospora arabidopsis*, and experiments using repair deficient mutants indicate that UV-induced DNA photoproducts are involved. In wild type plants UV-C-induced resistance is dose and time dependent and results suggest that in addition to UV photoproducts, an accumulation of endogenous oxidative DNA damage may also trigger resistance to the pathogen. To further examine the role of DNA damage we have analysed the effect of mutations in nucleotide excision repair, photoreactivation of cyclobutane pyrimidine dimers, ROS, flavonoid and callose production and components of the RAR1/SGT1 R protein-linked signalling complex on the resistance of Arabidopsis to the pathogen with or without pre-inoculation treatment with UV-C. The responses observed for the biotroph are now being examined for interactions with *Pseudomonas syringae pv. tomato* and the necrotrophs *Alternaria brassicicola* and *Sclerotinia sclerotiorum* to investigate the specific signalling pathways activated by UV exposure. We are examining UV-induced resistance using fluorescence microscopy, measurement of the activity of a suite of defence-related metabolites and whole genome gene expression analysis."
(a) School of Life and Environmental Sciences, Deakin University, Geelong, Victoria, 3217, Australia (b) School of Integrative Biology, The University of Queensland, St. Lucia, Queensland, 4072, Australia

P48053 *Arabidopsis* HARMLESS TO OZONE LAYER protein methylates a glucosinolate-breakdown product and functions in antibacterial resistance
Nagatoshi, Yukari (a) Nakamura, Tatsuo-presenter t-nakamu@ynu.ac.jp(a)
"Almost all the chlorine-containing gas emitted from natural sources is methyl chloride (CH_3Cl), which contributes to the destruction of the stratospheric ozone layer. Tropical and subtropical plants emit large amounts of CH_3Cl. A gene involved in CH_3Cl emission from *Arabidopsis* was previously identified and designated *HARMLESS TO OZONE LAYER* (designated *AtHOL1* in our study) based on the mutant phenotype. Our previous studies demonstrated that *AtHOL1* and its homologs, *AtHOL2* and *AtHOL3*, have S-adenosyl-L-methionine-dependent methyltransferase activities. However, the physiological roles of AtHOLs have yet to be elucidated. In the present study, our comparative kinetic analyses with possible physiological substrates indicated that all the AtHOLs have low activities towards chloride. AtHOL1 was highly reactive to thiocyanate (NCS^-), a

pseudohalide, synthesizing methylthiocyanate (CH_3SCN). We demonstrated *in vivo* that substantial amounts of NCS^- were synthesized upon tissue damage in *Arabidopsis* and NCS^- was largely derived from myrosinase-mediated hydrolysis of glucosinolates. Analyses with the T-DNA insertion *Arabidopsis* mutants (*hol1*, *hol2* and *hol3*) revealed that only *hol1* showed increased sensitivity to NCS^- in medium and a concomitant lack of CH_3SCN synthesis upon tissue damage. Bacterial growth assays indicated that the conversion of NCS^- into CH_3SCN increased antibacterial activities against *Arabidopsis* pathogens that normally invade the wound site. Furthermore, *hol1* seedlings showed an increased susceptibility towards an *Arabidopsis* pathogen. Here we propose that AtHOL1 is involved in glucosinolate metabolism and defense against phytopathogens. Moreover, CH_3Cl synthesized by AtHOL1 could be considered as a byproduct of NCS^- metabolism. "
(a) Graduate School of Environ. & Info. Sciences, Yokohama National University

P48054 Retrotransposon-mediated transcriptional activation of the rice blast resistance gene *Pit*
Hayashi, Keiko-presenter keko1@affrc.go.jp(a) Yoshida, Hitoshi (a)
"The plant genome contains a large number of disease resistance (*R*) genes that have evolved through diverse mechanisms. Here, we report that a long terminal repeat (LTR) retrotransposon contributed to the evolution of the rice blast resistance gene *Pit*. *Pit* confers race-specific resistance against the fungal pathogen *Magnaporthe grisea*, and is a member of the nucleotide-binding site-leucine-rich repeat family of *R* genes. Compared to the nonfunctional allele Pit^{Npb}, the functional allele Pit^{K59} contains four amino acid substitutions and has the LTR retrotransposon *Renovator* inserted upstream. Pathogenesis assays using chimeric constructs carrying the various regions of Pit^{K59} and Pit^{Npb} suggest that amino acid substitutions might have a potential effect in Pit resistance; more importantly, the upregulated promoter activity conferred by the *Renovator* sequence is essential for *Pit* function. Our data suggest that transposon-mediated transcriptional activation may play an important role in the refunctionalization of additional sleeping *R* genes in the plant genome."
(a) National Agricultural Research Center

P48055 "Water deficit modulates the response of grapevine susceptible to the Pierce's disease pathogen, *Xylella fastidiosa*"
Chung, Eunsook (a) Choi, Hongkyu -presenter hkchoi@dau.ac.kr(a) Iandolino, Alberto (c) Goes da Silva, Francisco (d) Lee, Jai-Heon (a) Cook, Douglas R. (b)
"Susceptible Vitis vinifera responds to Xylella infection with a massive redirection of gene transcription. This transcriptional response is characterized by increased transcripts for phenlypropanoid and flavonoid biosynthesis, ethylene production, adaptation to oxidative stress, and homologs of pathogenesis related (PR) proteins, and decreased transcripts for genes related to photosynthesis. In addition to highlighting potential metabolic and biochemical changes that are correlated with disease, the results suggest that susceptible genotypes respond to Xylella infection by induction of limited, but inadequate, defense response. A long-standing hypothesis states that Pierce's disease results from pathogen-induced drought stress, with the consequent development of disease symptoms. To test this hypothesis, we compared the transcriptional and physiological response of plants treated by pathogen infection, low or moderate water deficit, or a combination of pathogen infection and water deficit. Although the transcriptional response of plants to Xylella infection was distinct from the response of healthy plants to moderate water stress, we observed synergy between water stress and disease, such that water stressed plants exhibit a stronger transcriptional response to the pathogen. This interaction was mirrored at the physiological level for aspects of water relations and photosynthesis, and in terms of the severity of disease symptoms and pathogen colonization, providing a molecular correlation of the classical concept with the disease triangle."
(a) Dong-A University (b) Department of Plant Pathology, University of California, One Shields Avenue, Davis,CA 95616, USA (c) Monsanto Inc. 5th Ave. Davis, CA 95616, USA (d) Bio-Rad Inc. Hercules, CA 94547, USA

P48056 Functional analysis of a novel MAPKKK induced by *Fusarium* phytotoxin trichothecenes
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"Trichothecenes are a closely related family of phytotoxins produced by a phytopathogenic fungi. Type A trichothecenes, such as T-2 toxin, caused rapid and prolonged activation of MAPKs in *Arabidopsis*. However, MAP kinase cascade in the response to trichothecenes is unknown. Novel MAPKKK (MKD1) was identified as a subunit of an AtNFXL1 protein complex. The AtNFXL1 acts as regulator of the trichothecene-induced defense response. The *mkd1* mutant growing on a medium without trichothecenes showed no phenotype, whereas a resistant phenotype was observed in T-2 toxin-treated *mkd1* mutant. "
(a) Advanced Science Research Center, Kanazawa University

P48057 Jasmonate-dependent regulation of the galactinol synthase gene *AtGolS1* against *Botrytis cinerea* in the systemic resistance-induced Arabidopsis by root-colonization of *Pseudomonas chlororaphis* O6
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"Root colonization of the rhizobacterium, *Pseudomonas chlororaphis* O6, induced the expression of a galactinol synthase gene and resultant galactinol conferred systemic resistance in the leaves of cucumber against challenging fungal pathogen (Kim et al. 2008 Mol. Plant-Microbe Interac. 21: 1643-1653). The Arabidopsis-*Botrytis cinerea* system is introduced to understand signal transduction of the galactinol in the O6-mediated induced systemic resistance (ISR). Expression of the *AtGolS1* gene was specifically induced upon infection with the fungal pathogen *B. cinerea* among 10 independent Arabidopsis galactinol synthase (*AtGolS*) genes in the family, and only the gene expression was primed by O6 colonization against the pathogen in the leaves of Arabidopsis. Arabidopsis T-DNA insertion mutants at the *AtGolS1* gene compromised O6-mediated ISR against the pathogen suggesting important role of the gene in the ISR. The O6 colonization induced priming of the *AtGolS1* gene transcription as well as ISR in several Arabidopsis signaling mutants against the pathogen, but not in the *jar1-1* and *coi1* mutant lines. Exogenous jasmonate treatment induced the *AtGolS1* gene transcription in wild-type Col-0 plants, but salicylic acid and 1-aminocyclopropane-1-carboxylate did not. Therefore, the use of pathway-specific target genes as well as signaling mutants allowed us to elucidate that primed expression of the specific *AtGolS1* gene by O6 colonization is located in the jasmonate-dependent pathway to ISR in Arabidopsis against the *B. cinerea* infection. "
(a) Chonnam National university (b) Chunnam Techno College

P48058 Activation of a fungal-responsive MAPK cascade induces the biosynthesis of polyamine in *Arabidopsis*
Kim, Su-Hyun-presenter falsetear@hanmail.net(a) Jang, Eun-Kyoung (a) Kim, Kwang Sang (b) Zhang, Shuqun (c) Kim, Young Cheol (a) Cho, Baik Ho (a) Yang, Kwang-Yeol (a)
"Pathogen or elicitor recognition in plants triggers activation of the mitogen-activated protein kinase (MAPK) cascade, and two Arabidopsis MAPKs, MPK6 and MPK3, are known to be involved in signaling plant defense response to pathogens. However, little is known about the downstream target substrates or defense-related genes that are regulated by this cascade. Recently, we demonstrated that a pathogen-responsive MAPK cascade regulates polyamine synthesis, especially putrescine synthesis, through transcriptional regulation of the biosynthetic genes in tobacco. Polyamines in

plants are involved in various physiological and developmental processes including abiotic and biotic stress responses to conditions such as high osmotic stress, high salinity, low temperature and pathogen attack. In this study, we report that the expression of *ADC1* and *ADC2* in *GVG-NtMEK2^{DD}* transgenic *Arabidopsis* was rapidly induced following DEX treatment. Induction of putrescine was detectable in *GVG-NtMEK2^{DD} Arabidopsis* after DEX treatment, preceded by MPK6/MPK3 activation. In addition, the expression of these genes was partially compromised in *GVG-NtMEK2^{DD}/mpk6* and *GVG-NtMEK2^{DD}/mpk3* mutants following DEX treatment, which suggests that MPK6 and MPK3 play a positive role in the regulation of putrescine biosynthesis in *GVG-NtMEK2^{DD}* transgenic *Arabidopsis*."
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P48059 Synergistic regulation between ROS accumulation and ethylene production in response to biotic stress
Wi, Soo Jin-presenter akrp@sunchon.ac.kr(a) Park, Ky Young (a)
"To investigate the complexity between ROS and ethylene in response to biotic stress, the fungal pathogen, *Phytophthora parasitica* var. *nicotianae*, was treated in wild type and transgenic tobacco plants, in which each gene of ethylene or ROS production was down-regulated. *NtACS4* expression increased within 1 h, when ROS accumulation also increased rapidly, following inoculation with *Phytophthora*. The rapid and transient induction of *NtACS4* expression was responsible for the first ethylene peak, which was followed by a very large accumulation of *NtACS1* transcript from 48 to 72 h. This profile of ethylene production was similar with the pattern of ROS accumulation, determined by DAB staining and expression of profiles of isoform of NADPH oxidase, *RbohD* and *Rboh F*. Although the gene expression pattern of antioxidative enzymes such as *CuZnSOD* and cytosolic *APX* was also corresponded with those profiles, second peak of those transcripts were accumulated at post-inoculation from 36 h to 48 h, when was ahead of second peak of ethylene and ROS accumulation. After 72 h the amounts of those transcripts were significantly decreased, which were responsible for huge accumulation of ROS in pathogen-treated leaves. Therefore, it may be suggested that ROS accumulation at later stage was induced by a respectable decrease in gene expression of antioxidative transcripts, not by a direct increase in ROS production by NADPH oxidase. It may be also implied that the first peak of ROS accumulation, which can be named with oxidative burst, was significantly responsible for *de novo* production of ROS by NADPH oxidase. Also our data suggested that huge volume of ethylene production at later stage is signaled by second peak of ROS accumulation."
(a) Sunchon National University

P48060 Arabidopsis SGT1a and SGT1b Exhibit Molecular Chaperone Activity
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"Previously, it was proposed that RAR1 (required for Mla12 resistance) and SGT1 (suppressor of the G2 allele of Skp1) which formed a complex with Hsp90 play important roles in R gene-mediated plant disease resistance. We isolated and two SGT1 genes (AtSGT1a and AtSGT1b) from Arabidopsis, expressed in E. coli, purified the proteins, and analyzed their biochemical properties. In a size exclusion chromatography (SEC) analysis, AtSGT1b was eluted at the high molecular weight complex fractions, while AtSGT1a was eluted at the low molecular weight complex fractions. When holdase chaperone activity of the proteins was measured using malate dehydrogenase (MDH) as a substrate, AtSGT1b exhibited a relatively higher concentration dependent chaperone activity, while AtSGT1a showed a concentration independent chaperone-like activity. Chaperone activity assay using domain deletion mutant proteins indicated that the TPR and CS domains of AtSGT1a and AtSGT1b are important for the chaperone activity. Western blot analysis using polyclonal antibodies indicated that the expression of AtSGT1a is induced by heat but AtSGT1b shows rather constitutive expression. Our results indicate that AtSGT1a and AtSGT1b may play an important role in R gene-mediated plant disease resistance by acting as a chaperone though direct or indirect interactions with Hsp90. [Supported by EB-NCRC & BK21 program]"
(a) Division of Applied Life Science (BK21 Program), EB-NCRC and PMBBRC

P48061 Functional analysis of *NPR3* reveals a negative regulatory role in defense response during early flower development in *Arabidopsis*
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"Plants have evolved a sophisticated inter-connected web of defense mechanisms that involve the activity of the protein NPR1 as a central mediator of plant pathogen response signaling pathways. NPR1 is a member of the BTB/POZ domain containing protein family, and five *NPR1*-like genes exist in *Arabidopsis*. We explored the function of all six members in NPR gene family in *Arabidopsis* via database mining and promoter GFP fusions. One of these genes, *NPR3*, was shown to be highly expressed in unopened flower tissues, suggesting a unique role in flower development. An *Arabidopsis npr3* mutant, challenged with *Pseudomonas syringae* pv. tomato DC3000, showed normal silique development and 30-fold less bacterial growth compared to wild type plants revealing that *NPR3* acts as a negative regulator of plant defense response during early flower development. Although the level of *NPR1* is not affected, the basal and induced level of *PR1* is up regulated in *npr3* unopened flowers. Additionally, Bimolecular Fluorescence Complementation (BIFC) assay shows that NPR3 and TGA2 interact in cytoplasm of stable transgenic plants, suggesting that NPR3 repression may function by retaining TGA2 in the cytoplasm. Although there is no difference in the relative growth rate (RGR) between wild type and *npr3* mutants, the *npr3* mutant exhibits lower fitness manifested in less seed production, demonstrating the cost of constitutively expressed defense pathways. "
(a) The Huck Institutes of Life Sciences, The Pennsylvania State University (b) The Department of Horticulture, The Pennsylvania State University

P48062 OsMPK5 phosphorylates OsEIL1 and regulates ethylene signal transduction in rice
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http://www.ppath.cas.psu.edu/FACULTY/Yang.htm
"MAP kinase cascades play an important role in the regulation of plant growth and development as well as biotic and abiotic stress responses. Rice mitogen-activated protein kinase 5 (OsMPK5), an orthologue of *Arabidopsis* AtMPK3, was previously found to positively regulate rice tolerance to abiotic stresses, but negatively modulate disease resistance to pathogen infection. However, the stress signaling pathway(s) mediated by OsMPK5 remains to be elucidated. In this study, we demonstrate that OsMPK5 may act as a negative regulator of ethylene signaling by impinging on signal transduction between OsEIN2 and OsEIL1 in rice. Suppression of *OsEIN2* was shown to increase OsMPK5 mRNA, protein, and kinase activity. In vitro protein binding and kinase assays reveal that OsMPK5 interacts with and phosphorylates OsEIL1 in vitro. In addition, OsMPK5 protein or kinase activity is negatively correlated with the OsEIL1 protein level and downstream *OsERF1* and *OsPR5* expression in transgenic rice, suggesting that OsMPK5 mediates biotic and abiotic stress responses by negatively regulating OsEIL1 stability and ethylene signaling. Transgenic analysis showed that OsEIL1 positively regulated *OsERF1* and *OsPR5* expression and increased rice disease resistance against the rice blast infection."
(a) Pennsylvania State University

P48063 *Xanthomonas oryzae* pv. *oryzae* induces rice *HEN1* expression for disease susceptibility
Huang, Sheng (a) Yu, Hongman (a) Antony, Ginny (b) Liu, Bo (a) Frank, White (b) Yang, Bing-presenter byang@iastate.edu(a)
"*Xanthomonas oryzae* pv. *oryzae* (Xoo) depends on a type III secretion system to deliver substrate proteins, the so-called type III (T3) effectors, into host cells for pathogenesis of rice. A group of Xoo T3-effectors belong to transcription activator-like (TAL) T3-effector family. Each TAL effector transcriptionally activates a corresponding host gene for disease susceptibility in the manifestation of its virulence. Here, we report that one TAL T3-effector from Xoo strain PXO99 targets the rice small RNA processing pathway to enhance host susceptibility for bacterial blight. Microarray and northern hybridization analyses revealed that the rice gene *HEN1* (*OsHEN1*) was highly induced in PXO99 infection compared to a T3SS mutant. The induction is specifically associated with the presence of the TAL T3-effector gene *pthXo8* in PXO99. PthXo8 is an uncharacterized TAL effector with 19 repeats of 34 amino acids in central region, as well as the three nuclear localization motifs and transcription activation domain in the C-terminal portion, characteristic of TAL effectors from Xoo. Strains derived from PXO99 with a *pthXo8* mutation fail to induce *OsHEN1* and, concomitantly, have reduced virulence in susceptible rice plants. Re-introduction of *pthXo8* restores to the mutant the ability to induce *OsHEN1* upon infection and full virulence, indicating that *pthXo8* is a virulence factor. RNAi-mediated gene knock down of *OsHEN1* expression in transgenic plants resulted in reduced disease susceptibility to PXO99 infection, indicating induction of *OsHEN1* is associated with full bacterial virulence. Our results indicate *OsHEN1* is a disease susceptibility gene and as genetic disease vulnerability embedded in the small RNA-mediated host cellular functions that is specifically exploited by Xoo."
(a) Department of Genetics, Development and Cell Biology, Iowa State University (b) Department of Plant Pathology, Kansas State University

P48064 Purification and characterization of a D-mannose-binding lectin from the red clover
sasaki, izumi-presenter sasaki@oyama-ct.ac.jp(a) mastumoto, kaori (a)
"Broomrapes (*Orobanche* spp.) are holoparasites, which attack roots of various legumes. Lectins are widely distributed in plant, especially in legumes which control invaders, have been reported. A lectin was isolated and purified from the red clover, the host plant of parasitic plant *Orobanche minor*, by a combination of ammonium sulfate precipitation, affinity chromatography and ion-exchange chromatography. The purified lectin was homogeneous by SDS-PAGE with a molecular weight of approximately 34 kDa. It is thermally stable and shows maximum activities between pH 6 and 7.2. The lectin bound the ß-glucosidase of *O. minor* and exhibited binding affinity towards D-mannose. Since a high concentration of mannose in *O. minor*, this parasite could infect the host plants. "
(a) oyama national college of thechnology

P48065 Evaluation of lime sulfur and systemic acquired resistance activator for pear diseases control under laboratory and field conditions in korea
Min, Kwang-Hyun-presenter rokmc9540@naver.com(a) Lee, Yu Kyung (a) Cho, Young-Sik (b) Lee, Sang-Hyun (a) Kim, Wol-Soo (a) Kim, Young Cheol (a) Cho , Baik Ho (a) Yang, Kwang-Yeol (a)
"Pear diseases including scab and rust have been troubled in all pear producing regions. Fungicides were exclusively used to control the disease. Since consumers are demanding products of pesticide-free, the alternative ways are needed instead of conventional fungicide sprays. Here we introduce the efficacy of lime sulfur and a systemic acquired resistance activator for pear diseases control. The object of disease control using lime sulfur is to reduce primary inoculum on spring season. On 2008, fallen leaves from pear orchard infected by scab and other diseases were collected and treated with lime sulfur or water. Pear fruits on water-treated fallen leaves became severely infected, while they on lime sulfur-treated fallen leaves were fresh and uninfected after 10days incubation. The purpose of disease control using synthetic SAR activator, BTH, is to improve natural defense responses in pear. We have investigated the activation of mitogen-activated protein kinases pathway by BTH treatment. By quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR), we examined the expression of defense-related genes by BTH such as genes encoding pathogenesis-related (PR) protein and phenylalanine ammonia lyase (PAL). The results indicated that a possible involvement of *PR*, *PAL* and MAPK pathway in BTH-induced defense responses. "
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P48066 Pi5-mediated resistance to Magnaporthe oryzae requires two NB-LRR proteins
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"Rice blast, caused by the fungus *Magnaporthe oryzae*, is one of the most devastating diseases of rice. To understand the molecular basis of *Pi5*-mediated resistance to *M. oryzae*, we cloned the resistance (*R*) gene at this locus using a map-based cloning strategy. Genetic and phenotypic analyses of 2014 F2 progeny from a mapping population derived from a cross between *IR50*, a susceptible rice cultivar, and the *RIL260* line carrying *Pi5* enabled us to narrow down the *Pi5* locus to a 130-kb interval. Sequence analysis of this genomic region identified two candidate genes, *Pi5-1* and *Pi5-2*, which encode proteins carrying three motifs characteristic of *R* genes: an N-terminal coiled-coil (CC) motif, a nucleotide-binding (NB) domain, and a leucine-rich repeat (LRR) motif. In genetic transformation experiments of a susceptible rice cultivar, neither the *Pi5-1* nor the *Pi5-2* gene was found to confer resistance to *M. oryzae*. In contrast, transgenic rice plants expressing both of these genes, and generated by crossing transgenic lines carrying each gene individually, conferred *Pi5*-mediated resistance to *M. oryzae*. Gene expression analysis revealed that *Pi5-1* transcripts accumulate after pathogen challenge, whereas the *Pi5-2* gene is constitutively expressed. These results indicate that the presence of these two genes is required for rice *Pi5*-mediated resistance to *M. oryzae*."
(a) Graduate School of Biotechnology & Plant Metabolism Research Center (b) IRRI-Korea Office (IKO), National Institute of Crop Science (c) Rice Division, Yeongnam Agricultural Research Institute (d) Crop Environment and Biotechnology Division, National Institute of Crop Science (e) Department of Plant Pathology, University of California

P48067 Biosynthesis and regulation of 3-deoxyanthocyanidin phytoalexins induced during Sorghum-Colletotrichum interaction: Heterologous expression in maize.
Chopra, Surinder-presenter sic3@psu.edu(a) Gaffoor, Iffa (a) Ibraheem, Farag (b)
"Leaf blight is one of the main foliar fungal diseases of maize. The disease inoculum survives in the field debris and can repeatedly infect the maize plant at any stage during its life cycle. Although disease incidence can be reduced by tilling and rotating with non-cereal crops, identification of resistant germplasm is more promising. Unfortunately, most of the maize germplasm screened to date is susceptible to the disease. Sorghum bicolor, a close relative of maize produces a class of compounds (3-deoxyanthocyanidins) in response to fungal attack. These compounds have antifungal properties and confer resistance to leaf blight. Biosynthesis of these compounds is not very well understood but based on their structural similarities these compounds may be arising through the flavonoid pathway. Although maize produces numerous flavonoid compounds, it does not produce 3-deoxyanthocyanidins in leaves in response to fungal attack. We have identified candidate genes and transformed maize in an effort to engineer the biosynthesis of these antifungal compounds and thereby enhance resistance to leaf blight. We have also identified important cis-regulatory elements

in promoter of one of the Myb transcription factor genes. We have further characterized the response of transgenic maize lines when infected with the fungus that causes southern corn leaf blight. Profiling of several different flavonoid compounds using HPLC and LC-MS has been performed. Results showing induction of specific compounds will be presented."
(a) Pennsylvania State University (b) University of Illinois

P48068 Genetic screening and characterization of *pmr5* suppressors
Li, Yongqing-presenter yongqing@nature.berkeley.edu(a) John, Vogel (b) Bi-Huei, Hou (c) Shauna, Somerville (a)
" In Arabidopsis, mutation of *PMR5* (*Powdery Mildew Resistant 5*) confers resistance to the powdery mildew species *Golovinomyces cichoracearum* and *G. orontii*. This mutant also displayed a dwarf morphology and had enriched pectin in its cell walls. *PMR5* encodes a novel protein that belongs to a large family of plant-specific proteins of unknown function. Genetic study showed that *pmr5*-mediated resistance does not require signaling through either the salicylic acid or jasmonic acid/ethylene defense pathways. To study the molecular mechanisms of *pmr5*-mediated defense responses, a genetic screen for mutations that restore susceptibility to *G. cichoracearum* was carried out. Twenty *pmr5* suppressors were found that partially or fully suppressed resistance to powdery mildew. Of these, eight suppressor mutants resembled wild type in both morphology and susceptibility to *G. cichoracearum*. Nine kept the *pmr5*-like dwarf phenotype, while three mutants showed a more severe growth defect than the *pmr5* single mutant. Many of the suppressor mutations had pleiotropic effects on plant development including a change of flowering time or root growth. Further characterization and cloning of *pmr5* suppressors will provide knowledge of the molecular mechanisms of *pmr5*-mediated defense responses."
(a) UC Berkeley (b) Agricultural research services, USDA (c) Stanford University

P48069 WRKY53 Transcription Factor Is a Key Component in Flg22 Signaling
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"WRKY proteins, a family of transcription factors consisting of over seventy proteins, are implicated in regulating diverse cellular processes. However, the precise functions of most of them are not known. Here, we investigated the role of WRKY53 in flg22 peptide, a Pathogen Associated Molecular Pattern (PAMP), signaling in Arabidopsis. We show that the expression of *WRKY53* is induced by flg22 in an FLS2-dependent manner. Studies with a MAP kinase kinase (MAPKK) inhibitor suggest that the MAP kinase pathway might not be involved in flg22-induced expression of *WRKY53*. The induction of *WRKY53* expression by flg22 is reduced significantly in the presence of an inhibitor of the 26S proteosome, suggesting that proteolysis of a negative regulator might be involved in this activation pathway. In *wrky53-1* mutant plants, promoter activities of *WRKY53* and three other flagellin-induced genes were elevated in the absence of flg22. Furthermore, overexpression of WRKY53 in wild type or *wrky53-1* plants suppressed flg22 activation of its own promoter and three other promoters that are activated by flg22 whereas the activity of one flg22-induced promoter was enhanced. These results suggest that WRKY53 functions as a negative regulator of some and positive regulator of other flg22-induced genes. Infection studies revealed that *wrky53* plants are moderately more susceptible to pathogens and appeared to be compromised in flg22 induced resistance. In contrast, wild type or *wrky53* plants overexpressing WRKY53 showed elevated PR gene expression and reduced disease. GFP-WRKY53 fusion protein, as expected of a transcription factor, localized to the nucleus. Together, our results indicate that WRKY53 plays a key role in flg22 induced defense signaling. "
(a) Colorado State University (b) Duke University

P48070 Expression of nbs-LRR gene in Citrus plant infected with Xylella fastidiosa
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"The Brazilian citrus industry is responsible for 85% of the world concentrated orange juice production being the major world exporter for this product. One of the problems affecting the Brazilian citrus orchards is their vulnerability to pests and diseases, mainly due to the low genetic diversity of the commercial varieties used. Citrus Variegated Chlorosis (CVC) caused by Xylella fastidiosa is one of the most important diseases, causing large damages in the production and affecting all commercial sweet orange (Citrus sinensis L. Osb) varieties. However, it has been observed that mandarins (Citrus reticulata) are considered tolerant or resistant to this bacterium. This species is very important for studies on defense mechanisms as source of resistance genes. In sillico analysis comparing C. sinensis and C. reticulate EST libraries identified a gene that encodes a NBS-LRR type protein which is possibly involved in the recognition of a molecule from the bacteria or the plant triggering a signaling pathway that induces the expression of resistance genes. Hereof, the objective of this study was to verify the expression level of the NBS-LRR gene in sweet orange and mandarin plants inoculated with X. fastidiosa 9a5c strain through RT qPCR. As control, plants were inoculated with PBS buffer. After 14 days, PCR analysis with specific CVC primers confirmed X. fastidiosa infection, and RNA was isolated for the expression analysis. The expression level of the NBS-LRR gene did not change in sweet orange infected with X. fastidiosa, however we observed 10 fold increase in mandarin suggesting um possible involvement of the NBS-LRR protein in the defense mechanism of these plants. The copy number of this gene in those species is also under evaluation through Southern blot."
(a) Centro Universitario Herminio Ometto (b) Centro Apta Citros Sylvio Moreira

P48071 Response of Murcott tangor and Pera sweet orange to Citrus leprosis virus C (CiLV-C) and *Brevipalpus phoenicis* mites analyzed by 2DE
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"Citrus leprosis is transmitted by the tenuipalpidae mite *Brevipalpus phoenicis*, causing chlorotic or necrotic local lesions in leaves, fruits and stems of susceptible hosts. The control of the vector in Brazil costs around US$ 75 million per year. In this work we analyzed differentially expressed plant proteins in response to the mite feeding injury and the virus infection 48 hours after inoculation. The experiment consisted of three plants of each Murcott tangor and Pera sweet orange infested with viruliferous mites and three other plants of each genotype inoculated with non-viruliferous mites. The proteins were extracted with phenol from 3g of fresh leaf tissues. Isoelectric focusing was performed using 18cm 3-10pH non-liner immobilized pH gradient strips. Second dimension electrophoresis (SDS-PAGE) was performed according to Laemmli. For image and statistical analysis we used the software Image Master 2D platinum 7 (GE Healthcare). Both genotypes yielded around 15mg of proteins per gram of leaf and exhibited similar pattern in SDS-PAGE for the healthy controls. The 2DE gel analysis showed 712 spots for healthy Murcott tangor and 656 spots for healthy Pera sweet orange. The differentially expressed spots will be further identified by mass spectrometry. "
(a) Unicamp (b) Centro APTA Citros "Sylvio Moreira" (c) Embrapa Cassava and Tropical Fruticulture (d) UFRPe

P48072 Taro (*Colocasia esculenta*) transformed with wheat (*Triticum aestivum*) oxalate oxidase gene showed increased resistance to two taro pathogens *phytophthora colocasiae* and *sclerotium rolfsii*

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"Taro [*Colocasia esculenta* (L.) Schott], a tropical root crop, is declining in production in many areas of the world due to the spread of the oomycete pathogen *Phytophthora colocasia* and other diseases. To increase resistance to Taro Leaf Blight, the disease caused by this pathogen, taro cv. Bun long was transformed with the oxalate oxidase (OxO) gene from wheat (*Triticum aestivum* L.) using *Agrobacterium tumefasciens*. Transformation and expression of the OxO gene was confirmed using PCR, RT-PCR, Southern blot analysis, and OxO activity test. One independent line contained one gene insertion (g5) and a second independent line contained four copies of the gene. A laboratory bioassay using tissue-cultured plantlets that were inoculated with spores of *P. colocasiae* showed the complete arrest of this disease in transgenic line g5, apparently due to a hypersensitive response. In contrast, the pathogen killed non-transformed cv. Bun long after an average of eight days from inoculation. In a separate leaf disk bioassay using one-year-old potted plants that were inoculated with spores of *P. colocasiae* , it was confirmed that transgenic plants had increased resistance to this pathogen. In another bioassay to test fungal disease resistance to *Sclerotium rolfsii*, the transgenic line g5 exhibited increased disease resistance. These results demonstrated that genetic transformation of high oxalate-containing taro with an oxalate oxidase gene can confer increased resistance to both the oomycete pathogen, *P. colocasiae* and the fungal pathogen, *Sclerotium rolfsii*."
(a) Hawaii Agriculture Research Center (b) University of Hawaii

P48073 "Transgene-derived small RNAs in Papaya ringspot virus-resistant, Hawaiian Rainbow papaya"
Suzuki, Jon Y.-presenter jon.suzuki@ars.usda.gov(a) Tripathi, Savarni (a) Carr, James B (a) Ferreira, Stephen A. (b) Gonsalves, Dennis (a)
"Rainbow papaya, the first commercialized transgenic fruit crop was released in 1998 to combat *Papaya ringspot virus* (PRSV), an RNA virus that was ravaging the Hawaiian papaya industry in the 1990□$B!G□(Bs. Today, Rainbow represents the major planted acreage (> 70%) of papaya in Hawaii. Rainbow is a yellow-fleshed F_1 hybrid that is hemizygous for the transgene. Viral resistance is associated with the PRSV coat protein (*CP*) transgene derived from Hawaiian PRSV isolate HA 5-1 that was inserted in the original, transformed red-fleshed Sunset papaya, line 55-1, by particle bombardment. Previous studies have shown that papaya homozygous for the transgene had broader resistance to various geographic isolates of PRSV outside of Hawaii, yet steady state levels of *CP* transgene messenger RNA were lower as compared to hemizygous transgenic papaya. In addition, although the full length PRSV *CP* transgene was translatable, resistance was shown to be higher to those geographic isolates of PRSV with higher degrees of nucleic acid sequence homology between their *CP* genes and the 55-1 derived, *CP* transgene. Taken together, these evidences suggested involvement of transgene-derived posttranscriptional gene silencing (PTGS) and an RNA-mediated resistance to PRSV. Further information on the mechanism of transgene-derived silencing and its role in disease resistance in papaya will be beneficial for improved resistance through improved transgene design. With this aim, we investigated whether small RNAs, molecular hallmarks of PTGS could be detected from the *CP* transgene in Rainbow as well as SunUp, a cultivar homozygous for the transgene and present data on a sensitive, nonradioactive detection method for characterizing their expression patterns."
(a) USDA-ARS-Pacific Basin Agricultural Research Center, Hilo, Hawaii (b) Plant and Environmental Protection Sciences, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa

P48074 Suppression of root rot causing pathogens in *Capsicum annuum* by space competence and the activation of systemic resistance by a native Mexican isolate of binucleate *Rhizoctonia*
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"The root rot disease in pepper is one of the most important in this crop. Biocontrol is an alternative strategy to counteract the causal agents fungal pathogens, Avirulent strains of binucleate *Rhizoctonia* has emerged as useful biocontrol agents. A mechanism that explains the pathogen suppression is the activation of the systemic acquired resistance (SAR) in plant, a state of generalized resistance against a broader range of pathogens. In a previous study in *C. annuum*, we have found some defense response genes induced by this avirulent fungus. The object in this study is to analyze the colonization strategy in the root tissue in *C. annuum* of this binucleate *Rhizoctonia* isolate, alone or in competence with root rot causing pathogens; in addition, the molecular profile that characterize this plant-fungus benign interaction. The techniques used were scanning electronic and confocal microscopy, and subtractive hybridization. This binucleate *Rhizoctonia* strain colonize the root in *C. annuum* following the tissue topography in the root surface, taking advantage of the external intercellular fissures on the epidermis, and it reach the hypodermis but do not impact the parenchyma, with no evidence of necrosis in plant tissue, whereas others root rot causing pathogens grow profusely inter and intracellularly causing necrosis in epidermis and cortex. In co-inoculations, binucleate *Rhizoctonia* wins, colonizing early the plant tissue. In the molecular profile it was found 130 ESTs, 24% are defense genes, two align with SAR markers. In conclusion, in *C. annuum* this binucleate *Rhizoctonia* colonize the epidermis without necrosis, and exerts suppression against root rot causing pathogens by means of activation of SAR and by space competence in the colonization process in root tissue. "
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P48075 Leaf proteome analysis of plant-pathogen interaction: Citrus-*Alternaria alternata* tangerine pathotype
Stuart, Rodrigo M.-presenter rodrigostuart@centrodecitricultura.br(a,b) Bastianel, Marines (a) Bonatto, Jose M.C. (c) Labate, Carlos A. (c) Machado, Marcos A. (a)
http://www.centrodecitricultura.br
"The fungus *Alternaria alternata* tangerine pathotype affects mandarins and their hybrids causing the disease known as Alternaria brown spot. It affects twigs, young leaves and fruits; and the symptoms include brown to black necrotic spots surrounded by a yellow halo. Chlorosis and necrosis continue to expand along the veins due to the spread of the host-selective ACT-toxin produced by the fungus. Severe cases can lead to leaves and fruits abscission. In order to evaluate the plant response to the fungal infection in a proteomic level, young leaves (2-3cm length) of susceptible plants of Murcott tangor and tolerant plants of Pera sweet orange were inoculated with a conidial suspension of *A. alternata*. Healthy plants were used as experimental control. In order to normalize the patterns of protein expression before inoculation, plants were maintained for two weeks in a plant growth chamber at 27 Celsius degrees, 70% relative humidity in a L:D 12:12h photoperiod. Twelve hours after inoculation, leaves were collected and proteins were extracted with phenol from 3g of tissues and the isoelectric focusing was performed in polyacrylamide IPG strips (pH 3-10NL). 2DE images were analyzed using ImageMaster 2D (GE-Healthcare). The initial data exhibited 448 and 325 protein spots in healthy leaves of Murcott and Pera respectively. Twelve hours inoculated plants showed a reduction in the total number of spots detected for both Murcott (337 spots) and Pera (290 spots). Spots detected from inoculated leaves of Pera showed 78% matching to those from healthy leaves; inoculated Murcott leaves showed 82% matching to their healthy control. Complementary analysis are been performed to identify all the differentially expressed spots and their

identities."
(a) Centro APTA Citros "Sylvio Moreira" (b) Unicamp (c) Esalq/ USP

P48076 " Arabidopsis chitin inducible ATL2 Protein, a RING-H2 type E3-ligase, interacts with multiple transcription factors as a role in the defense response."
Jeon, Su Jeong-presenter allsu83@nate.com(a) Son, Geon Hui (a,b) Shin, Su Yeong (a) Kim, Hey Jin (a) Sim, Soon Ae (a) Wan, Jinrong (b) Yun, Dae-Jin (a) Gary, stacey (b) Hong, Jong Chan (a,b)
"Protein degradation by ubiquitylation is a common regulatory mechanism that controls a range of cellular processes such as cell division, differentiation, and defence responses. Ubiquitin ligases play a key role in determining the target specificity of protein degradation processes via the ubiquitin/26S proteasome pathway. In Arabidopsis thaliana genome more than 1,100 gene are thought to encode ubiquitin ligases. To study the role of E3 ligases in fungal defense signaling pathway we have analyzed the chitin elicitor responsive E3-ligase gene from ATH1 Affymetrix microarrays. 58 transcripts are identified in the list of chitin responsive genes that are up- and down-regulated by more than 1.5 fold. Among them RING-H2 type E3 ligases of ATL family are identified as a major class of chitin-induced genes. Proteins of ATL family are characterized as having a conserved transmembrane domain and RING-finger domain which are located in toward the N-terminus. The ATL2 is selected as representative of ATL proteins to study role of protein ubiquitylation in fungal defence responses as it is rapidly induced after exposure to chitin or inactivated crude cellulase preparations. To understand function of ATL2 we have screened interacting protein using yeast two hybrid screen and identified several transcription factors as a potential target of ATL2. These protein belong to transcription factors of bHLH, NAC and G2-like family. This result suggests that ATL2-transcription factor play a role in defence responses by degradation of specific ubiqutinylation target transcription factors. This research is supported by KOSEF grant (MOST: R01-2007-000-11232-0 KRF-2008-314-C00362), EB-NCRC and BK21 program. *corresponding author: 055-751-5960 e-mail: jchong@gnu.ac.kr "
(a) Geongsang National University (b) University of missouri-columbia

SESSION P49 – PLANT SYMBIONT INTERACTIONS

P49001 Cyanobacterial associations with bryophytes
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"Among plant-microbial associations, the cyanobacterial communities that grow epiphytically on mosses and liverworts have been the subject of relatively little research, in spite of their potentially symbiotic nature and their significant nitrogen-fixing capabilities (e.g., Dodds et al., 1995; Solheim & Zielke, 2003; DeLuca et al., 2002, Gentili et al., 2005; DeLuca et al., 2006; DeLuca et al., 2008). In this study, cyanobacterial-bryophyte communities from a boreal forest in Wisconsin were characterized based on morphotype data, cultures, and DNA sequence data, revealing that these associations are surprisingly common and diverse. Approximately 500 bryophyte samples were collected using a completely-randomized design during four weeks in 2007-2008 (summer, fall, winter, and spring). These yielded a diverse collection of approximately 50 species of mosses and liverworts, which were screened for cyanobacteria using epifluorescence microscopy. Because such associations are little-studied, previous estimates have suggested that they are rare. But at this site, the percentage of bryophyte samples hosting cyanobacteria varied from approximately 20% to 50% for summer, fall, and winter samples. A wide array of cyanobacterial morphotypes were detected, many with heterocytes potentially capable of nitrogen fixation. Overall, approximately 25% of all bryophyte samples contained heterocytous cyanobacteria, representatives of which were cultured and characterized using molecular phylogenetics. The composition of the cyanobacterial community and several potentially novel bryophyte-cyanobacterial associations will be reported, and the significance of these putative mutualisms to the evolution of later-evolved plant-microbe associations will be discussed. "
(a) University of Wisconsin at Madison

P49002 Next Generation SOLiD metagenomic sequencing of the greater duckweed Spirodela polyrhiza reveals novel symbiotic relationships
Michael, Todd P-presenter tmichael@waksman.rutgers.edu(a) Trumbull, Julia E (a) Wang, Wenqin (a) Zdepski, Ana (a) Lutz, Kerry (a) Kerstetter, Randall (a)
"The Lemnaceae, commonly known as Duckweeds, are the smallest, fastest growing and simplest of flowering plants, representing a high-impact biofuel feedstock that is ripe for exploitation. There are forty species representing five Genera in this tiny aquatic monocot family, *Spirodela, Lemna, Landoltia, Wolffia and Wolffiella*. *Lemna*) has long been a model of choice for biochemical studies and William Hillman carried much of the pioneering work in plant photoperiodism out using Lemna. The base chromosome number of this Family is 10, and the genome sizes have a 10-fold range (150 to 1500 mb) representing diploids to octaploids. Some of the current uses of Lemnaceae are a testimony to its scientific, commercial and biomass utility: basic research and evolutionary model system, toxicity testing organism, biotech protein factories, wastewater remediation, high protein animal feed, and carbon cycling. Currently we maintain the Duckweed Collection, which contains hundreds of sterile duckweed strains available for the general research community. We developed a new way to transform duckweed, which skips callus induction and regeneration, and only takes 1 week. Currently, the 150 mb *Spirodela polyrhiza* genome is being sequenced by the DEO-JGI through the Community Sequencing Program (CSP). We have used SOLiD Next Generation high-throughput sequencing to interrogate the symbiotic communities of bacteria in the *S. polyrhiza* meristematic pocket. One of the main bacterial species that we found associated with S. polyrhiza is a photosynthetic, nitrogen fixing *Bradyrhizobium*. We have assembled a novel strain of *Bradyrhizobium* and uncovered an important relationship using the deep sequencing technologies."
(a) Waksman Institute of Microbiology and Rutgers, The State University of New Jersey

P49003 Neotyphodium-graminoid interaction in a natural system: testing the mutualism theory
Cardou, Francoise-presenter cardou@ualberta.ca(a) Hik, David S (a)
"*Neotyphodium spp* are fungal endosymbionts of cool-season grasses that reproduce asexually by growing hyphae into the host's seed; their reproductive success is thus dependent on host fitness. The endophyte-grass relationship has traditionally been viewed as mutualistic, exchanging shelter and dispersal in return for services that may increase fitness such as herbivory deterrence or nutrient uptake. However, the consistent patterns observed in agronomic pastures have been difficult to confirm in natural systems, especially in the case of vertebrate herbivory deterrence. We investigated the infection dynamics of the perennial bunchgrass *Festuca altaica* across a stable long-term herbivory gradient caused by the native herbivore *Ochotona collaris* (collared pika). A previous screening at our study site showed infection (E+) frequency in *F. altaica* to be higher in high herbivory locations and lower where herbivory pressure is low. Our aim was to determine the respective role of biotic (herbivory) and abiotic (nutrient availability) stresses in mediating endophyte effect on plant fitness. We carried out a factorial design experiment combining four factors: infection, herbivory, fungicide and fertilizer. Leaf demography, vegetative growth and flower production were monitored (n=544) at 2 week intervals. We verified the stability of soil nutrient availability across the herbivory gradient by deploying 64 Plant Root Simulator probes (WesternAG Innovations). Analysis of preliminary morphological data shows no difference in the number of tiller per tussock between E+ and E- plants when herbivory is low, but E- tussocks had significantly more tillers at high herbivory. This may indicate that herbivory deterrence by the fungus represents a physiological weight to the plant."
(a) University of Alberta

P49004 A novel step towards increasing forage production in Alfalfa
Banerjee, Manas-presenter Manas.Banerjee@brettyoung.ca(a) Yesmin, Laila (a)
"Alfalfa, the queen of forage crops, has become very popular among the forages because of its productivity and high feed value. It has the greatest yield potential of any perennial forage legume grown in United States and Canada. On the other hand, dairy management has become an important concern due to the shortage of land for forage production especially with alfalfa growth. Hence, to capture the yield potential among the biological solution only the widespread practice at present is to use rhizobium inoculants for growing alfalfa (*Medicago sativa*) in North America. Nevertheless, in most cases using only rhizobium-based inoculant is not producing the expected yield due to already established optimum level of background rhizobium in the field. Realizing the need to increase production, an innovative biological but economically feasible approach has been revealed in the present study. A sulfur (S)-oxidizing plant growth promoting rhizobacteia (PGPR), *Delftia acidovorans* RAY209 was used in alliance with *Shinorhizobium meliloti* to increase efficiency of rhizobium as well as to augment the alfalfa productivity. Field research conducted in different parts of alfalfa growing areas in the US showed increase in herbage & yield can be obtained with this consortia based inoculum. Hence, this biological application has been shown to be a potential step forward and a novel means to increase alfalfa production."
(a) Research & Development, BrettYoung Seeds

P49005 Differential gene expression in the *Lolium pratense - Epichloe festucae* symbiotum during benign seed transmission and stromata formation
Mann, Lesley J-presenter Lesley.Mann@uky.edu(a) Schardl, Chris (a) Liu, Jinge (a) Hesse, Uljana (a)

"The *Lolium pratense - Epichloe festucae* symbiotum is a model system for symbioses of *Epichloe* and *Neotyphodium* species (endophytes) with C3 grasses, which receive great fitness benefits. The *E. festucae sexual* cycle is associated with a mildly pathogenic state, the formation of a stroma on a minority of tilers. To identify genes involved in disease development versus benign plant colonization, expression analysis was conducted using '454' pyrosequencing of mRNA from normal inflorescences and stromata of *E. festucae*-infected plants. In total, 1734 putative differentially expressed fungal genes were identified. To further examine expression of several of these, fungal mycelium and three tissues from four different *L. pratense-E. festucae* symbiota were extracted and used in RT-qPCR studies. $\Delta\Delta C_T$ analysis of the RT-qPCR data validated significant 454 results ($p < 0.05$) in most cases. For example, RT-qPCR showed that *sspA*, a predicted secreted protein gene, is over 1000-fold upregulated in inflorescences versus stromata. This gene, with its native promoter, has been cloned into an expression vector to generate a C-terminal translational fusion with an autoflorescent protein, and introduced into *E. festucae*. Perennial ryegrass has been inoculated with the transformed *E. festucae* to observe the expression of *sspA* in symbio via fluorescence microscopy. Other genes found to be differentially expressed will also be studied by in symbio localization. Δ"
(a) University of Kentucky, Department of Plant Pathology

P49006 Metabolism and Transfer of Nitrogen in the Arbuscular Mycorrhizal Symbiosis
Tian, Chunjie-presenter tiancj@msu.edu(a) Shachar-Hill, Yair (a)
"The Arbuscular Mycorrhiza is arguably the world's most important symbiosis. It brings together the roots of over 80% of land plant species and fungi of the Phylum Glomeromycota to great muttual advantage. Host plants benefit from improved uptake of soil nutrients such as phosphate and nitrogen. Nitrogen is the nutrient whose availability most commonly limits plant growth. Arbuscular mycorrhizal (AM) fungal hyphae take up both NO3- and NH4+ from the soil. Furthermore, AM fungi can increase the uptake of organic N sources. We are working to test and extend a model of nitrogen handling in the AM symbiosis in which N taken up by the fungus is incorporated into amino acids, translocated from the ERM to the intraradical mycelium (IRM) as arginine (Arg) and then broken down to release N but not C to the host plant. To provide evidence on the mechanism and regulation of N transfer from the fungus to the plant in the AM symbiosis, we have recently amplified several genes putatively involved in nitrogen transport and metabolism from the AM fungus Glomus Intraradices. The identity and potential roles of these genes are described in this presentation together with gene expression data to test the model and gain insight into the regulation of N nutrition during development and in response to environmental conditions."
(a) Plant Biology Department, MSU

P49007 Screening of symbiotic nitrogen fixation genes from *Bradyrhizobium japonicum* USDA110
Nomura, Mika-presenter nomura@ag.kagawa-u.ac.jp(a) Arunothayanan, Hatthaya (a) Dao, Tan Van (a) Itakura, Manabu (b) Minamisawa, Kiwamu (b) Tajima, Shigeyuki (a)
" *Bradyrhizobium japonicum* USDA110 is a gram negative soil bacterium that forms root nodules specifically on soybean (*Glycine max* L. Merr. cv. Akishirome). During nodule development, together with morphological alteration of cells, the bacteria undergo marked biochemical and physiological changes. Proteomics is an ideal tool for the dissection of plant microbe interactions. It provides a broad overview of the proteins produced by both partners during their constant signal exchanges. Early studies have reported that a large number of proteins are expressed as specific or up-regulated in *B. japoniucm* bacteroids. In the present study, to understand the bacteroid development in the nodule, we report the time-course analysis of bacteroid enhanced proteins using two-dimensional gel proteome techniques. In this study, 275 annotated protein spots were successfully identified. A large number of putative up-regulated proteins at 7 dpi were observed and typical examples for these up-regulated proteins are proteins related to transcription, translation, protein folding and degradation. Several specific proteins in the later period of bacteroid differentiation were also identified. From these proteome data, we have selected forty proteins that expressed at high level in the bacteroid and have made these single mutants. Soybeans were inoculated with these mutants, and were grown for 28 days. Acetylene reduction activity (ARA), and growth were determined. In the screen of these mutants, we found one mutant that decreased the nitrogenase activity of the nodules. This protein encoded hypothetical protein. We analyze the functions of the protein. "
(a) Kagawa University (b) Tohoku University

P49008 Transcriptome analysis of Induced systemic drought tolerance elicited by *Pseudomonas chlororaphis* O6 in *Arabidopsis thaliana*
Han, Song Hee-presenter molmol@hanmail.net(a) Cho, Song Mi (b) Oh, Sang A (a) Kim, Hyun Jung (a) Yang, Kwang-Yeol (a) Cho, Baik Ho (a) Kim, Young Cheol (a)
"Colonization of plant roots by *Pseudomonas chlororaphis* O6 induces tolerance in *Arabidopsis thaliana* to drought. Microarray analysis was performed using the 22,800-gene Affymetrix GeneChips to examine differential gene expression from plants colonizes with *P. chlororaphis* O6 with and without water withholding. Root colonization in watered plants increased transcript accumulation from genes associated with defense, response to activated oxygen species, and auxin- and jasmonic acid-responsive gene but decreased transcription factors associated with ethylene and ABA signaling. Withholding water revealed clusters of gene that were up-regulated in the *P. chlororaphis* O6-colonized plants that were down-regulated in the noncolonized plants displaying drought stress and vice versa. Transcripts of the jasmonic acid-marker gene, VSP-1 and pdf-1.2, the salicylic acid regulated gene, PR-1, and the ethylene-response gene, HEL, also were up regulated by root colonization with *P. chlororaphis* O6, but differed in their responsiveness to drought stress. These data show how gene expression in plants lacking adequate water can be remarkably influenced by microbial colonization leading to plant protection. "
(a) Department of Plant Biotechnology, Chonnam National University, Gwangju, Korea (b) Department of Floriculture, Chunnam Techno College, Jeonnam 516-911, Korea

P49009 Microbial diterminants from *Pseudomonas chlororaphis* O6 involved in induced systemic resistance against plant diseases and abiotic stresses
Park, Ju Yeon-presenter jjanggu282@hanmail.net(a) Cho, Song Mi (b) Kim, Hyun Jung (a) Yang, Kwang-Yeol (a) Cho, Baik Ho (a) Kim, Young Cheol (a)
"A plant growth promoting rhizobacterium, *Pseudomonas chlororaphis* O6, is able to induce systemic resistance against various plant pathogen, such as *Pectobacterium carotovorum* subsp. *carotovorum* SCC1, *Pseuddomonas syringae* pv. *tomato* DC3000, *Botrytis cinerea* and *Tobacco Mosic Virus* (TMV) and against abiotic stresses such as drought stress. 2R, 3R-butanediol produced by *P. chlororaphis* O6 was a key compound for induced resistance against *P. carotovorum* subsp. *carotovorum* SCC1 and drought stress. Exopolysacchride (EPS) produced by *P. chlororaphis* O6 also be able to elicit broad spectrum of systemic resistance against DC3000, *Botrytis cinerea, P. carovotorum* SCC1, TMV and drought stress. Tolerance to drought was correlated with reduced water loss in EPS-treated plants and with stomatal closure, indicated by size of stomatal aperture and percentage of closed stomata. Stomatal closure and drought resistance were mediated by EPS of *P. chlororaphis* O6. We conclude that the bacterial EPS from *P. chlororaphis* O6 was a major determinant in inducing resistance to drought and plant diseases, and are currently under investigating molecular

mechanisms involved EPS-mediated systemic resistance against drought and plant diseases. "
(a) Department of Plant Biotechnology, Chonnam National University, Gwangju, Korea (b) Department of Floriculture, Chunnam Techno College, Jeonnam 516-911, Korea

P49010 "Screening of Trichoderma spp. againt Phytophthora capsici, the oomycete pathogen in pepper (Capsicum annuum)."
Bae, Hanhong-presenter hanhongbae@hotmail.com(a) Roberts, Daniel P (b) Lim , Hyoun-Sub (b) Strem, Mary D (b) Ryu, Choong-Min (c) Bae, Hyeun-Jong (d) Bailey, Bryan A (b)
"Nineteen Trichoderma species, which are endophytes of cacao plants (Theobroma cacao) or are isolated from cacao forest soil, were screened for anti-Phytophthora capsici activity. The oomycete Phytophthora capsici is a causal agent in pepper (Capsicum annuum cv. Bugang) and leads to significant economic loss in worldwide. Anti-P. capsici activities were identified by mycoparasitism, antibiosis, volatiles, P. capsici inoculation assay, and genomics approaches. All nineteen Trichoderma isolates were proved to be the endophytic nature in pepper roots. The endophytic nature of Trichoderma in pepper root also was observed using transmission electron microscopy (TEM) and GFP-Trichoderma. Among nineteen Trichoderma isolates eleven isolates showed potent mycoparasitic activity against P. capsici. While seven Trichoderma isolates showed the control efficacy against P. capsici for antibiosis activity, Trichoderma caribbaeum var. aequatoriale strain DIS 320c (No. 11) showed the most potent control efficacy against P. capsici. DIS 320c volatile also showed the strong anti-P. capsici activity. Six Trichoderma isolates were selected based on various anti-P. capsici activities and were further analyzed in terms of biochemical and genomic aspects. Based on disease progress curve, two isolates (DIS 259j and 376f) showed the best protection ability against P. capsici. Microarray results showed that Trichoderma inoculation (48 hours) with DIS 259j and DIS 3767f altered expression of numerous genes in pepper roots. Quantitative real-time reverse transcriptase PCR (QPCR) was used to confirm the microarray results. These endophytic Trichoderma isolates can be used as biocontrol agents against P. capsici in the field."
(a) Yeungnam University (b) USDA-ARS-Plant Sciences Institute (c) Korea Research Institute of Bioscience and Biotechnology (d) Chonnam National University

P49011 Modification of *Pv*RACK1 expression inhibits nodule morphogenesis and development in *Phaseolus vulgaris*.
Islas-Flores, Tania (a) Guillen, Gabriel (a) Lara-Flores, Miguel (b) Bearer, Elaine (c) Sanchez, Federico (a) Villanueva, Marco A.-presenter marco@cmarl.unam.mx(d)
"RACK1 (Receptor for Activated C Kinase 1) is a highly conserved protein that is found both in animals and plants, and has seven WD-40 repeats that form a β-propeller structure. In animals RACK1 is over-regulated during angiogenesis and in human carcinoma, and can contribute to growth and tumor metastasis (Berns et al., FASEB J. 14: 2549-2558, 2000). The involvement of RACK1 in growth and proliferation in mammalian cells resembles the proliferation processes that must occur during nodule formation in plants; therefore, the nodulation process is a provoking model for studying RACK1 function in plants. We identified a *Phaseolus vulgaris* RACK1 homologue of 36 kDa with 7 WD-40 repeats and two PKC binding sites (*Pv*RACK1). This protein is encoded by a single gene in the *P. vulgaris* genome. It has a ubiquitous expression in root, nodules, stems, leaves and pods of bean plants; and a kinetic analysis of nodulation shows that its accumulation increases concomitant to the nodule development. The modification of *Pv*RACK1 expression (silencing and over-expression) yields nodulation phenotypes in which the root-nodule morphogenesis and development are profoundly affected. These results indicate that *Pv*RACK1 is functionally required in this process. T. Islas-Flores was supported by a Ph.D. fellowship from CONACyT (National Council of Science and Technology, Mexico)."
(a) Departamento de Biologia Molecular de Plantas, Instituto de Biotecnologia-UNAM, Cuernavaca, Morelos, Mexico (b) Centro de Ciencias Genomicas-UNAM, Cuernavaca, Morelos, Mexico (c) Pathology and Laboratory Medicine, Brown University, Providence, RI (d) Unidad Academica Puerto Morelos, Instituto de Ciencias del Mar y Limnologia-UNAM, Puerto Morelos, Quintana Roo, Mexico

SESSION P50 – POLLEN BIOLOGY

P50001 Analysis of the Arabidopsis 4CL-like ACYL-CoA SYNTHETASE5 gene and co-expressed genes reveals an ancient biochemical pathway required for pollen development and sporopollenin biosynthesis.
Douglas, Carl J-presenter cdouglas@interchange.ubc.ca(a) Kim, Sung Soo (a) Friedmann, Michael (a) de Azevedo Souza, Clarice (a) Kombrink, Erich (b) Koch, Stefanie (b) Kienow, Lucie (b) Schneider, Katja (b) Colpitts , Che (c) Suh, Dae-Yeon (c) Quilichini, Teagen (a)
"Formation of pollen and spore walls requires the deposition of sporopollenin, a poorly characterized mixed aliphatic/aromatic polymer with ester and ether linkages that contributes to the protective and tough exine layer. We discovered that the Arabidopsis 4-coumarate:CoA ligase (4CL)-like enzyme Acyl-CoA synthetase5 (ACOS5) is absolutely required for pollen development. An acos5 mutant is sterile, devoid of visible pollen grains, and lacks sporopollenin or exine. Phylogenetic analysis revealed that ACOS5 genes are conserved in land plants (eg, poplar, rice, tobacco, and Physcomitrella). ACOS5 is transiently and exclusively expressed in tapetum cells, while ACOS5 is an acyl-CoA synthetase with highest activity against medium chain hydroxy-fatty acids. In silico co-expression analyses identified Arabidopsis genes encoding potential enzymes and transporters that could work with ACOS5 to generate and secrete sporopollenin monomers. Previous studies, and our reverse genetic analyses of co-expressed genes such as a dihydroflavonol-4-reductase-like gene, polyketide synthase (PKS) genes, and a transporter gene, revealed that mutants in these co-expressed genes are also compromised in male fertility and sporopollenin deposition. Phylogenetic analyses showed that these genes are conserved in land plants including Physcomitrella, and the Arabidopsis and Physcomitrella PKS enzymes have similar in vitro biochemical activities and could use ACOS5-generated starter molecules to produce polyketides incorporated into sporopollenin. This work illuminates the outlines of an ancient but previously uncharacterized pathway involved in biosynthesis of the monomeric constituents of the sporopollenin polymer, one of the most robust cell wall matrices known in plants. "
(a) Department of Botany, University of British Columbia (b) Department of Plant-Microbe Interactions, Max Planck Institute for Plant Breeding Research (c) Department of Chemistry & Biochemistry, University of Regina

P50002 Lipid Raft-Mediated Internalization of Arabidopsis Pollen-Specific Receptor Kinase PRK2a Regulates Polarized Growth of Pollen Tubes through Spatiotemporal Activation of small GTPase ROP
Zhang, Yan-presenter mpizyanzhang@berkeley.edu(a,b) He, Junmin (b,c) McCormick, Sheila (a,b)
"The plant-specific Rho GTPase, ROP, controls pollen tube polarity through restricted localization of its GTP-bound active form at the apical plasma membrane. The dynamic tube growth requires spatiotemporal control of ROP, for which a feedback loop composed of RopGAP and RhoGDI was reported. However, little was known about a feed-forward loop that, by analogy, should operate in parallel. An Arabidopsis receptor- like kinase PRK2a acts as a scaffolding protein to recruit the activator of ROP, RopGEF, to the plasma membrane. Here we show that PRK2a co-localized with endosome markers in ring-shaped cytosolic compartments when over-expressed. A sorting motif (YXXφ) in the carboxy-terminus of PRK2a is essential for its interaction with one of the five Arabidopsis μ;2 adaptin subunits in yeast and for its sorting/recycling in pollen tubes. The

internalization of PRK2a did not involve clathrin but was inhibited by the over-expression of a non-phosphorylatable version of Caveolin1, suggesting the involvement of lipid rafts. Such lipid raft-mediated PRK2a internalization may in turn be regulated by ROP activity, because over-expression of a dominant-negative or a constitutively active ROP disturbed the distribution of Lyn24GFP, a fluorescent probe labeling a certain raft population. That the dynamics of PRK2a are critical for the spatiotemporal activation of ROP was further demonstrated by a synthetic molecular approach: over-expression of the PRK2a cytoplasmic domain, which specifically binds to GTP-bound ROP, resulted in the inhibition of tube growth, likely by depleting endogenous RopGEF. We propose a model in which the dynamics of a PRK2a feed-forward loop regulate the spatiotemporal localization of ROP during pollen tube growth. "
(a) Plant Gene Expression Center, United States Department of Agriculture/Agricultural Research Service (b) Department of Plant and Microbial Biology, University of California at Berkeley (c) School of Life Sciences, Shaanxi Normal University, Xi'an 710062, China

P50003 Palynological Analysis of Ancient Domestic Turkey Droppings from the American Southwest
Nott, BreAnne M-presenter bmnott@gmail.com(a) Kemp, Brian M (a) Jones, John G (a) Lipe, William (a)
"Domestication and introduction of domesticated plant and animal species has fundamentally impacted human evolution. Changes in social structure, economy, social processes and lifestyle all occur with the introduction of domestication and have significant impacts on human history. Despite the importance of domestication, identifying domestication in archaeological sites is often futile. Molecular and palynological analysis (i.e. pollen analysis) of 1600-2100 year old turkey droppings (coprolites) from the Turkey Pen Ruin site can be used to infer domestication by reconstructing turkey diet. Preliminary data suggest that the analyzed droppings may have originated from both wild and domestic birds and indicate that the site was used seasonally for turkey husbandry. Recent genetic evidence of other coprolites from the site suggested they all originated from domestic birds alone (Kemp 2008). Using palynological data and molecular tools, diet and molecular origins of each turkey dropping was concluded. A series of expectations were then created in order to infer domestication from diet and genetic data. "
(a) Washington State University

P50004 Propidium iodide staining of de-methylated pectin in the lily pollen tube cell wall.
Lubeck, Eric-presenter esl04@hampshire.edu(a) Rounds, Caleb (b) Winship, Lawrence (a) Hepler, Peter (b)
"Propidium Iodide(PrPI) fluorescence allows for the dynamic quantification of free carboxylic acid residues in the pectin-rich apical cell wall of *L. formosanum* pollen tubes. Lily pollen tube cell wall growth is confined to the highly methylesterified apical pectin cell wall through control of lateral wall tensile strength via Pectin Methyl Esterase (PME) enzymes. Enzymatic pectin demethylation in the sub-apical cell wall results in a dramatic increase in wall stiffness through gelation with free Ca^{2+} cations. The hydraulic pressure of the pollen tube forces the weaker highly methylesterified apical cell wall to irreversibly expand. Pollen tube PrPI apical fluorescence was found to vary in a log-linear relationship with the extracellular concentration of Ca^{2+} and PrPI, indicating a competition for free anionic residues in the pectin cell wall. PME(30U/mL) addition to plasmolyzed pollen tubes caused apical PrPI florescence to increase in a parabolic fashion until all available methyl-esterified pectin was consumed. Florescence increased in a uniform fashion throughout the apical cell wall, rapidly declining in an exponential gradient along the lateral cell wall until reaching a background level at approximately 5µ;m from the apex. This observation appears to corroborate previous reports suggesting that pectin is highly-methylated in the apical cell wall and progressively demethylated through the shank via enzymatic activity. PrPI functions as an inexpensive fluorescent probe to dynamically quantify pectin demethylation in plasmolzyed and growing pollen tubes. Future research should allow for the real-time monitoring of PME induced cell wall morphogenesis and microbial infection through PrPI fluorescence of free carboxylic acid residues in all pectinacious cell walls. "
(a) Hampshire College (b) UMASS Amherst

P50005 Cation/proton transporters are key players in pollen tube guidance
Lu, Yongxian-presenter yxlu@umd.edu(a) Chanroj, Salil (a) Sze, Heven (a)
http://www.cbmg.umd.edu/faculty/sze/sze.html
"Understanding the molecular & cellular bases of fertilization in plants is critical for enhanced reproduction and crop seed production. Successful fertilization depends on accurate delivery of sperms to the ovule by the pollen tube. Guiding signals from the female cells are being identified, though how the pollen senses and responds to the signals are largely not known. We are testing the role of two related genes CHX-A and CHX-B from a family of predicted cation/proton exchangers that are expressed preferentially in pollen (Sze et al. 2004 Plant Physiol 136: 2532). Single T-DNA insertion mutants (*aa* or *bb*) showed in vitro pollen germination and tube growth similar to that of wild-type. However, male gene transmission was specifically blocked when both genes are defective. When wild-type pistil was pollinated with a limited number of pollen grains from *Aabb* plants, seed set was reduced to half of that from *AAbb* plants, suggesting *ab* pollen is infertile. In vivo pollen tube growth was monitored using mutants expressing the GUS reporter. *aB* pollen tube grew inside the transmitting tissues of the female reproductive organ, turned towards the ovule and discharged its content into the embryo sac. However, the double mutant pollen tube grew mainly inside the transmitting tissue and failed to target the ovule. To our knowledge, this is the first transporter mutant with a defect in pollen tube guidance. The results suggest that loss of these transporters disrupts the perception and/or transduction of female guidance signals to shift the axis of polar tip growth. GFP-tagged CHX-B was localized to endomembranes in the pollen tube. Studies are underway to determine the cellular and molecular bases of this phenotype. (Supported by DOE BES grant to HS)"
(a) University Of Maryland

P50006 "The Arabidopsis *PIRL1* & *PIRL9* genes are essential for pollen mitosis, growth, and viability and do not functionally overlap with related *PIRLs*"
Forsthoefel, Nancy-presenter forsthn@whitman.edu(a) Dao, Thuy P (a) Simeles, Barbara (a) Vernon, Daniel M (a)
"Plant Intracellular Ras-group-related LRRs (PIRLs) are a plant-specific family of nine leucine-rich repeat proteins related to animal and fungal LRRs that act in Ras signaling complexes. Analysis of T-DNA knockouts has revealed that *PIRL1* & *PIRL9* are functionally redundant and essential for male gametophyte development. *Pirl9;pirl1/PIRL1* plants fail to transmit the double mutant allele combination through pollen, and double mutant pollen appear shrunken and are inviable. Pollen defects are fully penetrant and segregate as a post-meiotic phenotype in a *qrt1* background. SEM and developmental profiles by DIC and brightfield microscopy indicate that *pirl1,pirl9* pollen establish a normal exine pattern but arrest during anther stages 9-10, failing to fully expand or achieve rounded morphology. DAPI staining indicates that prior to the appearance of morphological defects, mutant microspores do not complete pollen mitosis and consistently fail to produce a clearly-defined second nucleus. Aniline blue staining patterns in mutant pollen also differ from wild type. PIRL1 & PIRL9 form a closely related sub-family with PIRL2 & PIRL3, and RT-PCR gene expression surveys show that all members of this group are widely expressed during both vegetative and reproductive development. To investigate additional potential *PIRL* functional redundancy, we constructed double mutants lacking various combinations of these four genes. Double mutant plants were recovered and no defects in allele transmission or pollen development were observed. Together, these results demonstrate that *PIRL1* and *PIRL9* are essential for pollen mitosis and development, and show that functional redundancy with related genes in this *PIRL* sub-family is not evident in double knock-

outs. *Supported by NSF award 0616166*"
(a) Whitman College

P50007 HAP2(GCS1) is a sperm-expressed component of a deeply conserved fertilization mechanism
Johnson, Mark A-presenter Mark_Johnson_1@Brown.edu(a) Wong, Julian L (a) Frank, Aubrey C (a) Chang, Alexander (a) Leydon, Alexander R (a)
http://brown.edu/Departments/Molecular_Biology/pgl/
"Pollen tubes carry two sperm to the female gametophyte where one fuses with the egg to form the embryo and the other fuses with the central cell to form the endosperm. This process of double fertilization is at the core of seed crop production and is as mysterious as it is fascinating. We know very little about the mechanisms required for interactions between sperm and the female gametes that lead to fusion and initiation of development. We discovered *hap2-1* in a screen for mutations in Arabidopsis that disrupt the function of the male gametophyte. *hap2-1* pollen tubes are defective in pollen tube guidance, but deliver sperm to ovules at reduced frequencies. However, *hap2-1* sperm are completely incapable of fertilizing either the egg or the central cell. Recently, it has been show that the HAP2 ortholog is essential for gamete fusion in the green alga, *Chlamydomonas reinhardtii*, and in the protozoan parasite, *Plasmodium falciparum*. These results, combined with the conservation of HAP2 in plants and other organisms, lead us to propose that HAP2 is an anciently conserved component of a widely used fertilization mechanism. Our primary hypothesis is that HAP2 is directly involved in mediating fusion of the sperm and female gamete plasma membranes. We are taking a number of approaches to understand the biochemical function of HAP2, which has a transmembrane domain but no other domains of known function. Genetic dissection of HAP2 suggests that the large region of HAP2 N-terminal to the transmembrane domain, predicted to be outside of the sperm cell, is essential for function. Analysis of the C-terminal portion of the protein indicates that the charge of amino acid side chains is critical for function. We will discuss models for HAP2 function based on these results."
(a) Brown University

P50008 "Interaction of a cysteine protease with PRALF, a peptide growth regulator is a potential determinant of pollen tube growth in tomato"
Chalivendra, Subbaiah C.-presenter schalive@lamar.colostate.edu(a) Day, Irene (a) Martini, Dyllon (a) Bedinger, Patricia (a)
"We reported previously that *Sl*PRALF, a pollen-specific RALF (Rapid Alkalinizing Factor) in tomato (*Solanum lycopersicum*), is inhibitory to pollen tube growth when added to the pollen germination medium (PGM). Further, this inhibitory effect is transient and pollen tubes become refractory to exogenous PRALF within an hour after pollen tube emergence (Covey et al., communicated; Abst. M2603: Plant Biology 2008). We are analyzing the mode of PRALF action on tube growth *in vitro*, as well as its potential involvement in pollen tube growth in pistils. Immunolocalization of PRALF in pollen tubes indicated that the protein is localized to the growing tip. Protein blot analysis showed that pollen, germinated or ungerminated, contains only the pre-proPRALF and lacks the processed (or active) form. In contrast, only the active peptide is detectable in the PGM, although at very low steady state concentrations (2-5 pmoles/ 10^6 pollen grains). This indicates that PRALF is expressed during pollen tube growth, processed and secreted into the medium, where it appears to be rapidly degraded. Consistent with this proposal, the proteolytic activity of the PGM was found to increase somewhat after the first h of germination. Our recent Y2H screen with *Sl*PRALF as the bait identified a putative secreted cysteine protease with a granulin-domain (*Sl*PCysPro), as a potential interacting partner of the peptide. We are further confirming this interaction by pull-down & bimolecular fluorescence complementation assays. We are also looking at the *in vivo* implications of these protein associations in RNA_i transgenics. We propose that the dynamics of *Sl*PCysPro and *Sl*PRALF within & outside the growing tip may regulate the growth of pollen tubes. "
(a) Colorado State University

P50009 A lily pollen-specific gene encodes a novel cytoskeleton-binding protein
Wang, Co-Shine (a) Wang, Sung-Mo -presenter s0921718@mail.ncyu.edu.tw(a) Hsu , Yi-Feng (a)
"The *LLP13* clone was identified from a suppression subtractive cDNA library that constructed from mRNA isolated at the desiccation stage of lily (*Lilium longiflorum*) anthers. The full length of *LLP13* is 2799 bp in which an open reading frame of 2424 bp encodes 807 amino acid residues. The protein has a calculated molecular mass of 91 kDa and contains a hydrophobic signal peptide at the N-terminus. The protein shares 34% identity with an intermediate filament binding protein in *Oryza sativa*. Two pieces of fragments without containing the signal peptide, LLP13N and LLP13C (313 and 337 amino acids, respectively), were chosen and overexpressed in *E. coli*. The purified proteins were injected into rabbits. The titer of LLP13C antiserum is ten-fold higher than that of LLP13N. An immunoblot of total protein indicated that a protein with molecular mass larger than 97 kDa was detected either by LLP13N or by LLP13C antiserum. The tissue-specificity and temporal expression of the *LLP13* gene was characterized by Northern and immunoblot analyses. The LLP13 protein remained its level of accumulation even after 24-h germination suggesting that the protein may play a critical role in tube growth. Particle bombardment of GFP-LLP13 and LLP13-GFP showed that the LLP13 protein produced a bending pollen tube with serious twist. GFP-LLP13 and LLP13-GFP fused proteins were located both on the tip and on the filament of pollen tubes, suggesting that the LLP13 protein is likely a cytoskeleton-binding protein. "
(a) Graduate Institue of Biotechnology, National Chung Hsing University

P50010 An anther-specific gene encoding *cis*-prenyltransferase in *Lilium longiflorum*
Liu, Ming-Che-presenter rufeselma@hotmail.com(a) Chen, Jing-Ping (a) Wang, Co-Shine (a)
"A *cis*-prenyltransferase gene, *LLA66*, was identified from a suppression subtractive cDNA library at the phase of microspore development in lily (*Lilium longiflorum*) anthers. The method of 5'-RACE-PCR was used to obtain the full-length of *LLA66* cDNA with a size of 1185 bp. The *LLA66* cDNA contains an open reading frame of 927 bp encoding a polypeptide of 308 amino acids with a calculated molecular mass of 35.7 kDa. Sequence alignment revealed that the LLA66 protein belongs to a family of *cis*-prenyltransferases. Northern blot analysis indicated that the *LLA66* gene was specifically expressed at the phase of microspore development in the lily anther. The *LLA66* mRNA was also detected in the microspore at the phase of microspore development. *In situ* hybridization with DIG-labeled antisense riboprobe of the *LLA66* showed strong signals localized at the tapetum layer of anthers walls. A fragment of 1084 bp that contains several pollen-specific and hormone responsive elements was identified from the promoter region of *LLA66* gene by TAIL-PCR. The ORF fragment of *LLA66* gene was cloned into pET-32a, overexpressed in *E. coli*, and purified using Ni^{2+}-nitrilotriacetic acid agarose. The anti-LLA66 antiserum was raised against the LLA66 protein overexpressed in *E. coli*. To determine the enzyme activity of LLA66, the recombinant LLA66 was overexpressed in *E. coli* and extracted with protein extraction buffer containing 10 mM 3-(1-pyridinio)-1-propanesulphonate (NDSB201) which is one type of non-detergent sulphobetaines. Consequently, the soluble form of LLA66 protein was successfully obtained. After the digestion with thrombin protease, the His-tagged LLA66 was used to test enzyme activity. To look insight into the structure of the protein, the soluble form of LLA66 will be crystallized. "
(a) Graduate Institute of Biotechnology, National Chung Hsing University

P50011 Functional characteization of bZIP transcription factor in Arabidopsis pollen
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"Haploid male gametophyte, the male partner in sexual reproduction of flowering plants plays a key role in plant fertility and crop production. Our ability to control and guide this process represents an effective tool for crop breeding and genetic optimalisation. We have a very limited understanding of the regulatory mechanisms that have evolved to specify the gametophytic developmental programs. Therefore, it is necessary to identify transcription factors that are part of such haploid regulatory networks. Here we focus on bZIP transcription factors playing critical roles also in plants. We report the functional characterization of Arabidopsis thaliana AtbZIP34 that is expressed in both gametophytic and surrounding sporophytic tissues during flower development. T-DNA insertion mutants in AtbZIP34 show pollen morphological defects that result in reduced pollen germination efficiency and slower pollen tube growth both in vitro and in vivo. Light and fluorescence microscopy revealed misshapen and misplaced nuclei with large lipid inclusions in the cytoplasm of atbzip34 pollen. Scanning and transmission electron microscopy revealed defects in exine shape and micropatterning and a reduced endomembrane system. Several lines of evidence including the AtbZIP34 expression pattern and the phenotypic defects observed suggest a complex role in male reproductive development that involves a sporophytic role in exine patterning, and a sporophytic and/or gametophytic mode of action of AtbZIP34 in several metabolic pathways, namely regulation of lipid metabolism and/or cellular transport. Acknowledgment Authors gratefully acknowledge the financial support from the Grant Agency of the Czech Republic (grant 522/09/0858) and Ministry of Education of the Czech Republic (grant LC06004). "
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P50012 Chemical genetic analysis of pollen tube glycobiology: β-galactosidases are essential for penetration of the pistil.
Kern, David M.-presenter david_kern@brown.edu(a) Wright, Laurel D. (a) Becker, Jason P. (b) Song, Shang (b) Rossi , Lauren L (b) Qin, Yuan (c) Palanivelu, Ravi (c) Basu, Amit (b) Johnson, Mark (a)
"When a pollen grain contacts a receptive stigma it extends a polar pollen tube that rapidly grows through pistil tissue and targets an ovule for fertilization. This process depends on dynamic synthesis, breakdown, and recognition of complex carbohydrate molecules. Glycobiology has emerged as a field aimed at understanding the functions of carbohydrates; however, progress has been hampered because carbohydrates are chemically complex, their structures are indirectly encoded by the genome, and the enzymes that govern them are often redundant. Fortunately, pollen tube growth provides a fantastic model system for interdisciplinary approaches to glycobiology: Pollen grains are amenable to chemical screens and genetic tools facilitate analysis of pollen mutant phenotypes in vitro and in the pistil. We created a fluorescence-based assay of tobacco pollen tube growth that allowed us to screen a library of putative β-galactosidase (β-gal) inhibitors. β-gals are abundant in pollen, but their roles are currently unknown. We found four compounds with consistent inhibitory activity on pollen tube growth and are using these inhibitors as probes to identify cellular targets. These results led us to use reverse genetics in Arabidopsis to test whether β-gal activity is required for pollen tube growth. We used microarray analysis to identify a pair of β-gals that are abundantly and specifically expressed in pollen and pollen tubes. Single mutants have mild effects; however, double mutants significantly diminish tube growth in vitro and block the ability of pollen tubes to penetrate the stigma. Future experiments will be aimed at determining the subcellular localization of this pair of β-gals and whether the molecules identified in our chemical screen inhibit their enzymatic activity."
(a) Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University (b) Department of Chemistry, Brown University (c) Department of Plant Sciences, University of Arizona

P50013 "Zm908p11, encoded by a pollen-specific gene with short open reading frames, is essential for pollen development and germination in tobacco and Arabidopsis"
Yu, Jingjuan-presenter jingjuanyu@gmail.com(a) Wang, Dongxue (a,b) Liu, Peng (a,c) Li, Chengxia (a,d) Zhao, Qian (a) Ao, Guangming (a)
"A pollen specific gene *Zm908* was isolated from a genomic library of maize (*Zea mays.* L.) using Zm401p10 cDNA (Wang et al. Functional Plant Biology 2009, 36:73-85) as a probe. The *Zm908* cDNA contains short open reading frames (sORFs) and the longest ORF encodes a nuclei-localized small protein with 96 amino acids (designated Zm908p11). The examination of a promoter-GUS construct in transgenic tobacco plants revealed that the *Zm908* is preferentially expressed in pollen, which is consistent with the results of Northern blot and RT-PCR. For analysis of the function of *Zm908*, transgenic plants harboring sense and antisense copies of *Zm908* cDNA driven by *CaMV35s* were generated. Ectopic expression of *Zm908* resulted in meiosis disaccord, low pollen viability and deviant pollen germination in tobacco anther, sunken exines and low pollen germination rate in Arabidopsis in addition. Moreover, Zm908p10 showed the same function as Zm908, whereas the mutants of the ORF caused the loss of function. These studies strongly suggest that Zm908 has an essential role in pollen development, and function as the product of the longest ORF. "
(a) State Key Laboratory for Agro-biotechnology,College of Biological Sciences,China Agricultural University, China (b) Department of Biology, Stanford Univ. Standford, CA 94305-5020, USA (c) Department of Plant Sciences and Pathology, Montana State Univ. MT 59715, USA (d) Department of Plant Sciences, University of California, Davis, CA 95616, USA

P50014 The rice LOW SEED SETTING 1 encodes a TPR-like protein and is involved in pollen and seed development
Li, Zhigang (a) Hu, Qian (a) Luo, Hong-presenter hluo@clemson.edu(a)
"The tetratrico peptide repeats (TPR) are degenerate 34-amino acid motifs present in tandem in a large number of proteins that have been identified in all kingdoms. TPR-containing proteins either form active multiprotein complexes with partner proteins through TPR-mediated specific interaction or act as co-chaperones involved in the folding of a growing set of substrates, impacting a variety of biological processes. In plants, although TPR proteins have been reported to be involved in hormone signaling, photosynthesis, their involvement in other cellular functions is still largely unknown. We have identified a novel TPR-like protein-encoding gene, LOW SEED SETTING 1 (LSS1), involved in pollen and seed development in rice. The LSS1 protein is localized in the Golgi and other vesicles in cytoplasm. T-DNA insertional mutant lls1 exhibited low pollen viability leading to poor seed setting. Suppression of LSS1 by RNAi accentuated the LSS1 loss-of-function phenotypes in the T-DNA insertion line, while ectopic over expression of LSS1 cDNA in the homozygous mutant fully restored the wild-type phenotype. These data suggest that a novel TPR protein is involved in pollen development, greatly impacting plant seed setting."
(a) Clemson University

P50015 "Influence of abiotic stress, flower morphology, and pollen dehydration sensitivity on cotton out-crossing potential"
Burke, John J-presenter john.burke@ars.usda.gov(a)
"Genetic diversity in reproductive abiotic stress tolerance has been reported for cotton [*Gossypium hirsutum* (L.)] based upon the percentage of anther dehiscence of mature pollen in adverse environments. This study investigated the abiotic stress tolerance of mature pollen and identified

genetic variability among six cotton lines. Similar high temperature sensitivities were observed for the SG248, STV474, DP565, NM67, Acala maxxa, and Phy72 pollen. Genetic diversity in pollen viability was observed following a 6.5 h exposure to 25% relative humidity (RH). NM67, DP565, and SG246 exhibited less inhibition of pollen germination than STV474, Acala maxxa and PHY72. Similar pollen water contents were observed for all lines. Genetic diversity in pollen tube length development at 25% RH compared with 80% RH was observed. Acala maxxa and Phy72 pollen produced tube lengths of 35-40% of controls at 80% RH, while STV474, SG248, DP565, and NM67 exhibited tube lengths 50-60% of controls. Pollen water uptake studies showed faster uptake in PHY72 and AM than the other lines. Competitive pollinations showed faster germination of PHY72, AM and SG248 pollen compared to STV474, DP565 and NM67. These findings show genetic differences in cotton pollen sensitivities to water uptake and water loss. Field studies of cotton pollen gene flow under irrigated and dryland conditions show the importance of abiotic stress, flower morphology, and pollen dehydration sensitivity on out-crossing potential."
(a) USDA-ARS Cropping Systems Research Laboratory

P50016 Investigating Pollen Function in *Arabidopsis LRX8* and *LRX9* Double Mutants
Denney, Ashley S.-presenter asdenney@rams.colostate.edu(a) Bedinger, Patricia A. (a)
"Fertilization in higher plants requires pollen tube growth through female tissue. Pollen-expressed LRX (Leucine-rich Repeat eXtensin) proteins are found within pollen tube cell walls and may be important for pollen tube growth. In *Arabidopsis thaliana*, there are four pollen-specific *AtLRX* genes that group as two closely related pairs by sequence comparison; *AtLRX8* and *AtLRX9* form one pair and *AtLRX10* and *AtLRX11* form another pair. *Arabidopsis* lines with T-DNA loss-of-function insertion mutations in individual pollen *AtLRX* genes (*AtLRX8*, *AtLRX9*, *AtLRX10*, and *AtLRX11*) were obtained through the Salk Institute. Pollen is unaffected in homozygous mutants of any individual line. This study was undertaken to test the effect of mutations in the related gene pair, *AtLRX8* and *AtLRX9*, on pollen function. To assess this, we developed a PCR genotyping assay to identify wild-type and mutant alleles for *AtLRX8* and *AtLRX9*. Select F2 plants with one wild-type allele in either *AtLRX8* or *AtLRX9* were selfed and crossed as pollen parent onto male sterile plants. Progeny above were genotyped by PCR. Here we show successful transmission of both mutant alleles with recovery of unique *Atlrx8|Atlrx9* pollen-contributed progeny, implying functional redundancy between *AtLRX8|AtLRX9* and *AtLRX10|AtLRX11* gene pairs. Microscopic examination and *in vitro* germination are currently underway with *Atlrx8|Atlrx9* pollen relative to Columbia pollen, as well as tetrad analysis of select F2 pollen in *quartet* background. We plan to further investigate the roles of the remaining pollen-specific *LRX* genes, *AtLRX10* and *AtLRX11*, in pollen function. Understanding gene function in *Arabidopsis* as it concerns fertilization has implications in the genetics of sexual plant reproduction as well as potentially agriculture."
(a) Colorado State University

SESSION P51 – PRIMARY METABOLISM

P51001 Closed and Open Form Crystal Structures of *Glycine max* Homoglutathione Synthetase
Galant, Ashley-presenter a.l.galant@gmail.com(a,b) Arkus, Kiani (b) Zubieta, Chloe (b) Cahoon, Rebecca (b) Jez, Joseph (a,b)
"In plants, glutathione serves as a general reductant, a sequestration agent for heavy metals, and as a detoxicant for xenobiotics. Glutathione is synthesized by a two-step ATP-dependent pathway. In the first step, glutamate-cysteine ligase catalyzes the formation of γ-glutamylcysteine from glutamate and cysteine. This intermediate product then further reacts with glycine via glutathione synthetase (GS) to produce glutathione. However in some plants, notably legumes, glycine is replaced with b-alanine; this alternate reaction is catalyzed homoglutathione synthetase (hGS) and results in the production of homoglutathione instead of glutathione. Here we present the x-ray crystal structures of soybean hGS in two states: an apoenzyme (open) form (2.0 angstrom resolution) and a closed form in complex with homoglutathione and ADP (1.9 angstrom resolution). These structures shed light on domain movements occurring within the protein during its catalytic cycle. Comparison with human and yeast GS structures suggests that two amino acid differences allow for accommodation of a larger substrate in hGS than GS. Site-directed mutagenesis of Leu466 and Pro467 within a conserved active site loop provides insight into the determinants of substrate specificity for b-alanine (hGS) versus glycine (GS) in these related enzymes."
(a) Department of Biology, Washington University, St. Louis, MO (b) Donald Danforth Plant Science Center, St. Louis, MO

P51002 Biochemical Characterization of Arabidopsis thaliana β-Cyanoalaine Synthase
Juergens, Matthew T-presenter mjuergens05@webster.edu(a) Cahoon, Rebecca E (a) Jez, Joseph M (a,b)
"Plants generate cyanide (CN-), a potent toxin affecting the electron transport chain, during the synthesis of ethylene. For CN- detoxification, β-cyanoalanine synthase (CAS) uses pyridoxal phosphate as a cofactor to react cysteine and CN- to make β-cyanoalanine. This reaction is similar to that catalyzed by O-acetylserine sulfhydrylase (OASS), which catalyzes the formation of cysteine from hydrogen sulfide and O-acetylserine. Arabidopsis thaliana CAS and OASS are ~61% similar in amino acid sequence. To better understand how these two enzymes evolved specificity for each reaction, we have expressed and purified recombinant CAS and OASS. OASS shows a 71-fold preference for O-acetylserine versus cysteine and a 7900-fold preference for sulfide over CN- as a nucleophile. In contrast, CAS is 39-fold more efficient with cysteine over O-acetylserine and displays a 12-fold preference for CN-. Spectroscopic analysis of CAS and OASS demonstrates that each enzyme utilizes an α-aminoacrylate reaction intermediate, although CAS and OASS bind different substrates, cysteine and O-acetylserine, respectively. The structural and functional basis for the evolution of substrate specificity in these enzymes is under investigation."
(a) Donald Danforth Plant Science Center (b) Department of Biology Washington University

P51003 Gene expression profiles in field-grown perennial ryegrass under different defoliation practises
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"Perennial ryegrass (*Lolium perenne* L.) is a highly significant pasture plant in all temperate pastoral regions, with its popularity largely attributed to its ability to grow large amounts of high quality feed for livestock. While different defoliation practises influence regrowth, the complex biological processes within the plant that are affected are largely unknown. In New Zealand, $6m^2$ field plots of ryegrass were defoliated three times at the 1-leaf stage of regrowth or once at the 3-leaf stage to 20, 40 or 60 mm height. All swards were then regrown to the 3-leaf stage before a final defoliation to their treatment heights. Relative to those previously defoliated at the 1-leaf stage, defoliation at the 3-leaf stage increased herbage regrowth. Defoliating swards to 20 or 40 mm increased herbage regrowth compared with those defoliated to 60 mm. Frequent defoliation reduced post-defoliation stubble water-soluble carbohydrate (WSC) content, but the effect had dissipated by the 1-leaf stage of the subsequent regrowth period. Stubble WSC content declined with increasing defoliation severity, and this trend remained throughout the regrowth period. Gene expression profiles of 14 genes involved in photosynthesis/carbohydrate metabolism/transport were analysed in samples of leaf and stem tissue collected the day after defoliation, and at the 1-, 2-, and 3-leaf stages of regrowth, to explain some of the WSC changes in source and sink tissues. To the best of our knowledge, no study has attempted to analyse gene expression in field-grown perennial monocots. Before we can harness the power of biotechnology to commercialise perennial grasses for pastoral/biofuel use, it is imperative that we develop an in-depth understanding of gene

expression in these plants grown in the field."
(a) DairyNZ Ltd. (b) Tasmanian Institute of Agricultural Research, University of Tasmania (c) Pastoral Genomics, ViaLactia Biosciences

P51004 A Plant-like Methyltransferase Pathway for Phosphocholine Biosynthesis in the Parasitic Nematode *Haemonchus contorticus*
Lee, Soon Goo-presenter slee@danforthcenter.org(a,b) Haakenson, William (c) Kumaran, Sangaralingam (b) McCarter, James P. (c) Williams, D. Jeremy (c) Hresko, Michelle C. (c) Jez, Joseph M. (a,b)
"Phosphatidylcholine (PtdCho) is a structural component and one of the most abundant phospholipids in animal, plant, and some prokaryote cell membranes. In plants and *Plasmodium falciparum*, the formation of PtdCho requires a single enzyme, phosphoethanolamine N-methyltransferase (PEAMT), which catalyzes a triple sequential methylation reaction of phosphoethanolamine (pEA) to produce phosphocholine (pCho), which is a precursor of PtdCho. Recent studies suggest that *C. elegans* synthesizes phosphocholine through the action of two PEAMT enzymes, PMT-1 and PMT-2. To determine if PMT homologs are also functional in a parasitic nematode, we isolated two PMT-like cDNAs from *Haemonchus contortus*. The two PMT enzymes were overexpressed in *E. coli* and purified by affinity and size-exclusion chromatographies. Initial kinetic studies show that HcPMT-1 only catalyzes the conversion of pEA to phospho-monomethylethanolamine (pMME), which is the first step in the PMT-pathway. The last two steps, the methylation of P-MME to phospho-dimethylethanolamine (pDME) and of pDME to pCho, are sequentially catalyzed by HcPMT-2. The results suggest that the two PMT enzymes perform the multiple methylations in the pathway in contrast to the multifunctional PMT from plants and Plasmodium that use a single enzyme to catalyze the reactions, indicating a functional evolution for the nematode PMT. Future work will examine the structural difference between the plant PEAMT and two PMT from the parasitic nematodes through the crystallization of each enzyme. Because the PMT homologs are not found in mammals and are highly conserved in multiple parasitic nematodes, the identification of the structures and inhibitors targeting PMT may prove valuable in human and veterinary medicine and agriculture. "
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P51005 Molecular and functional characterization of the plastid-localized phosphoenolpyruvate enolase (ENO1) from Arabidopsis thaliana
Prabhakar, Veena-presenter veena.prabs@gmail.com(a) Loettgert, Tanja (a) Bell, Kirsten (a) Fluegge, Ulf-Ingo (a) Haeusler, Rainer E (a)
"The Arabidopsis thaliana gene At1g74030 codes for a putative plastid phosphoenolpyruvate (PEP) enolase (ENO1). The recombinant ENO1 protein exhibited enolase activity and its kinetic properties were determined. ENO1 is localized to plastids and expressed in most heterotrophic tissues including trichomes and non-root-hair cells, but not in the mesophyll of leaves. Two T-DNA insertion eno1 mutants exhibited distorted trichomes and reduced numbers of root hairs as the only visible phenotype. The essential role of ENO1 in PEP provision for anabolic processes within plastids, such as the shikimate pathway, is discussed with respect to plastid transporters, such as the PEP/phosphate translocator."
(a) Institute of Botany, University of Cologne

P51006 CP12 co-ordination of thioredoxin regulated enzymes is crucial for plant development
Raines, Christine A-presenter rainc@essex.ac.uk(a) Howard, Thomas P (a) Singh, Prashant (a)
"The small redox sensitive chloroplast protein, CP12, has been shown to regulate the activity of two thioredoxin f regulated enzymes, NADP-GAPDH and PRKase in the photosynthetic carbon reduction (Calvin) in responses to changes in light intensity. This regulatory mechanism involves the formation of a multiprotein complex mediated by oxidized CP12. The redox state of the CP12 protein is regulated by thioredoxin f providing a mechanism to link Calvin cycle function with the availability of energy from the electron transport chain. Unexpectedly, CP12 antisense plants have a severe growth phenotype with abnormal leaf and floral morphology, reduced apical dominance and, in some lines, the flowers are sterile. However, photosynthesis in these plants is reduced by only maximum of 25- 30% and PRKase and GAPDH activities are similar to that in wild type plants. It is highly unlikely that these changes could account for the dramatic differences in morphology in these plants. Interestingly the activities of two additional thioredoxin regulated chloroplast enzymes, malate dehydrogenase and glucose-6-phosphate dehydrogenase, are different in the antisense plants compared to wild type plants. Carbon allocation was also altered in the antisense plants and steady state levels of both starch and sucrose were reduced by 50%. In addition oxoglutarate levels were significantly reduced as were the levels of the aromatic amino acids. Interestingly changes in levels of polyamines were evident which could be related to the abnormal leaf morphology. Our data have revealed that CP12 plays a central role in mediating thioredoxin modulation of chloroplast enzymes in order to maintain a balance between metabolism and the availability of NADPH. "
(a) University of Essex

P51007 Reaction mechanism of phytochromobilin synthase HY2 from *Arabidopsis*
Chiu, Fang-Yi-presenter doris714@gate.sinica.edu.tw(a) Chen, Hsiu-Chen (a) Tu, Shih-Long (a)
"Phytochromes are a major class of photoreceptors that regulate plant growth and development. Their activity depends on the correct assembly of the apophytochrome and chromophore precursor. The immediate precursor of the phytochrome chromophore, phytochromobilin, is synthesized in plastids by the phytochromobilin synthase. Phytochromobilin synthase is encoded by the HY2 (LONG HYPOCOTYL 2) gene in Arabidopsis, catalyzes the ferredoxin-dependent reaction of phytochromobilin formation. Mutations in HY2 have been shown to severely affect light-mediated plant growth and development due to the loss of all functional phytochromes. HY2 is a member of ferredoxin-dependent bilin reductase family of enzymes, which catalyze the synthesis of linear tetrapyrrole chromophores important for the growth and development of photosynthetic organisms. Our approaches including spectroscopic analysis, site-directed mutagenesis, and structural homology modeling have shown the catalytic reaction of HY2 proceeds with a radical mechanism. Several potential proton donating residues in the active site of HY2 are involved in the catalytic steps. We are also working on the interaction between the electron donor ferredoxin and HY2. Preliminary data showed that their interaction mainly involves the salt bridges in-between charged residues on both proteins. A mechanistic prediction of HY2 reaction from ferredoxin interaction to phytochromobilin production will be presented."
(a) Institute of Plant and Microbial Biology, Academia Sinica

P51008 A novel plant-type ferredoxin-dependent bilin reductase from *Physcomitrella patens*
Chen, Yu-Rong-presenter cyr302@gate.sinica.edu.tw(a) Wu, Chia-Chen (a) Tu, Shih-Long (a)
"Phytobilins are open-chain tetrapyrrole molecules that mainly function as chromophores of light-harvesting phycobiliproteins and light-sensing phytochromes in phototrophs. In the phytobilin biosynthesis pathway, biliverdin IXalpha is converted to different phytobilins by members of ferredoxin-dependent bilin reductase (FDBR) family with distinct double bond reduction activities. It has been shown for a long time that *HY2 (LONG*

HYPOCOTYL 2) is the only FDBR which encodes a phytochromobilin:ferredoxin oxidoreductase in land plants including ferns and mosses. However, we have lately found another homolog of FDBRs in *Physcomitrella patens*, a model species of mosses. In this study, we demonstrated that the novel plant-type FDBR has the same enzymatic activity as PebA, a FDBR generally present in cyanobacteria and algae that converts biliverdin IXalpha to 15,16-dihydrobiliverdin. Thus, we named the novel FDBR as PpPEBA. According to the results of transient assay and gene targeting, both PpPEBA and PpHY2 are localized in chloroplasts. At gene expression level, *PpPEBA* is highly expressed at gametophore. Additionally, homologs of PpPEBA were also found in *Selaginella mollendorffii* (fern), *Micromonas* strain RCC299 (green alga) and *Ostreococcus* (green alga). These results indicate that the PebA is universally present in lower photosynthetic organisms, such as cyanobacteria, algae, mosses and ferns. We believe this discovery will provide a new insight into the mechanism of light-harvesting or light-sensing in plant evolution. We also obtained gene-targeting knockout mutants of *PpPEBA* recently. Physiological functions of *PpPEBA* will be investigated and presented in the meeting."
(a) Plant and Microbial Biology, Academia Sinica

P51009 Alterations in Respiration Rate and Glycolytic Intermediates in Wounded Sugarbeet Roots
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"Wounding of sugarbeet roots causes an increase in respiration rate, which contributes to postharvest sucrose losses. Although respiration is estimated to cause 60 to 80% of postharvest sucrose losses, the mechanisms that regulate respiration rate in wounded sugarbeet roots are not well known. To identify mechanisms of respiratory control for wounded sugarbeet roots, root respiration was characterized with respect to early glycolytic substrates and their related enzymes. Glycolytic substrates and enzymes were characterized in wounded roots at the site of injury and in unwounded tissue, allowing localized and systemic changes to be determined. Respiration increased in wounded roots after 3 days storage. Fructose increased in wounded tissue compared to unwounded control roots or the uninjuried tissue from wounded roots. There were also localized and systemic increases in glucose 6-P and fructose 6-P in wounded roots. Glucose 1-P increased in the unwounded tissue of wounded roots but was unchanged in wounded tissue. Triose phosphate and fructose 1,6-BP decreased in wounded tissue compared to unwounded control roots. This decrease could be due to their utilization as substrates in the elevated respiration rate of wounded tissue. Hexokinase and fructokinase activities increased in wounded tissue after 2 and 4 days. However, phosphofructokinase activity transiently increased in systemic tissue of wounded roots and transiently declined in wounded tissue. The increase in glucose 6-P and fructose 6-P corresponded with the activation of hexokinase and fructokinase in wounded tissue. Changes in glycolytic intermediates and enzymes in wounded tissue suggest that glycolysis is altered in response to wounding, possibly to provide additional substrates for elevated root respiration."
(a) Northern Crop Science Lab., ARS, USDA

P51010 Unique primary metabolism during seed germination of root parastic plants
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http://www.bio.eng.osaka-u.ac.jp/pl/index.html
"Parasitic plants cause serious damage to agriculture worldwide. A novel strategy for the parasitic weed control is desired for economical and humanitarian reasons. Since the life cycle of parasitic weeds is significantly different from that of host plants, understanding of the parasite-specific biological events is important for design of selective control strategies. We focused on the germination process of parasitic weeds to find biological events specific to these species. Metabolic profiling revealed that activation of metabolism occurred only after a synthetic strigolactone GR24 treatment in seeds of *Orobanche minor*. One of the identified metabolites, allantoin was found to decrease immediately after GR24 treatment. Acetohydroxamate (AHA), an allantoin catabolism inhibitor, reduced the germination rate of *O. minor*. This reduction was recovered by additional treatment with excess amounts of allantoin catabolites, such as urea and ammonium. Moreover glutamine was increased in parallel with the allantoin degradation. In Arabidopsis and other plants seeds, arginine-rich storage proteins are pooled as a nitrogen source and arginine is remobilized during germination but we could not detect arginine in O. *minor* seeds. From these result, we hypothesize that allantoin is pooled in *O. minor* seeds as a nitrogen source. Gentianose was also found to decrease by GR24 treatment. Gentianose is a trisaccharide consisted of two glucoses and a fructose. The amounts of glucose and fructose significantly increased after GR24 treatment indicating these were supplied with the hydrolysis of gentianose. An inhibitor of gentianose decreased the germination rate and also the amounts of glucose and fructose. These results indicate the uniqu primary metabolism during their seed germination."
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P51011 Metabolomics of a single organelle reveals metabolic dynamism in an alga *Chara corallina*
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http://labs.psc.riken.jp/mart/English/index.html
"Metabolomics is the most reliable analytical method for understanding metabolic diversity in single organelles derived from single cells in various biological conditions. On the basis of past studies, different organelles are closely associated with well-defined roles (such as storage function in vacuole). Therefore, metabolites are believed to be localized to different organelles in a highly-specific manner. In general, cell volume is too small for the detection of metabolites in single organelles using current techniques. The analysis of metabolites in single organelles has consequently presented a significant challenge. In this study, we have identified 55 metabolites in a single vacuole and in cytoplasm isolated from a single giant internodal cell of an alga *Chara corallina* by metabolomic analysis using CE-MS (capillary electrophoresis-mass spectrometer). Identified metabolites were categorized into 2 groups according to distribution; vacuole-type and cytoplasm-type. Additionally, fluctuations of metabolites in vacuolar and cytoplasmic compartments were observed under changing light conditions. Our metabolomics approach provides novel insights on metabolic dynamics in a single organelle derived from a single cell. This study confirms for the first time that metabolites exhibit organelle-specific localisation within single cells, and reveals a previously unknown biological function for the vacuole."
(a) RIKEN Plant Science Center (b) Department of Biology, Faculty of Science, Kobe University (c) Graduate School of Pharmaceutical Sciences, Chiba University

P51012 Chloroplastic phospho*enol*pyruvate carboxylase of rice plays an important role in the nitrogen assimilation
Masumoto, Chisato (a) Miyazawa, Shin-Ichi (a) Fukuda, Takuya (a) Miyao-Tokutomi, Mitsue-presenter mmiyao@affrc.go.jp(a)
"Phospho*enol*pyruvate carboxylase (PEPC) catalyzes the CO_2 fixation in C4 and CAM photosynthesis and has an anaplerotic function of replenishing the TCA cycle with intermediates in C3 plant leaves. Although PEPC has long been considered to be cytosolic, rice has a plant-type PEPC targeted to the chloroplast, Osppc4. *Osppc4* is highly expressed in mesophyll cells of leaf blade and leaf sheath, and also in roots and spikelets albeit at low levels. The physiological functions of Osppc4 were investigated by comparing non-transgenic rice with transgenic rice, in which *Osppc4* expression was knocked down by the RNAi technique. The knockdown reduced the maximum PEPC activity, measured in the presence of glucose 6-phosphate, to about 70%, an indication that Osppc4 accounted for about one third of total PEPC protein in the leaves. A remarkable phenotype of the knockdown lines was the growth inhibition, which was more marked when ammonium was used as the nitrogen source than nitrate. Photosynthetic

characteristics examined by gas-exchange measurements were not at all affected. The growth analysis indicated that the lamina area and the nitrogen uptake rate were significantly reduced by the knockdown, suggesting the suppressed nitrogen uptake and/or assimilation. Changes in levels of organic acids and amino acids confirmed this hypothesis and suggested that the ammonium assimilation by the GS-GOGAT cycle was suppressed due to the reduced level of malate in the knockdown lines."
(a) National Institute of Agrobiological Sciences

P51013 "Deficiency in a cytosolic ribose-5-phosphate isomerase causes chloroplast dysfunction, late flowering and premature cell death in Arabidopsis"
Mou, Zhonglin-presenter zhlmou@ufl.edu(a) Xiong, Yuqing (a) DeFraia, Christopher (a) Zhang, Xudong (a)
"The oxidative pentose phosphate pathway (oxPPP) is part of central metabolism, consisting of two distinct stages: the oxidative stage and the nonoxidative stage. The nonoxidative stage of the oxPPP generates carbon skeletons for the synthesis of nucleotides, aromatic amino acids, phenylpropanoids and their derivatives, which are essential for plant growth and development. However, it is not well understood how the nonoxidative stage of the oxPPP contributes to plant growth and development. Here we report the characterization of Arabidopsis T-DNA knockout mutants of the *RPI2* gene (At2g01290), which encodes a cytosolic ribose-5-phosphate isomerase (RPI) that catalyzes the reversible interconversion of ribulose-5-phosphate and ribose-5-phosphate in the nonoxidative stage of the oxPPP. Although recombinant Arabidopsis RPI2 protein exhibits marked RPI enzymatic activity, knockout of the *RPI2* gene does not significantly change the total RPI activity in the mutant plants. Interestingly, knockout of *RPI2* interferes with chloroplast structure and decreases chloroplast photosynthetic capacity. The *rpi2* mutants accumulate less starch in the leaves and flower significantly later than wild type when grown under short-day conditions. Furthermore, the *rpi2* mutants display premature cell death in the leaves when grown at an above-normal temperature. These results demonstrate that a deficiency in the nonoxidative stage of the oxPPP has pleiotropic effects on plant growth and development and causes premature cell death."
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P51014 Overexpression of *ZmDof1* in rice alters carbohydrate and nitrogen partitioning
Kurai, Tomohiro-presenter aceout@mail.ecc.u-tokyo.ac.jp(a) Wakayama, Masataka (a) Aoki, Naohiro (a) Ohsugi, Ryu (a)
"Due to the negative environmental effects of excessive nitrogen input, increasing nitrogen use efficiency is an important approach to achieve sustainable development. Nitrogen metabolism is inseparably connected to carbon metabolism and regulated tightly, therefore transcription factor which can regulate multiple gene expression may function better. Dof1 is a plant specific transcription factor, which has shown increased plant growth under low-nitrogen condition in *Arabidopsis thaliana*. In our experiment, the effect of introduction of *ZmDof1* in *Oryza sativa* (cv. Nipponbare) is examined. *ZmDof1* cDNA, driven by the maize ubiquitin promoter, was introduced into rice by means of Agrobacterium. T-DNA insertion and *ZmDof1* expression was confirmed by PCR. Genomic southern blot to 22 T_1 lines revealed 1-5 copy insertion. Using 4 Dof1 lines and vector control (Dof1 is replaced by mGFP), a hydroponic experiment was conducted under 2 nitrogen regimes, 10ppm (control) and 2.5ppm (low-N). T_3 seedlings were grown for 14 days under deionized water. 16 uniform plants of each line were grown for 46 additional days under 2 nitrogen solutions then sampled. *ZmDof1*-expressing rice plants showed normal growth and phenotype. The dry weight of both shoots and roots were significantly increased. Total N contents also increased, yet the NC ratio remained the same. T/R ratio of Dof1 rice significantly decreased. Organic acid profile in leaves and amino acid profile in roots changed considerably. Soluble protein and chlorophyll concentration as well as PEP Carboxylase activity in leaves increased. These results indicate Dof1 alters both nitrogen and carbon metabolism. This work was supported in parts by grants from the Program for Promotion of Basic Research Activities for Innovative Biosciences. "
(a) Graduate School of Agricultural and Life Sciences, The University of Tokyo

P51015 Regulation of cysteine synthesis in soybean
Gordon, Courtney D-presenter cmorriss@ufl.edu(a) Kirst, Mariana E (a) Vyas, Dhiraj (b,a) Harmon, Alice C (a)
"Sulfur metabolism is essential for plant growth and production of high nutritional quality food. Cysteine biosynthesis is the terminal step of sulfur assimilation, and it lies at the junction between sulfur and nitrogen metabolism and feeds into the biosynthesis of the protective compounds (homo)glutathione and phytocheletin. Cysteine is synthesized from serine by the enzymes serine acetyltransferase (SAT) and O-acetylserine (thiol) lyase (OASTL). In Arabidopsis, cysteine synthesis is regulated by metabolites which control the reversible formation of a complex between SAT and OASTL, called the cysteine synthase complex (CSC). Our results for three SATs and two OASTLs from soybean show that, as in Arabidopsis, formation of CSC enhances SAT activity while it inhibits OASTL activity. However, the plastidic/cytosolic SAT (GmSerat2;1) was less able than the cytosolic SAT (SAT1) to inhibit OASTL. GmSerat2;1 has a phosphorylation site unique to this isoform, and the mutant GmSerat2;1S378D mimics constitutive phosphorylation in that it is insensitive to feedback inhibition by cysteine. GmSerat2;1S378D did not inhibit OASTL activity to the same degree as wild type isoforms. In addition, in assays of the ability of CSC to synthesize cysteine from serine, complexes containing GmSerat2;1S378D showed the highest activity relative to those containing wild type enzyme. These experiments suggest that phosphorylation supports higher production of cysteine, not only by relieving feedback inhibition of GmSerat2;1, but also by lowering inhibition of OASTL in the CSC. This research is supported by USDA NRI CREES Award 2006-35318-17392 to ACH. "
(a) Program in Plant Molecular and Cellular Biology and Department of Biology, University of Florida, Gainesville, Florida (b) Indian Institute of Integrative Medicine (CSIR), Jammu, India

P51016 The role of sucrose-cleaving enzymes in controlling storage organ growth and development.
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http://www.cef-cfr.ca/index.php?n=Membres.AnthonyGandin
"Recent studies on *Erythronium americanum* indicate that this spring geophyte produces a much larger bulb at low than at warmer temperatures, a unique case amongst temperate species. Although foliar senescence is delayed at low temperature, longer leaf life duration alone cannot explain the temperature effects. Sucrose-cleaving enzymes are known to regulate sugar entrance into the different biochemical pathways, and could thus influence starch accumulation, the main component of bulb biomass. We investigated whether these key enzymes could explain the temperature effects on bulb growth in *E. americanum*. Plants were grown at three temperatures: 18/14°C, 12/8°C and 8/6°C. Cell wall, acid and neutral invertase activities along with sucrose synthase activity were monitored in the bulb throughout the season. Enzymes increased in activity sequentially during sink growth; comparisons between temperatures indicated an earlier induction of these enzymes at warmer temperatures. Cell wall invertase activity peaked during the first days of bulb initiation while cell division and elongation were occurring. A few days later, acid and neutral invertase activities increased while starch accumulates. Acid invertase activity was higher at lower temperatures, suggesting a vacuolar accumulation of carbohydrates. This accumulation could avoid the induction of early leaf senescence. During bulb development, invertase activities slowly decreased whereas sucrose synthase activity, on the other hand, reached its maximum a few days before leaf senescence. It appears that the delayed activation of sucrose synthase at lower temperatures increased the duration of the cell elongation phase, leading to larger cells and an increased capacity to accumulate

carbohydrates within the bulb."
(a) Nancy-University, UMR1137 Ecologie et ecophysiologie forestieres (b) Laval University, Departement de Biologie & Centre for Forest Research

SESSION P52 – PROTEIN MODIFICATION & TURNOVER

P52001 Chlorophyllase regulation during citrus fruit color break: Dual N and C-terminal processing of Chlorophyllase precursor within the plastid membranes lead to the active mature enzyme
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"Chlorophyllase (Chlase) was recently shown to be the rate limiting enzyme in chlorophyll catabolism in vital tissues, regulated at both the transcriptional and posttranslational levels. Dramatic chlorophyll catabolism occurs during the chloroplast-chromoplast transition characteristic of citrus fruit color break, however, the regulatory mechanism is still not deciphered. Using in-situ immunofluorescence, we demonstrate a strong correlation between Chlase accumulation and chlorophyll breakdown in ethylene treated lemon peel plastids. We show that Chlase initially accumulates as a ~35 kDa precursor, which is subsequently processed to a ~32 kDa mature enzyme. Our most recent data shows that the mature Chlase is the result of dual posttranslational processing at both N and C termini, which has rarely been documented in plants and is not known for plastid proteins. MS analysis of purified citrus Chlase reveals that cleavage at the N-terminus occurs at either of 3 consecutive amino acid positions (19/20/21), while cleavage at the C-terminus removes a short carboxy terminal extension. Both N and C terminal processing events, leading to the mature enzyme, occur within the plastid membranes, suggesting involvement in post-translational regulation of enzyme activity in-vivo. Indeed, the N-terminus of the enzyme was found not to be essential for plastid targeting, and its removal results in a more active enzyme in-vivo. The significance of the dual N and C-terminal processing events, as post-translational regulatory steps in chlorophyll degradation, will be discussed. "
(a) Institute of Plant Sciences, The Volcani Center, ARO, Israel (b) Faculty of Agriculture, Food, and Environmental Quality Sciences,Hebrew University of Jerusalem, Israel (c) Centre for Proteomics and Systems Biology, Manitoba University, Canada

P52002 Thermodynamic studies of a plant specific type-II thioredoxin in Arabidopsis
Kim, Sang Gon-presenter Sang_Kim@baylor.edu(a,c) Yong Hun , Chi (c) Seung Sik, Lee (c) Jong-Sun , Lee (a) Sang Yeol , Lee (c) Sung-Kun , Kim (a,b)
"AtTDX is an enzyme from *Arabidopsis thaliana* that is composed of two domains, with a thioredoxin-like C-terminus and an N-terminal tetratricopeptide (TPR)-repeating domain (AtTDX). This enzyme has been shown to function as both a thioredoxin and a chaperon. A deletion experiment that removes the TPR domain has shown an increase in the reduction of thioredoxin-dependent proteins, but a loss of the chaperon property. The thermodynamic basis of AtTDX has been investigated in the presence and absence of the TPR domain using various redox titrations, i.e., titration of a thiol-specific binding monobromobimane, the intrinsic alternation of tryptophan microenvironment, and a thiol-specific binding mal-PEG titration. For the both cases, the titrations show the presence of a dithiol/disulfide couple in the enzyme with midpoint potential values of approximately -290 mV at pH 7.0. Site-directed mutagenesis was used to identify the redox active sites. The two cysteines present in the typical thioredoxin conserved amino acid sites (WCGPC) which may play a role in the electron transfer mechanism of the reduction process have been mutated. The cysteines present in the redox active sequence have been mutated (i.e., Cys304Ser, Cys307Ser, and Cys304/307Ser) to identify the electron entry point of the enzyme. The thioredoxin-like domain was separately cloned, expressed and purified. The results of the redox titration of the domain support the evidencethat the redox values arise from the disulfide bond formed by the two cysteines."
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P52003 Understanding ubiquitination in plant stress signaling
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http://www.genetics.ac.cn/xywwz/Faculty/XieQi.htm
"Ubiquitination plays important roles in plant adapting itself to internal and external signals to regulate plant growth and development. The model plant Arabidopsis (*Arabidopsis thaliana*) genome size is about 120 Mbs and it contains near 30,000 predicted genes, in which approximately 5% of the Arabidopsis proteome is predicted to be involved in the ubiquitination/26S proteasome pathway. Recent developments in plant stress biology have demonstrated that numbers of plant E3 complexes involved in both biotic and abiotic stress signaling. The majority of these predicted proteins have identity to conserved domains found in E3 ligases from other eukaryotic systems. Since E3 ligases are key factors to decide the substrate specificity, we are currently focusing on one group of them, the RING finger E3 ligases in plant. RING finger proteins are composed of big family with near 500 members in Arabidopsis. By microarray proteomics analysis we found that numbers of Arabidopsis RING finger E3 ligases responding to different stress treatments and involved plant biotic and abiotic stress signaling. We applied genetic and molecular analysis to detect the functions of numbers of RING finger E3 ligases in Arabidopsis, as well as in the other model plant rice (*Oryza sativa*). We will focus to discuss the function of two novel RING finger E3 ligases, AtKPPC1 and SDIR1 in biotic and abiotic stress signaling, respectively. Our results demonstrated that plant RING finger E3 ligases play key roles in plant adaptation to different internal external signals to control plant growth and development. In addition we will present a novel approach by applying an efficient transient expression system in tobacco to study the ubiquitination *in vivo* in plant."
(a) Chinese Academy Of Science Institute Of Genetics and Developmental Biology

P52004 High-throughput measurement of protein turnover in plants using stable isotope labeling coupled with LC-MS/MS analysis
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"A novel statistical modeling method allows automated measurement of protein turnover rate (T1/2). Using RuBisCO as a reference protein, Arabidopsis was labeled with ^{15}N or $^{13}CO_2$ in automated chambers for 3 weeks before chasing under unlabeled conditions. To identify peptides using existing databases, labeled samples were spiked with unlabeled control before SDS-PAGE, trypsin digestion and LC-MS/MS. The MS spectra were processed using an algorithm written mostly in R. A linear regression step was added to remove chemical noise and modeling spectra of ^{15}N labeled peptides as mixed beta-binomial distributions and fitting via statistical maximum likelihood estimation proved successful for calculating both labeling ratios and abundance of dynamically changing peptide isotopomer distributions. Using this algorithm, we identified and calculated the T1/2 of 17 RuBisCO peptides in approximately 1 min. The T1/2 varied from 0.96 to 1.49 days based on the labeling ratio change of newly synthesized peptides, and 1.81 to 2.56 days based on the abundance changes of either new or old peptides. The variations in T1/2 are likely related to amino acid composition. Spectrum fitting using a beta-binomial model did not give consistent results for the $^{13}CO_2$ labeled peptides, probably due in part to the wide isotopomer distributions of partially labeled peptides. However, estimation could still be made using a simple mixed binomial model. The T1/2 of

$^{13}CO_2$ labeled RuBisCO based on abundance changes from 7 common peptides was estimated at 2 days, which was close to the ^{15}N method. A web-based program will be available soon for easy access to the methods. Ultimately this approach will be optimized for proteome-wide T1/2 measurements that should provide insight into protein dynamics in plants. "
(a) Dept. Horticultural Science, U of MN, Saint Paul, MN (b) School of Statistics, U of MN, Saint Paul, MN (c) Center for Mass Spectrometry and Proteomics, U of MN, Saint Paul, MN (d) Dept. Plant Biology, U of MN, Saint Paul, MN 55108

P52005 Using $^{2}H_2O$ to measure turnover rates of plant proteins
Yang, XiaoYuan-presenter yangx973@umn.edu(a,c) Chen, Wen-Ping (b,c) Hegeman, Adrian D (a,b) Gray, William M (a,c) Cohen, Jerry D (b,c)
"Using 30% $^{2}H_2O$ with Arabidopsis, the labeling efficiency of amino acids were variable, from ~60-100% of theoretical. The half-lives ($T_{1/2}$) of Ala, Asp, Glu and Ser were less than 2 h; Gly, His, Met, Phe, Pro, Trp and Val showed a $T_{1/2}$ of 3~15 h; and Leu, Ile, Tyr and Lys exhibited a $T_{1/2}$ greater than 24 h. The feasibility for assessing protein turnover was examined using the CAND1 protein and LC-MS/MS. We found a calculated $T_{1/2}$ of ~35 h using an incorporation chase and ~26 h when measured in a dilution chase experiment. Half-lives were variable among different CAND1 peptides, from 28 to 40 hours during incorporation chase and 16 to 39 hours during dilution chase. This variation was likely a reflection of peptide amino acid composition and the variable $T_{1/2}$ of the distinct amino acid pools. To characterize responses to growth on $^{2}H_2O$, we employed microarray analysis to identify genes whose expression was altered. Microarray data indicated that relative few genes were altered by a 7-day $^{2}H_2O$ treatment or a 4 h H_2O recovery; ~ 3% of expressed genes were affected by a 4 h $^{2}H_2O$ treatment and many of them are typical for a response to stress. Some stress response genes were also down regulated by a 4 h H_2O recovery. Thus, where stress-related gene expression is important in the process being studied, stress responses to the labeling conditions may make $^{2}H_2O$ labeling less suitable than other labeling statagies. Nevertheless, a $^{2}H_2O$ labeling system is easy to to implement in order to measure either protein or small molecule turnover rates and is especially useful with seedlings where other strategies are difficult to implement. "
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P52006 Characterization of a plasma membrane-localized E3 ligase mcCPN1 interacting with ARGONAUTE 4 from halophyte ice plant
Yen, Hungchen E-presenter heyen@dragon.nchu.edu.tw(a) Li, Chang-Hua (a) Chen, Yu-Chan (a) Jou, Yingtzy (b)
"McCOPINE1 (mcCPN1), identified from halophyte ice plant (*Mesembryanthemum crystallinum* L.), contains two conserved domains, N-terminal copine A domain and C-terminal RING (really interesting new gene)-finger domain (HCa type). Sequence analysis also revealed that a potential N-terminal myritoylation site and immunostaining confirmed a plasma membrane localization of mcCPN1.Constitutive expression of *mcCPN1* was found in salt-stressed roots and leaves of 6-wk-old ice plant; whereas protein accumulation of mcCPN1 was salt-induced suggesting that mcCPN1 is regulated at translational or/and post-translational level. RING-finger proteins usually possess E3 ligase catalytic activity executing the last step of ubiquitination in recognizing and ubiquitinating substrate proteins. The potential E3 ligase activity of mcCPN1 was examined using in vitro ubiquitination assay. The result showed a formation of poly-ubiquitin chain when mcCPN1 was used as E3. Yeast two-hybrid screening revealed that mcCPN1 interacted with a large set of protein including ARGONAUTE 4, a protein involved in gene silencing. The involvement of mcCPN1 in salt-tolerant mechanism of ice plant is possibly by way of recognizing and ubiquitinated a set of salt stress-related proteins. "
(a) National Chung Hsing University (b) National Pingtung University of Science and Technology

P52007 "ClpPR subunits of the plastid ClpPR protease complex have differential contributions to embryogenesis, plastid biogenesis, and plant development "
Kim, Jitae-presenter jk378@cornell.edu(a) Rudella, Andrea (a) Rodriguez, Verenice R. (a) Zybailov, Boris (a) Olinares, Paul D. (a) van Wijk, Klaas J. (a)
"The plastid ClpPR protease complex in *Arabidopsis* consists of five catalytic ClpP and four non-catalytic ClpR subunits. An extensive analysis of the *CLPR* family and *CLPP5* is presented to address this complexity. Null alleles for *CLPR2* and *CLPR4* showed delayed embryogenesis and albino embryos, with seedling development blocked in the cotyledon stage; this developmental block was overcome under heterotrophic conditions, and seedlings developed into small albino to virescent seedlings with serrated leaves and light-sensitive chloroplast development. In contrast, null alleles for *CLPP5* were embryo lethal. Overexpression of *CLPR3* fully complemented a *CLPR1* mutant, but not *CLPR2* or *CLPR4* null alleles; limited *CLPR3* transcription in *clpr1-1* prevents full substitution explaining its phenotype. Additional overexpression experiments showed that ClpR1,2,4 proteins could not substitute each other. Double mutants of weak *CLPR1,2* alleles were seedling lethal, showing that a minimum concentration of different ClpR proteins is essential for Clp function. Microscopy and large scale comparative leaf proteome analyses of a *CLPR4* null allele demonstrate a central role of Clp protease in chloroplast biogenesis and protein homeostasis. The lack of transcription feedback regulation within the *CLPPR* gene family suggests that control of Clp activity occurs through Clp complex assembly and substrate delivery."
(a) Cornell University

P52008 A Novel Proteomics Approach to Identify Heat-Induced SUMO Protein Conjugates
Miller, Marcus J-presenter miller7@wisc.edu(a) Saracco, Scott A (a) Vierstra, Richard D (a)
"The covalent attachment of Small Ubiquitin-like MOdifier (SUMO) proteins to other intracellular proteins is an essential regulatory process in most eukaryotes. Despite this fact, little is known about the function of SUMO or the identity of most of its targets. In addition, one of the more striking phenomena associated with the SUMO pathway, its dramatic up-regulation in response to stress, remains unclear. Whereas most of the SUMO pool is in the free form under non-stressed conditions, various abiotic stresses (e.g., heat shock and chemical insults) induce its attachment to other cellular proteins in a rapid yet reversible reaction. To help catalog proteins affected by SUMOylation in Arabidopsis, we have developed an affinity method to enrich for the SUMO conjugates in plants, which can then be identified by tandem mass spectrometry. This method involves the complete replacement of the SUMO1 and SUMO2 isoforms with various tagged forms using an artificial SUMO1 transgene to rescue the normally lethal *sum1-1 sum2-1* double mutant. The transgenic SUMO was also modified to contain an arginine near the C-terminal glycine. This novel trypsin cleavage site can then be detected by mass spectrometry as a footprint on the target peptide bearing a short C-terminal segment of SUMO attached via an isopeptide bond to the modified lysine. Using this affinity approach, we hope to develop an in-depth list of Arabidopsis proteins subjected to SUMOylation *in vivo*, especially those targeted during stress. "
(a) Department of Genetics, University of Wisconsin, Madison, Wisconsin

P52009 ^{15N}Effects on *Chlamydomonas reinhardtii*
Geraets, Ryan-presenter rdgeraets@jacks.sdstate.edu(a) Kollars, Brett (a) Sauer, Marie-Laure (a) Cohen, Jerry (b) Sutton, Fedora (a)

"Non-radioactive isotopes can be used to efficiently label a wide range of macromolecules. Steady state levels of proteins as determined by immuno-blotting and other techniques do not necessarily provide the level of detail needed to better understand the roles of proteins in various processes. Thus, the overall goal for this project was to develop a method of globally measuring plant protein turnover rates. One of the first tasks towards achieving the overall goal was to determine which of the various non-radioactive isotopes had a high efficiency of labeling amino acids and thus proteins. Our group was responsible for such determinations using *Chlamydomonas reinhardtii*. The primary objective of our study was to develop a ^{15N}-labeled Tris-Acetate-Phosphate (TAP) media in which *Chlamydomonas reinhardtii* can successfully grow, without eliciting stressed physiological responses. Morphological, physiological, and RT-PCR data indicate that we have successfully developed ^{15N}-labeled TAP that does not stress Chlamydomonas reinhardtii. Other research from our laboratory also included examining the incorporation of ^{15N} into free amino acids and proteins as well as the use of the non-radioactive isotope Deuterium (^{2H})."
(a) Plant Science Department, South Dakota State University (b) Department of Horticulture Science, University of Minnesota

P52010 Arabidopsis ubiquitin ligases in growth and development
Callis, Judy-presenter jcallis@ucdavis.edu(a) Hsia, Mandy (a)
"Eucaryotic organisms utilize the ubiquitin system to regulate many cellular processes such as cell cycle, signal transduction, transcription and chromatin remodeling. The ubiquitin pathway is comprised of activities required for covalent attachment of the protein ubiquitin, its recognition and finally, removal. Three distinct activities, E1, E2, and E3 are typically required for ubiquitination of substrate proteins. E3, the ubiquitin ligase, is often the key component for substrate specificity and is a large gene family. For example, there are over 450 RING-type E3 ligases in Arabidopsis thaliana, of which only a small percentage have been characterized. To begin to understand the physiological function of RING-type E3s, phenotypic screens of T-DNA insertion mutants in RING domain containing proteins have been conducted. Homozygous T-DNA insertions in either of two related RING proteins called BRIZ1 and BRIZ2 result in seeds with abnormal germination and arrested development seedling development. Expression of BRIZ from a transgene rescues the arrested phenotype, demonstrating that loss of BRIZ caused the mutant phenotype. Because loss of either protein results in the same phenotype, the two proteins are not functionally redundant and appear to work in the same pathway. Both GST-BRIZ1 and 2 are active E3s in in vitro ubiquitination assays. In vitro binding assays with recombinant proteins demonstrate that BRIZ1 and BRIZ2 interact and the C-terminal coiled coil domain present in both proteins is necessary and sufficient for binding. Treatment of briz seeds with phytohormones and phytohormone synthesis inhibitors implicates hormone response pathways in BRIZ function. Further results on the role of BRIZ proteins on germination will be discussed."
(a) UC-Davis

SESSION P53 – PROTEIN TARGETING & VESICULAR TRAFFICKING

P53001 The Arabidopsis ankyrin repeat-containing protein 2A is an essential molecular chaperone for the biogenesis of a class of membrane-bound proteins and it plays an important role in plant growth and development
Kuppu, Sundaram-presenter sundaram.kuppu@gmail.com(a) Shen, Guoxin (a) Venkataramani, Sujatha (b) Narendra, Savitha (c) Qiu, Xiaoyun (a) Kolli, Sasi (a) Zhang, Hong (a)
"Peroxisomes are eukaryotic organelle without its own genome; consequently all peroxisomal proteins are post-translationally targeted to peroxisomes. Biogenesis of peroxisomal matrix proteins is better understood, whereas biogenesis of peroxisomal membrane-bound proteins is less understood. The Arabidopsis ankyrin repeat-containing protein 2A (AKR2A) was found to interact with the peroxisomal membrane-bound ascorbate peroxidase 3 (APX3), and this interaction involves the C-terminal sequence of APX3, i.e. a transmembrane domain plus a few basic amino acid residues, that resembles the mPTS, a targeting signal for some peroxisomal membrane-bound proteins. The specificity of the AKR2A-APX3 interaction hints at a possibility that AKR2A regulates APX3s biogenesis, because binding of AKR2A to APX3s mPTS could prevent APX3 from forming aggregates after biosynthesis. Analysis of three AKR2A mutants indicates that AKR2A is required for APX3s stability in plant cell. Furthermore, reduced expression of AKR2A by using RNA interference technique also leads to reduced steady-state level of APX3 and significantly reduced APX3 targeting to peroxisomes in plant cells. In addition, AKR2A mutants display abnormal phenotypes and delayed flowering, indicating that AKR2A plays important roles in plant growth and development. Given the fact that AKR2A also binds specifically to an mPTS -like sequence in several chloroplast outer membrane proteins, AKR2A appears to be a general chaperone in plant cells. The AKR2A-binding mPTS should therefore be re-defined as a membrane protein targeting signal, and AKR2A is an essential chaperon that binds specifically to the mPTS in a group of membrane-bound proteins and regulates their biogenesis in plant cells. "
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P53002 The role of Toc complex interactions in regulating chloroplasts protein import
Lee, Jeonghwa-presenter jeonghwa@nsm.umass.edu(a) Schnell, Danny J (a)
"Nucleus-encoded chloroplast proteins are synthesized in the cytosol with N-terminal cleavable transit peptides. Preprotein import into the organelle occurs post-translationally through protein complexes located in the outer and the inner envelope membrane. The outer membrane Toc complex controls the import process by binding to the transit peptides of preproteins and initiating transport into the organelle. The high fidelity of the protein import process is maintained by the specific recognition of the transit peptide by the coordinate activities of two Toc receptors, Toc34 and Toc159. The receptors bind transit peptides and regulate the entry of preproteins into the organelle via their intrinsic GTPase activities. Structural and biochemical studies suggest that GTP-regulated dimerization of the Toc receptors functions as a gate to control access of preproteins to the membrane transport machinery of the chloroplast envelope. To test this hypothesis, we are using a combination of in vitro and in vivo approaches in Arabidopsis thaliana to examine the gating hypothesis. We show that specific mutations that disrupt receptor dimerization in vitro reduce the rate of protein import in transgenic Arabidopsis compared to wild type receptor. The mutations do not affect the GTPase activities of the receptors. Interestingly, these mutations do not disrupt initial preprotein binding at the receptors, but they reduce the efficiency of the transition from preprotein binding to membrane translocation. These data indicate that dimerization of receptors has a direct role in protein import, and supports a model in which GTP hydrolysis by the receptors is coupled to conformational changes that initiate membrane translocation of chloroplast preproteins. "
(a) University Of Massachusetts

P53003 The Rab GTPase RabA4d regulates pollen tube tip growth in *Arabidopsis thaliana*
Szumlanski, Amy L.-presenter aszumla@umich.edu(a) Nielsen, Erik (a)
"In plants, fertilization is mediated by pollen tubes. These highly polarized cells have a rapid growth rate which is supported by a tip-focused delivery of membrane and cell wall components and is balanced by a retrieval of excess membrane by endocytosis. Both of these processes are regulated by the Rab family of GTPase proteins. The RabA4 subfamily consists of RabA4b and four other closely-related genes. RabA4b is tip-localized in growing

root hair cells and is important for the tip growth of these cells. To determine whether any RabA4 subfamily members were expressed in pollen, their expression patterns were tested in roots, stems, leaves, flowers, and pollen. Of the RabA4 subfamily only RabA4d was expressed in pollen. Additionally, RabA4d was expressed only in mature flowers and pollen. Subcellular localization of EYFP-RabA4d revealed that, like RabA4b in root hairs, the pollen-specific RabA4d was tip-localized, and this localization correlated with pollen tube elongation. Disruption of RABA4D led to the formation of bulged pollen tubes with a reduced rate of elongation in vitro. Expression of EYFP-RabA4d restored a WT phenotype to these cells. In vivo, disruption of RABA4D resulted in a male-specific transmission defect. Analysis of siliques from crosses using pollen from a heterozygous mutant and a WT female showed that most of the seeds at the base of the silique are WT, indicating that raba4d pollen tubes have defective growth and/or guidance in vivo. Observations in vivo showed that raba4d pollen tubes displaying aberrant growth in the ovary and reduced guidance at the micropyle. These results indicate that RabA4d function is necessary for the regulation of pollen tube growth, and may play important roles in the perception of guidance cues. "
(a) University of Michigan

P53004 Lipid pumps required for endocytosis and formation of secretory vesicles
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"Vesicle budding in eukaryotes depends on the activity of lipid translocases that generate membrane bilayer lipid asymmetry and in this way initiate changes in membrane curvature which lead to budding. We present evidence that in a higher plant, a member of the P4-ATPase subfamily (*aminophospholipid ATPase3; ALA3*) is involved in lipid flipping across Golgi membranes and is required for initial formation of secretory vesicles. The *ALA3* gene of *Arabidopsis thaliana* show highest activity in root tips. Plants carrying mutations in *ALA3* show impaired growth of roots and shoots. The growth defect is accompanied by failure of the root cap to release border cells involved in the secretion of slime required for root penetration of the soil. Further, *ala3* mutants are devoid of slime vesicles, which normally bud off from the Golgi and contain secreted polysaccharides and proteins. ALA3 function requires interaction with members of a novel family of membrane proteins, ALIS1 to ALIS5 (for ALA-Interacting Subunit). *In planta*, like ALA3, ALIS1 localizes to the Golgi and is expressed in root peripheral columella cells. We propose that the ALIS1 protein is a beta-subunit of ALA3 and that this protein complex forms an essential part of the Golgi machinery required for secretory processes during plant development. We have subsequently identified ALA2, which also interacts with ALIS1. The ALA2/ALIS1 complex resides in the plasma membrane and is involved in flipping phosphatidylserine from the outer to the inner plasma membrane leaflet. We propose that the ALA2/ALIS1 complex is required for invagination of the plasma membrane during initial endocytosis."
(a) University of Copenhagen (b) University of Nevada (c) Humboldt University, Berlin

P53005 Transcytosis of PIN2 in arabidopsis is regulated by protein phosphatase 2A and PID kinase
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"PIN proteins, whose polarized deployment is essential for polar auxin transport, have recently been shown (in arabidopsis) to be targeted in part by transcytosis. The polarity shift of basally localized PIN proteins was shown to be induced by the fungal toxin, brefeldin A (BFA), through a GNOM-ARF GEF pathway. Here, we report that BFA affects PIN2 localization via protein phosphatase 2A (PP2A) and pinoid (PID) kinase. Long-term incubation in low concentration (10µ;M) BFA reduced root elongation and gravitropism and caused an irreversible shift in the polarity of basally localized PIN2 but not of PIN1. The altered PIN2 appears functional, insofar as the BFA treatment increased the rate of shoot-ward polar auxin transport. These responses (inhibition of elongation and gravitropism, altered PIN2 polarity) were induced by lower BFA concentrations (1 to 3µ;M) in protein phosphatase 2A (PP2A) mutants. In the mutant line complemented with PP2A-GFP, 10 µ;M BFA interfered with targeting of the reporter in cortical cells, as indicated by slower recovery from photobleaching, and intracellular localization. In contrast, BFA treatment did not alter the sub cellular localization of PID but enhanced its expression. Previously, this kinase has been shown, when over-expressed, to shift the polarity of PIN2 in the root cortex. Our results suggest that basal localization of PIN2 in the root cortex depends on a phosphorylation balance and further that BFA enhances the kinase activity, reversing the polarity of PIN2 and thereby altering auxin flux in the root. "
(a) Iwate University (b) Kobe University (c) University of Massachusetts, Amherst

P53006 Characterization of Nuclear/Nucleolar Localization of Arabidopsis Ribosomal Protein RPL23a
Prasad Savada, Raghavendra-presenter prasadaralasurali@gmail.com(a) Bonham-Smith, Peta C (a)
"Ribosomes are responsible for protein synthesis in all living organisms. Cytoplasmic ribosomal subunits, comprised of rRNAs and ribosomal proteins (r-proteins) are assembled in the nucleolus, within the nucleus. Ribosomal subunit assembly is dependent on correct and efficient targeting of r-proteins to the nucleus and into the nucleolus. Failure can be lethal to the organism. Nuclear localization of a protein is generally mediated by one or more stretches of basic amino acids, the nuclear localization signal (NLS), that interacts with importins, for transport into the nucleus. As many r-proteins do not contain canonical NLSs, they may also be co-transported into the nucleus with other nuclear-targeted proteins. We are investigating the nuclear/nucleolar localization of large subunit r-protein RPL23a, one of the 81 r-proteins of Arabidopsis, encoded by a gene family of two members; RPL23aA and RPL23aB. In prokaryotic ribosomes, RPL23a orthologues are positioned at the exit of the peptide tunnel and as such are thought to be involved in ribosome docking and protein translocation at the ER. RPL23aA is essential for normal plant development, whereas RPL3aB is not. Both isoforms have a conserved putative bipartite NLS - ^{10}**KKAD**PKA**KALK**^{20}. In addition, RPL23aA has three and RPL23aB has two canonical monopartite NLSs (K-K/R-X-K/R). Site-directed mutagenesis studies have shown that the conserved putative bipartite NLS has no effect on nuclear/nucleolar localization of RPL23aA. A role for the putative monopartite NLSs is currently being studied. The possibility of co-transport of RPL23aA and -B into the nucleus and eventually to the nucleolus through interaction with other proteins like nucleolin, that shuttle between cytoplasm, nucleus and nucleolus, will be studied."
(a) Department of Biology, College of Arts and Science, University of Saskatchewan

P53007 Role of small GTP- binding proteins in nodule formation and development in model legume plant *Medicago truncatula*
Memon, Abdul R-presenter Abdulrezzak.Memon@mam.gov.tr(a) Yuzbasioglu, Elif A (b) Keskin, Birsen C (a)
http://www.mam.gov.tr
"Legumes (Fabaceae) are one of the most economically important crop families in the world and are characterized by their unique ability to fix atmospheric nitrogen through a symbiotic relationship with soil bacteria. These bacteria, collectively termed Rhizobia and which include genera such as Rhizobium, Sinorhizobium, Bradyrhizobium, Mesorhizobium, and Azorhizobium, form specialized organs within the plant, and nitrogen fixation occurs via the conversion of N_2 into NH_3 by bacterial nitrogenases.The mutualistic interactions provide a plentiful supply of nitrogen to the plants that in turn results in very high protein levels in legumes.Therefore, legumes have been assimilated as a major dietary source of protein for both humans and animals. Legumes also provide nitrogen to the soil, thus reducing the need for exogenous fertilizers. Legumes are also a unique source of natural products such as isoflavonoids, alkaloids, and saponins, many of which have documented antimicrobial and pharmacological properties. Based on the

above traits, legumes have significant economic and ecological value. Small GTP-binding proteins play essential regulatory role in multitude of cellular processes such as vesicle-mediated intracellular trafficking, signal transduction, cytoskeletal organization, cell division in plants and animals. *Medicago truncatula* is the first model leguminous plant with a completed genome sequence available. We have analyzed the role of ADP-ribosylation factor (ARF) and SAR1 both on gene expression and protein level. Our data clearly show the importance of ARF and SAR1 in rhizobium infection process and in early stages of nodule formation in Medicago truncatula. "
(a) Genetic Engineering and Biotechnology (b) Istanbul University, Biology Department

P53008 Tox1 domains in type 2 prolyl 4-hydroxylases function as cis-Golgi targeting domain in plants
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http://www.agr.kyushu-u.ac.jp/prweb2/b06/mat/index.html
"The first step of plant-specific protein O-glycosylation is the hydroxylation of proline residues catalyzed by prolyl 4-hydroxylases (P4Hs). To investigate this modification, we have been analyzing P4Hs in tobacco. Plant P4Hs are divided into two classes; type 1 P4H containing N-terminal type II signal anchor and type 2 P4H with N-terminal signal peptide. From tobacco, we cloned three full-length cDNAs for type 2 P4H (*NtP4H2.1*, *NtP4H2.2*, *NtP4H2.3*). All the encoded proteins contain N-terminal signal peptide, catalytic domain and C-terminal Tox1 domain, the function of which is unknown. Membrane fractionation analysis revealed that NtP4H2.2 distributes in fractions containing Golgi apparatus. NtP4H2.2 fused with secretory derivative of GFP (spGFP) distributed a punctate pattern and was co-localized with a cis-Golgi marker protein, NtP4H1.1-mRFP, in tobacco BY-2 cells. These and other results indicate that NtP4H2.2 is a membrane-associated protein localized predominantly in the cis-Golgi. We then analyzed the function of Tox1 domain. The expression of spGFP-Tox1 domain from NtP4H2.2 directed the GFP signal to the place where NtP4H1.1-mRFP localized. After the BFA treatment of the cells, spGFP-Tox1 distribution changed from the Golgi to the ER. The homology search with tobacco type 2 P4H revealed that proteins containing a Tox1 domain are widely distributed in plant kingdom, from higher plants to Algae. spGFP fused with a Tox1 domains of *Arabidopsis* or rice also localized in Golgi apparatus in BY-2 cells. Likewise, spGFP fused with that of tobacco also localized in Golgi apparatus in *Arabidopsis* epidermal cells. These results indicate that Tox1 domain is the cis-Golgi targeting domain and its function is conserved at least in higher plants."
(a) Faculty of Agriculture, Kyushu University (b) RIKEN Plant Science Center

P53009 "Angiosperm exocyst participates in cell division, growth and differentiation "
Zarsky, Viktor-presenter zarsky@ueb.cas.cz(a,b) Cole, Rex (c) Cvrckova, Fatima (a) Fowler , John (c) Drdova, Edita (b,a) Kulich, Ivan (a) Fendrych, Matyas (a,b) Hala, Michal (b) Synek, Lukas (b) Pecenkova, Tamara (a,b) Hana , Toupalova (b,a) Potocky, Martin (b)
"Regulation of exocytosis is a primary mechanism for controlling morphogenesis of plant cells enclosed within the cell walls.Most plant cells exhibit several distinct plasma membrane domains, established and maintained by membrane recycling. Rop GTPase regulatory module is central for initiating certain of these exocytically active domains in the plant cell cortex. We have recently described in Arabidopsis and tobacco a likely effector for Rop GTPases: the tethering complex called the exocyst, which may be involved in the first contact between secretory vesicles and their target plasma membrane domain. Due to the possibility of exocyst-Rop interaction and the multiplicity of exocyst Exo70 subunits, this complex may also be a part of the core organizer(s) of membrane recycling domains. Arabidopsis mutants in exocyst subunits show pleiotropic defects in cell morphogenesis. For example, pollen tube germination and polar growth are seriously compromised, growth of root hairs and stigmatic papilae is impaired, cell elongation in roots and hypocotyls is reduced. Mutant plants are smaller with reduced apical dominance and fewer flower organs,implying dysfunction in the meristem. Co-purification of exocyst subunits in chromatography, and co-localization in growing pollen tube tips, shows that the exocyst is a conserved eukaryotic complex involved in the regulation of cell polarity. We will present our current work on the roles of the Arabidopsis exocyst in cell division, polar auxin transport and seed differentiation. Lab of V.Z. was supported by the Ministry of Education, Youth and Sports MSMT of the Czech Republic (MSMT Kontakt ME841, MSMT LC06034 REMOROST and MSM0021620858). The work in the lab of J.F. was supported by the US National Science Foundation (IBN-0420226). "
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P53010 Coordination of plastid protein import and nuclear gene expression by plastid-to-nucleus retrograde signaling pathway
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"Expression of nuclear-encoded plastid proteins and import of those proteins into plastids are indispensable for plastid biogenesis. One possible cellular mechanism that coordinates these two essential processes is retrograde signaling from plastids to the nucleus. However, the molecular details of how this signaling occurs remain elusive. Using the *ppi2* mutant of *Arabidopsis thaliana*, which lacks the atToc159 protein import receptor, we demonstrate that the expression of photosynthesis-related nuclear genes is tightly coordinated with their import into plastids. Down-regulation of photosynthesis-related nuclear genes is also observed in mutants lacking other components of the plastid protein import apparatus. Genetic studies indicate that the coordination of plastid protein import and nuclear gene expression is independent of the accumulation of Mg-protoIX and the activity of ABI4 pathway. Instead, it may involve the transcription factor AtGLK. The expression level of *AtGLK1* is tightly correlated with the expression of photosynthesis-related nuclear genes in mutants defective in plastid protein import. Furthermore, overexpression of *AtGLK1* in the *ppi2* mutant partially restores photosynthesis-related nuclear gene expression. Taken together, we suggest that AtGLK1 may act as a positive regulator that coordinates plastid protein import and nuclear gene expression in response to the functional state of plastids."
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P53011 The analysis of the movement of the targeting signal on the precursor protein during the transition of early translocation intermediates formed during protein import into chloroplasts
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"The majority of nuclear genome encoded chloroplastic proteins are synthesized as precursors with chloroplastic targeting signals, transit peptides, at their amino-termini and translocate through the translocons, the Toc and Tic complexes, in the outer and the inner envelope membranes, respectively. Protein import process into chloroplasts consists of two energy-dependent steps: docking and translocation. During the docking step, precursors irreversibly bind to chloroplasts to form the early translocation intermediates under stringent energy conditions, i.e. low concentrations of GTP and/or ATP, and low temperatures. In contract, translocation requires a higher level of ATP (>1 mM) at higher temperatures. It has been demonstrated that depending on the temperature and the requirement for ATP, different types of early-intermediates are present, for which the extent of precursor protein translocation differs [Inoue and Akita (2008) *J. Biol. Chem.* **283**, 7491-7502]. However, the issue of whether the

surrounding environment for each precursor differs at each intermediate stage has not been investigated. Therefore we have applied a site-specific photo-crosslinking strategy to examine the interactions between the transit peptide and polypeptides in close proximity at different stages in early-intermediates. We identified various crosslinked products, one of which contains Toc75. The generation of these products was dependent on the position of the transit peptide upon modification by the crosslinker, as well as the intermediates formed under different energy conditions. These results indicate that the transition of early-intermediates is accompanied with the movement of the transit peptide within the intermediates [Inoue and Akita (2008) *Arch. Biochem. Biophys.* **477**, 232-238]."
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P53012 Altered Root Hair Polarity of the *Arabidopsis thaliana agd1* Mutant is Associated with Defects in Various Components of the Tip Growth Machinery
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http://www.noble.org/PlantBio/Blancaflor/index.html
"Knockouts to *AGD1*, encodes a class I ADP-ribosylation factor-GTPase activating protein, result in root hairs with wavy and bifurcated tip growth. To understand the role of AGD1 in the polar growth of root hairs, we used live cell imaging approaches to evaluate how various components of the tip growth machinery are affected in the mutant. The growing *agd1* root hairs showed bundles of endoplasmic microtubules and actin filaments extending into the extreme tip. The wavy phenotype and pattern of cytoskeletal distribution in root hairs of *agd1* partially resembled that of an armadillo-repeat containing kinesin (*ARK1*) mutant. We found that cytoplasmic calcium ($[Ca^{2+}]_{cyt}$) tip oscillations, RabA4B and Rop2 targeting and vacuolar membrane dynamics were modified in root hairs of *agd1*. Tip focused $[Ca^{2+}]_{cyt}$ gradients was shown to persist in *agd1* root hairs, but the frequency of the oscillations was significantly dampened in the root hairs. RabA4B occasionally dissipates at the tips of *agd1* root hairs. In addition, the shifting of RabA4B and ROP2 localization was observed to coincide with a change in root hair growth direction. Organelle trafficking as revealed by a Golgi marker was slightly inhibited and Golgi stacks frequently protruded into the extreme root hair apex of *agd1* mutants. Transient expression of GFP-AGD1 labeled punctate bodies that partially colocalized with the endocytic marker, FM4-64, while ARK1-YFP associated with microtubules. Brefeldin A rescued the phenotype of *agd1* indicating that the altered activity of an AGD1-dependent ARF contributes to the various phenotypes. We propose that AGD1 and ARK1 are components of converging signaling pathways that impact cytoskeletal organization and membrane trafficking to specify growth orientation in Arabidopsis root hairs."
(a) The Samuel Roberts Noble Foundation

P53013 *Os*Tudor-SN is required in rice (*Oryzo sativa* cv.kitaake)
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"The RNAs for the rice storage proteins are transported and localized to specific subdomains of the endoplasmic reticulum (ER). Specifically, prolamine RNAs are targeted to the ER that bound the prolamine intracisternal inclusion whereas glutelins RNAs are localized to adjacent cisternal ER. The localization of these RNAs requires specific *cis*-elements located in the coding and 3 untranslated region and are transported as particles on cytoskeletal dependent process. A major cytoskeletal-associated RNA binding protein is *Os*Tudor-SN which binds storage protein RNAs as assessed by immunoprecipitation/RT-PCR and microarray analyses. Binding of the prolamine RNA occurs through the 3 untranslated sequences. *Os*Tudor-SN co-localizes with GFP-tagged RNA particles suggesting a role in RNA transport. RNAi plants show a pronounced reduction in *Os*Tudor-SN transcripts in developing seeds, along with a marked reduction in prolamine RNA and protein levels. Analysis of developing RNAi seed sections by immnofluorescence microscopy shows that the reduction in *Os*Tudor-SN gene expression significantly reduces the number of prolamine PBs and not the size of these organelles. Overall, these results support a direct role for *Os*Tudor-SN in RNA transport and localization. Proteins that interact with *Os*Tudor-SN are presently being studied by yeast two-hybrid analysis. Supported *by the National Research Initiative (NRI) Plant Biology: Gene Function and Regulation USDA Cooperative State Research, Education and Extension Service (CSREES, grant number 2006-35301-17043)*."
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P53014 New regulatory mechanisms at toc159 in the chloroplast protein import machinery
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http://www2.unine.ch/physiologievegetale
"Chloroplast protein import and biogenesis are essential for plant energy and biomass production. The Toc- (translocon at the outer membrane of the chloroplast) and Tic- (translocon at the outer membrane of the chloroplast) complexes constitute the quantitatively most important preprotein import pathway into the chloroplast. The Toc core complex consists of two membrane bound and surface exposed preprotein receptor GTPases, Toc159 and Toc33 associating with a protein translocating channel, Toc75. Together, Toc159 and Toc33 form a GTP-regulated gate at the entry of channel. We will show new data on how GTP regulates translocation at the outer membrane. But not only GTP also phosphorylation is a known regulator of translocation at the level of the Toc-complex. Here, we will report on the hyper-phosphorylation of domains of Toc159 and on the identification of kinases involved. Literature : 1. Wang, F., Agne, B., Kessler, F. and Schnell, D.J. 2008. The role of GTP binding and hydrolysis at the atToc159 preprotein receptor during protein import into chloroplasts. J. Cell Biol. 183: 87-99. 2. Rahim, G., Bischof, S., Kessler, F. and Agne, B. 2009. In vivo interaction between atToc33 and atToc159 GTP-binding domains demonstrated in a plant split-ubiquitin system. J. Exp. Bot. 60: 257-267. 3. Agne, B. and Kessler, F. (2009) Protein transport in organelles: the Toc complex way of preprotein import. FEBS J. 276: 1156-1165. 4. Agne, B., Infanger, S., Wang, F., Hofstetter, V., Rahim, G., Martin, M., Lee, D.W., Hwang, I., Schnell, D.J. and Kessler, F. 2009. A Toc159 import receptor mutant, defective in hydrolysis of GTP, supports preprotein import into chloroplasts. J. Biol. Chem. (in press) http://www.jbc.org/cgi/doi/10.1074/jbc.M804235200 "
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P53015 "An *Arabidopsis thaliana* mutant in the UDP-glucose glycoprotein: glucosyltransferase, a key enzyme in quality control at the endoplasmic reticulum, shows a root phenotype."
Tapia, Rodrigo (a,b) Reyes, Francisca (a,b) Orellana, Ariel-presenter aorellana@unab.cl(a,b)
"The presence of a single terminal glucose residue on N-linked oligosaccharides of newly synthesize polypeptides is used as a signal for folding assistance in the endoplasmic reticulum (ER) lumen. The enzyme responsible for the recognition of misfolded proteins and the addition of the glucose residue is the UDP-glucose glycoprotein: glucosyltransferase (UGGT). Thus, this enzyme is a key step in the quality control process that occurs in the ER to ensure the proper folding of proteins. Little is known about the role of this enzyme in multicellular eukaryotes and even less is known in plants. Therefore, we decided to analyze the role of this enzyme in the model plant *Arabidopsis thaliana*. First, we detected biochemically the UGGT activity and found that was present in ER enriched fractions from *Arabidopsis*. The analysis of N-linked oligosaccharides produced by UGGT, confirmed the specificity of the enzyme. The *Arabidopsis* genome contains only one putative gene encoding for this enzyme. Mutants in UGGT are smaller than wild type. Roots seem to be the more affected organ, coinciding with the higher content of the mRNA for UGGT. Microscopic analysis reveals that root

architecture was altered. Moreover, mutant plants are less tolerant to stress. These results suggest an important role for UGGT in root cell elongation probably due to an alteration in protein secretion. Supported by Fondecyt 1070379, PCB-MN ICM P06-065-F, PFB-16 "
(a) Center of Plant Biotechnology, Universidad Andres Bello (b) Millenium Nucleus in Plant Cell Biotechnology

P53016 Purification and proteomic analysis of plant prevacuolar and early endosomal compartments
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"We have recently identified multivesicular body (MVB) as prevacuolar compartment (PVC) and trans-Golgi network (TGN) as an early endosome (EE) in plant cells. These PVC/MVB and TGN/EE were defined by the vacuolar sorting receptor (VSR) proteins and the rice secretory carrier membrane protein 1 (OsSCAMP1) respectively, whereas the same VSR-positive PVCs might mediate the vacuolar transport of both lytic and storage protein reporters in plant cells. To further study the molecular mechanisms of PVC/EE biogenesis and PVC/EE-mediated protein trafficking in the plant secretory and endocytic pathways, we have developed protocols to isolate PVC and EE from Arabidopsis suspension cultured cells for proteomic analysis. Both confocal and immunogold EM studies demonstrated that the isolated fractions were highly enriched in MVB and TGN. Nano LC-MS-MS analysis of the purified PVCs and EEs has identified more than 300 proteins from each of these organelles. Further molecular, cellular, biochemical and genetic studies have also been carried out on selective PVC/EE proteins to determine their subcellular localization and roles in mediating protein trafficking and organelle biogenesis in the plant secretory and endocytic pathways. Preliminary results indicate that some of these novel PVC/EE proteins localize and function in PVC or EE organelles in plants. Supported by grants from RGC and CUHK Schemes B/C. References: 1. Miao Y et al., (2008) The Plant Journal 56:824-839 2. Lam SK et al., (2008) Plant Physiology 147:1637-1645 3. Lam SK et al., (2007) Trends in Plant Science 12:497-505 4. Lam SK et al., (2007) Plant Cell 19:296-319 5. Miao Y et al., (2006) Plant Physiology 142:945-962 6. Tse YC et al., (2006) Plant Physiology 142:1442-1459 7. Tse YC et al., (2004) Plant Cell 16:672-693 "
(a) Department of Biology, The Chinese University of Hong Kong

P53017 The A-domains of the Toc159 family of chloroplast preprotein receptors are intrinsically unstructured protein domains
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"Most chloroplast proteins are encoded in the nucleus and translated in the cytosol with an N-terminal transit peptide, which is recognized by the receptors of the Toc complex. The primary chloroplast preprotein receptors in *Arabidopsis thaliana* are the Toc159 family of receptors (atToc159, -132, -120, and -90). These receptors directly bind to transit peptides, and have some preprotein substrate selectivity, which is conferred by an unknown mechanism. Members of the family share a tripartite domain structure; the amino acid sequences are most variable among the family members within their N-terminal acidic (A-) domains, suggesting that this domain may contribute to the functional specificity of the receptors. The overall objective of this study was to gain insight into the function of the A-domain by taking a structural approach. The A-domains of the Toc159 family are predicted to be largely disordered. Circular dichroism and fluorescence spectroscopy analyses of recombinant A-domains of atToc159 and atToc132 are consistent with their classification as intrinsically unstructured proteins (IUPs). This holds true under both physiological and extreme conditions of temperature and pH. IUPs are commonly involved in protein-protein interactions, suggesting that the A-domain may interact with other components of the Toc complex and/or directly with transit peptides, which could provide a mechanism for conferring substrate specificity. These possibilities will be tested, and our most current data will be presented and discussed."
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P53018 RNA localization in plants
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"Rice seeds contain two major types of storage proteins, prolamine and glutelin, which are sequestered in specialized organelles derived from the endoplasmic reticulum (ER) or vacuole called protein bodies. Proper protein sorting to these different compartments requires targeted localization of their mRNAs to distinct subdomains on the cortical ER via the cytoskeleton. Prolamine RNA is transported to ER-derived protein bodies (PB-ER) which contain prolamine proteins, while glutelin RNA is targeted to the cisternal-ER (cis-ER). The latter protein is then transported in dense vesicles to protein storage vacuoles via the Golgi. *Cis*-acting RNA localization signals within prolamine and glutelin RNA are required for transport to these distinct subdomains of the ER. In an effort to elucidate the RNA transport machinery, we have sought to identify RNA binding proteins (RBPs) that interact with the RNA *cis*-elements. *Os*Tudor-SN is a cytoskeleton-associated RBP that binds both prolamine and glutelin RNAs and co-localizes with prolamine RNA transport particles. Downregulation of *Os*Tudor-SN by RNAi leads to a reduction in prolamine RNA and protein. Using two-dimensional difference gel electrophoresis (2D DIGE), over 20 protein spots were found to be differentially expressed in developing seeds of the RNAi plants compared to wild type and identified proteins include possible RBPs. Together, these results aim to provide insight on the mechanism of RNA localization in rice. These studies were supported by grants from the National Science Foundation."
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P53019 Tic40 is important for reinsertion of proteins from the chloroplast stroma into the inner membrane
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"Chloroplast inner-membrane proteins Tic40 and Tic110 are first imported from the cytosol into the chloroplast stroma, and subsequently reinserted from the stroma into the inner membrane. However, the mechanism of reinsertion remains unclear. Here we show that Tic40 itself is involved in this reinsertion process. When precursors of either Tic40 or a Tic110 C-terminal truncate, tpTic110-Tic110N, were imported into chloroplasts isolated from a *tic40*-null mutant, soluble Tic40 and Tic110N intermediates accumulated in the stroma of *tic40*-mutant chloroplasts, due to a slower rate of reinsertion. We further show that a larger quantity of soluble Tic21 intermediates also accumulated in the stroma of *tic40*-mutant chloroplasts. In contrast, inner-membrane insertion of the triose-phosphate/phosphate translocator was not affected by the *tic40* mutation. Our data suggest that multiple pathways exist for the insertion of chloroplast inner-membrane proteins."
(a) Institute of Molecular Biology, Academia Sinica

P53020 Filling the gap between cytoskeletal remodeling and membrane trafficking in the regulation of tip growth in plants
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http://www.noble.org/PlantBio/Blancaflor/index.html
"To achieve its final form and orientation, a plant must coordinate the growth of its individual cells. Crucial to this function is the establishment of polarity where cell wall precursors and membrane material are targeted to specific regions of the cell. This differential sorting of cellular components is largely responsible for many of the diverse cell and organ shapes that comprise the plant body. To study factors that define cell shape in plants, we

use Arabidopsis root hairs as a model system. Root hairs elongate by a process known as tip growth where expansion is restricted to a small region of the cell leading to the formation long tubular cells. To facilitate the process of tip growth, a number of interconnected signaling pathways come into play. For instance, vesicles carrying cell wall precursors and membrane material are directed to the tip of the cell via the cytoskeleton. Through a forward genetic screen we identified an ADP ribosylation factor (ARF)-GTPase activating protein (GAP) as a new molecular player that could function as a signaling scaffold between several components of the root hair tip growth machinery. When the gene encoding this ARF-GAP protein (*AGD1*) is mutated, root hairs display a wavy growth pattern instead of the straight growth pattern, typical of wild-type plants. The cytoskeleton, cytoplasmic calcium tip oscillations, tip targeting of other small GTPases, vacuolar membrane dynamics and the distribution of fluorescent phosphoinositide sensors were altered in root hairs of *agd1*. Our data indicate that AGD1 could serve as an important link through which several components of the root hair tip growth machinery interact. "
(a) The Samuel Roberts Noble Foundation

P53021 Targeting of a chloroplastic inner envelope membrane protein carrying a putative targeting signal
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"Two pathways have been proposed for the targeting of proteins to the inner envelope membrane of chloroplasts. One is the 'stop-transfer' pathway, the other is 'post-import' pathway. The latter pathway requires the second cleavage of the targeting signal, indicating the presence of inner envelope-targeting signal. Serine/proline-rich domain of Tic40, attached to the chloroplastic targeting signal, has been demonstrated to play a role as the inner envelope-targeting signal. By searching in the Arabidopsis genomic database for membrane proteins possibly targeting to the chloroplastic inner envelope membrane, we found the gene whose translational product carried the putative chloroplastic targeting signal, followed by the domain rich in particular amino acid residues, the featured amino acid sequence (FAAS). To determine the function of the FAAS as the potential inner envelope-targeting signal, we constructed the series of deletion mutants of this translational product. Using an in vitro import assay, we showed that this translational product was imported into chloroplasts by double processing steps, while the FAAS-deleted mutant produced the single processed form. This indicated that the FAAS is involved in the second processing step. "
(a) The United Graduate School of Agricultural Sciences, Ehime University (b) Faculty of Agriculture, Ehime University

P53022 Regulation of the Arabidopsis ER stress response by membrane-bound bZIP transcription factors
Koizumi, Nozomu-presenter nkoizumi@plant.osakafu-u.ac.jp(a,b) Tajima, Hiromi (b) Fedoroff, Nina V (c) Iwata, Yuji (c,b)
"Disruption of protein maturation in the endoplasmic reticulum (ER) causes a protective response such as induction of the ER chaperones. This phenomenon is termed as the ER stress response. AtbZIP60 is a transcription factor with a trans-membrane domain that regulates the ER stress response in Arabidopsis. Over-expression of a cDNA encoding a truncated form of AtbZIP60 lacking the C-terminal trans-membrane enhanced expression of BiP and calnexin, whereas over-expression of a cDNA encoding the full-length AtbZIP60 did not, suggesting that proteolytic cleavage is necessary for its activation. Full length AtbZIP60 was localized to the ER membrane under unstressed conditions. Its cleaved N-terminal fragment was detected in the nucleus of tunicamycin-treated cells. A number of ER stress-responsive genes were much less strongly induced by ER stress in a knockout mutant of AtbZIP60 than in wild type. However, many genes were equally induced in the mutant and wild type indicating that alternative transcription factor is involved in the ER stress response. AtbZIP28 was identified as a candidate. Its truncated form also has an ability to enhance promoter of ER chaperone genes as well as AtbZIP60. GFP fused with AtbZIP28 was detected in the ER without ER stress; however, GFP fluorescence was translocated to the nucleus after addition of the ER stressor. Bioinformatic analyses indicated that AtbZIP60 and AtbZIP28 can form hetero-dimers, as well as homo-dimers. Since both transcription factors activate ER stress-responsive genes, the homo- and hetero-dimers may activate different subsets of genes. Double knockout mutant of AtbZIP60 and AtbZIP28 is under observation."
(a) Osaka Prefecture University (b) Nara Institute of Science and Technology (c) Pennsylvania State University

P53023 Protein Targeting to the Thylakoid Membrane
Froehlich, John E.-presenter froehli5@msu.edu(a) Keegstra, Kenneth (a)
"Most chloroplastic proteins are encoded in the nucleus, synthesized in the cytoplasm as higher molecular weight precursors, and imported posttranslationally across the two envelope membranes. Because chloroplasts have six distinct subcompartments to which a protein may be targeted, chloroplast assembly is a complex problem. One important aspect of understanding chloroplast assembly is understanding how nuclear-encoded proteins of the thyalkoid membranes are able to reach their proper destination. These proteins must first be transported across the envelope membrane before being inserted into the thylakoid membrane. What ensures that these critical thylakoid proteins are targeted to the correct membrane? Presently, the mechanism by which nuclear-encoded thylakoid membrane proteins cross the IEM unimpeded as they journey to the thylakoids is not well understood. For this investigation, we have examined the sorting mechanism involved in the proper targeting of known integral thylakoid and inner envelope membrane proteins. Using different model proteins that are known to reside in the thylakoid membrane or in the inner envelope membrane and standard molecular biology techniques, we have attempted to identify essential targeting determinants that cause these proteins to either be halted at the inner envelope membrane or to be transported across it into the stromal space, where subsequent targeting events can direct these proteins to the thylakoid membrane. We anticipate that the information gleaned from our comparative analysis, will result in the identification of unique differences between the TMDs of thylakoidal membrane proteins and the TMDs of IEM proteins. (The following work is funded by DOE grant no. DE-FG02-91ER20021) "
(a) MSU-DOE Plant Research Laboratory

P53024 Secretory carrier membrane protein 2 localizes in trans-Golgi network-derived cluster of vesicles moving separately from the Golgi apparatus
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http://labs.psc.riken.jp/gdrg/English/index.html
"Secretory proteins and extracellular glycans are transported to the extracellular space during cell growth. These materials are carried in secretory vesicles generated at the trans-Golgi network (TGN). Analysis of the mammalian post-Golgi secretory pathway demonstrated the movement of separated secretory vesicles in the cell. Using secretory carrier membrane protein 2 (SCAMP2) as a marker for secretory vesicles and tobacco (*Nicotiana tabacum*) BY-2 cell as a model cell, we characterized the transport machinery in plant cells. A combination of analyses, including electron microscopy of quick-frozen cells and four-dimensional analysis of cells expressing fluorescent-tagged SCAMP2, enabled the identification of a clustered structure of secretory vesicles generated from TGN that moves in the cell and eventually fuses with plasma membrane. This structure was termed the secretory vesicle cluster (SVC). The SVC was also found in *Arabidopsis thaliana* and rice (*Oryza sativa*) cells and moved to the cell plate in dividing tobacco cells. Thus, the SVC is a motile structure involved in mass transport from the Golgi to the plasma membrane and cell plate in plant cells."
(a) RIKEN Plant Science Center (b) Kyushu University (c) Niigata University

P53025 Molecular and subcellular changes in response to transgenic expression of a lysine-rich fusion protein in rice seeds
Chun Wai, Yu-presenter ambiv@ymail.com(a) Sai Ming Samuel, Sun (a)
"In an attempt to increase the content of lysine, the first limiting essential amino acid in rice, we inserted the cDNA encoding a lysine-rich protein (LRP) from winged bean (*Psophocarpus tetragonolobus* L.) into the basic subunit of the gene encoding rice glutelin 1 protein (Gt1) and introduced the fusion construct into rice, generating the Gt1-LRP-fusion transgenic rice lines. While marked enhancement in lysine content was achieved, we observed that significant amount of the 57-kD proglutelin precursor and correspondingly lower levels of the acidic- and basic-subunits of glutelin were accumulated in the transgenic rice when compared with the wild type. In addition, the transgenic rice seeds showed notable chalkiness. In further studies, we found that the levels of RNA and protein of the ER chaperones luminal binding protein (BiP) and protein disulfide isomerase (PDI) in the Gt1-LRP-fusion lines were also significantly increased. Through transmission electron-microscopy, we observed the appearance of morphological different protein bodies (nPB). Proglutelin, prolamin and ER chaperones such as BiP and PDI were identified in the nPB. These findings suggest that proglutein, prolamin, PDI and BiP aggregate in the nPB of the seeds of Gt1- LRP fusion lines and the abnormal aggregation of proglutelin and prolamin in nPB may have contributed to the chalky phenotype (This research has been supported by the UGC-AoE Center for Plant and Agricultural Biotechnology grant and the PVMR project grant of the Bill and Melinda Gates Foundation)."
(a) Department of Biology, The Chinese University of Hong Kong

P53026 "Construction of a novel ERAD substrate, AtCPY*-GFP, in plant cells. "
Yamamoto, Masaya-presenter sunstar@biochem.chem.nagoya-u.ac.jp(a) Kawanabe, Mitsuyoshi (a) Hayashi,, Yoko (a) Endo, Toshiya (a) Nishikawa, Shuh-ichi (a)
"The quality control system of the endoplasmic reticulum (ER) ensures that only folded proteins are transported to the downstream organelles. Misfolded proteins produced in the ER are degraded by the ERAD (ER-associated degradation), which transports the misfolded proteins to the cytosol for degradation by the ubiquitin-proteasome system. Recent genome sequencing revealed that Arabidopsis thaliana has orthologs of the ERAD machineries identified in yeast and mammalian cells. To better understand the ERAD mechanisms in plant cells, we attempted to construct a novel ERAD substrate. The mutant form of carboxypeptidase Y (CPY*) is one of the best characterized ERAD substrates in yeast cells. *A. thaliana* has an ortholog of yeast CPY (AtCPY). Expression of a GFP-tagged AtCPY (AtCPY-GFP) in *A. thaliana* culture cells showed its vacuolar localization. Here we constructed a new ERAD substrate in plant cells, AtCPY*-GFP, by introducing a mutation orthologous to that of CPY* in yeast into AtCPY-GFP. AtCPY*-GFP expressed in *A. thaliana* culture cells was not transported out of the ER, but degraded in a proteasome-dependent manner. However, we found that, while the ER-Golgi transport is required for degradation of CPY* in yeast cells, inhibition of ER-Golgi transport did not affect degradation of AtCPY*-GFP in plant cells. An N-linked glycan is shown to be essential for degradation of CPY* in yeast cells. AtCPY*-GFP has one N-linked glycan, but a glycosylation-site mutant of AtCPY*-GFP was degraded by the ERAD as efficiently as AtCPY*-GFP. In order to reveal whether these differences are attributed to the substrate proteins or the cells used for the analyses, we are now analyzing degradation of AtCPY*-GFP in yeast cells."
(a) Dept. of Chemistry, Grad. Sch. of Sci. Nagoya Univ.

P53027 Sorting of seed storage proteins in the rice endosperm
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"During rice (*Oryza sativa*) seed development, the rough endoplasmic reticulum (ER) synthesizes large amounts of disulfide-containing storage proteins. Oxidative protein folding is necessary for sorting the storage proteins. The proglutelins acquire intramolecular disulfide bonds before transported to the protein storage vacuole via the Golgi apparatus, whereas prolamins, a major group of cereal storage proteins, are sequestered within the ER and polymerized through intermolecular disulfide bonds, forming the protein body. Protein disulfide isomerase (PDI) is a highly abundant resident of the ER lumen that is found in all eukaryotic cells. PDI is a multifunctional member of dithiol-disulfide oxidoreductases involved in the formation of the correct pattern of disulfide bonds in most secretory proteins. In higher plants, genomic database searches of rice, Arabidopsis (*Arabidopsis thaliana*), and maize (*Zea mays*) have identified a family of PDI-like (PDIL) proteins which contain two redox-active thioredoxin domains, PDIL1;1-1;4 and PDIL2;1-2;3. Phylogenetic analysis showed that plant PDIL2;3 proteins form their own branch together with human P5 and seem likely to be separated from PDIL1;1 proteins at the early stage of plat evolution. We investigated roles of *Oryza sativa* (Os) PDIL1;1 and OsPDIL2;3 in sorting the storage proteins. We will present confocal image analyses of DsRed-fused OsPDIL1;1 and GFP-fused OsPDIL2;3 and effects of *OsPDIL1;1* knockout and *OsPDIL2;3* RNAi knockdown on sorting the storage proteins in the rice endosperm. "
(a) National Institute of Agrobiological Sciences (b) Yamaguchi Prefectural University

P53028 Spatial expression patterns of PATL1 and PATL3 suggest functional redundancy
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"The Patellin (PATL) gene family of Arabidopsis consists of 6 members which are characterized by a variable N-terminal domain, followed by domains found in proteins involved in membrane traffic (Sec14 and GOLD). The best characterized member of the family, PATL1, is a phosphoinositide-binding protein that localizes to the expanding and maturing cell plate. Phylogenetic analysis has divided the PATLs into four clades, suggestive of both distinct and overlapping roles for the different PATL family members. We are analyzing spatial gene expression patterns as part of an effort to determine which of the PATLs are functionally redundant and which may play distinct roles. In both PATL1::GUS and PATL3::GUS transgenic plants, β-glucuronidase staining was observed in vascular tissue, hydathodes, guard cells and trichomes of young leaves. Interestingly this pattern is similar to that observed in DR5::GUS plants, consistent with a connection to auxin signaling. Highly significant coexpression of PATL1 and PATL3 was also found in the ATTED-II microarray coexpression database (MR=2). These results indicate that PATL1 and PATL3 may be functionally redundant, a hypothesis which is being tested through analysis of PATL knockout mutants. "
(a) Wellesley College

P53029 Biochemical and Genetic Analyses of Arabidopsis TOC complex.
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"The chloroplast, like mitochondria, needs to import most of its proteins from the surrounding cytosol. It is surrounded by a double membrane called the envelope, which contains the stroma, and the thylakoid membranes. Embedded in the envelope are the TOC and TIC complexes (translocon outer and inner envelope membrane of the chloroplast respectively). Several components of the TOC and TIC complexes have been identified. For some components in Arabidopsis we have two or more homologues. In our interest, the Toc159 protein import receptor has four homologues; atToc159, atToc132, atToc120, and atToc90. It is known that atToc159 supports accumulation of photosynthetic proteins while atToc132 and atToc120 support the import of non-photosynthetic housekeeping proteins. However, the role of atToc90 is not known. The atToc90 protein associates with the chloroplast surface and with the Toc complex a similar to what atToc159 is doing, suggesting that it has a function in chloroplast protein import. In this study, we are trying to determine the functions of the atToc90 by studying a series of toc90 single, double and triple mutants alongside with atToc90 over-expression lines. Affymetrix microarray experiments, suggested a possible role for atToc90 during senescence. Based on this observation, we carried out experiments to investigate whether the toc90 knockout mutants and the over-expression lines have any differences

regarding leaf senescence. In a separate project, we are studying another receptor, Toc34, which has two homologues in Arabidopsis; atToc33 and atToc34. By tagging the atToc33 and atToc34 we are able to purify different TOC complexes from transgenic plants. In this way, we expect to elucidate the association preferences of the various TOC component isoforms. "
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SESSION P54 – QUANTITATIVE TRAITS

P54001 Elemental processes controlling soybean seed composition
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"Complex quantitative traits governed by many genes can be dissected into more elemental processes. The final concentration of protein in soybean seeds (SPC), for example, is determined by a combination of accumulation rates and durations of the major storage components: protein, oil and carbohydrate. As such, similar values for SPC can result from a variety of developmental and metabolic strategies within the maternal and zygotic tissues. To identify elemental processes that might determine high SPC, we examined genetic variation in seed development traits in a population of 100 F2:3 progeny lines constructed from parents differing widely in seed composition. We identified two developmental strategies that achieved the same high level of SPC within this population of segregating lines. One subset maintained protein content constant while decreasing accumulation of other seed components. A second subset increased seed protein accumulation. These lines are being screened for SSR markers associated with high SPC to identify genomic regions determining these two unique strategies. In a related study, we evaluated the association between seed composition and assimilate supply per seed. In both experimental lines and elite varieties, high SPC was associated with near saturating levels of assimilate supply per seed during seed filling. The more favorable source-sink ratio, however, was due to reduced seed set. We hypothesize different suites of genes determine these alternative strategies to achieve high SPC, which are not necessarily linked to those associated with reduced oil synthesis or seed yield. If so, identifying genes controlling these high SPC strategies may allow breeders to overcome the commonly observed negative correlation between SPC and grain yield."
(a) Iowa State University

P54002 Dissecting Malting Quality QTL in Barley using *Ac/Ds*Transposons
Tan, Han Qi (a) Singh, Jaswinder-presenter jaswinder.singh@mcgill.ca(a)
"Barley is the major cereal grain grown for malt needed by the brewing industry. Improvement for malting quality, a complex multi-component trait, is difficult because of its quantitative nature and low heritability. A series of efforts have been undertaken to genetically dissect this trait and to localize individual quantitative trait loci (QTL) on barley chromosomes. One major QTL complex, QTL2 mapped on the short arm of chromosome 4H, affects several malting quality parameters. The maize *Ac/Ds* transposon system is proven to be an effective approach for gene identification and cloning in heterologous species. Using this system, single-copy *Ds* insertion lines (TNPs) were generated in barley to identify genes and their function. Coupled with availability of extensive genomic resources, a robust platform is available for effective use of transposon approaches for isolating, characterizing and mapping genes in barley. The recent successful demonstration in barley of *Ds* transposition at significant frequencies over multiple generations and its preference to re-insert near the original site of excision and into genic regions facilitates targeted mutagenesis. We are utilizing this transposon system to saturate malting quality QTL regions to identify and characterize genes associated with malting in the QTL2. *Ds* elements were re-activated by crossing them with *Ac*TPase-expressing plants. New *Ds* transpositions have been identified by DNA blotting and *Ds* tagged genes are being cloned using inverse PCR. This effort of saturation mutagenesis with *Ds* transposons will lead to a better understanding of malting quality traits and candidate genes that display quantitative variation. "
(a) McGill University

P54003 Genetic control of phenolic antioxidants biosynthesis in chicory leaves
Bahri, Meriem-presenter meriem.bahri@ed.univ-lille1.fr(a) Hance, Philippe (a) Hilbert, Jean-Louis (a) Hendriks, Theo (a)
"Roots of industrial chicory (*Cichorium intybus* L.) are processed to obtain products used in pharmaceutical, food and feed industry but their foliage is not harvested and their waste might cause environmental and phytohygienic problems. Studies have demonstrated that chicory by-products could form a natural source of antioxidants, known to reduce the risk of cancer and vascular diseases. Breeding of chicory as a source of natural antioxidants requires an understanding of the genetic control of the metabolism of these molecules. This requires a high through-put method to extract and identify the molecules of interest. We have developed a new method that simplifies sampling and extraction, and reduces the variability that can be induced by different (inexperienced) manipulators. This miniaturized protocol was applied on a F1 progeny of 200 chicory genotypes, previously used for the construction of a molecular genetic map for chicory, each represented by 5 clones in 5 randomized plots. HPLC analyses and DPPH tests were applied on the extracts to determine the concentrations of MS-identified phenolic compounds (i.e. caftaric, chlorogenic, and chicoric acid) and total antiradical activity, respectively. The results indicated the presence of genetic variability and transgression of these traits in the progeny. We will also show the results of the application of QTL (Quantitative Trait Loci) analysis and candidate gene mapping, as first steps towards the identification of genes involved in the regulation of antioxidant production in chicory."
(a) laboratoire SADV - UMR USTL/INRA 1281 - Universite de Lille1

P54004 Study on bio-fortification of rice bran tocotrienol
Murata, Kazumasa-presenter kazumasa.murata@pref.toyama.lg.jp(a) Phumon , Sookwong (b) Kiyotaka, Nakagawa (b) Akira, Shibata (b) Toshiyuki, Kimura (c) Teruo, Miyazawa (b)
"Rice bran has been known to contain some bio-active compounds such as tocotrienol (T3, unsaturated vitamin E) and tocopherol (Toc, saturated vitamin E). In recent years, T3 has gained much attention for its several physiological properties that differ somewhat from those of Toc. T3 shows better anti-oxidative, anti-hypercholesterolemic, anti-cancer and neuroprotective activities than those of Toc. Moreover, T3 has been demonstrated to suppress pathological angiogenesis, which is an important stage in progression of many severe disorders (i.e., diabetic retinopathy, rheumatoid arthritis and cancers). These beneficial effects suggest T3 as a rice bran constituent with a wide variety of health benefits. Despite the potential significance of T3, knowledge on how to apply rice bran T3 for health promotion has been poorly understood. Therefore, we have been investigated utilization and nutritional application of rice bran T3. About 300 kinds of rice bran samples collected around the world were determined for their T3 and Toc. As results, there was a wide variation of T3 contents in rice bran samples. Some varieties such as Milyang23 were found as T3-rich varieties, whereas.Koshihikari, the most major Japanese variety, did not contain much level of T3. The findings suggest that cross-breeding of Koshihikari with Milyang23 would result a higher distribution of T3 content and some of their progenies would be expected to be rich in T3 content. Their F_2 progenies could be useful in clarifying T3 biosynthesis in rice plants by using a QTL analysis. As results, five putative QTLs responding T3 production were found on chromosome 1 (*qT3-1*), chromosome 6 (*qT3-6-1* and *qT3-6-2*), and chromosome 9 (*qT3-9-1* and *qT3-9-2*). "

(a) Toyama Prefectural Agricultural Forestry & Fishery Research Center (b) Tohoku University (c) National Agricultural Research Center for Tohoku Region

P54005 Characterization of genotype-environment interactions from the analysis of QTLs across environments
Yang, Rong-Cai-presenter rong-cai.yang@ualberta.ca(a,b) Ham, Bong Joo (a)
"The presence of genotype-environment interactions (GE) leads to imperfect genetic correlation between measurements of the same trait in different environments, thereby limiting our ability to identify superior breeding lines or best cultivars across the environments. Our presentation investigates if the imperfect genetic correlation is associated with occurrence of quantitative trait loci (QTLs) that were common (shared) between the environments. Such an association was evaluated from the analysis of genetic correlations and QTL distributions between yields of pairs of 16 environments for a barley doubled haploid (DH) population. Each pair would fall into one of four Scenarios in the 2 x 2 contingency table: high (> 0.6) genetic correlation with QTL sharing (scenario A), high genetic correlation without QTL sharing (scenario B), low (< 0.6) genetic correlation with QTL sharing (scenario C) and low genetic correlation without QTL sharing (scenario D). The numbers of environment pairs under scenarios A, B, C and D were 9, 8, 27 and 76, respectively. Further partitioning of the covariance due to individual shared QTLs not only confirmed the expected occurrence of scenarios A and D, but also enabled us to explain the unexpected existence of scenarios B and C. Scenario B was more likely due to the accumulation of undetectable shared QTLs with small effects and/or linked QTLs. On the other hand, Scenario C was likely due to the cancelling effect of shared QTLs and /or linked QTLs with opposite signs. This study points out the need for examining joint contributions of all QTLs affecting yield or any other quantitative trait to the magnitude of genetic correlation between environments before a definite conclusion on the nature of GE can be reached."
(a) University of Alberta (b) Alberta Agriculture and Rural Development

P54006 Construction of a linkage map of DNA markers and QTL analysis of root thickness in *Raphanus sativus*
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"Radish (*Raphanus sativus* L. n=9) is one of the major vegetative crops in Asia. Radish called daikon has some unique morphological or physiological traits. One is root thickness. In order to identify the genes controlling this trait, we performed QTL analysis using an inbred line of 'Aokubi' as a root thickening type and an inbred line of 'Sayatori' as a non-thickening type. One F_1 hybrid was produced by crossing 'Aokubi' with 'Sayatori', and seeds of F_2 generation were obtained by self-pollination. Diameters of grown roots of 159 F_2 plants were measured at 61 DAS (days after sowing). Root thickness showed continuous distribution with a bell shape. For genotyping of F_2 individuals, we produced 154 DNA markers (SCAR, PCR-RFLP and dot-blot-SNP) polymorphic between the parents. For designing primers of dot-blot-SNP markers, we took advantage of the Radish database (http://radish.plantbiology.msu.edu) for DNA sequence information and the MAEZATO system to predict an exon/intron-junction and design primer sequences not including intron sequences. These markers were used for genotyping of 93 F_2 individuals and 61 DNA markers including 39 dot-blot-SNP markers were available for constructing a genetic map with 9 linkage groups covering 754.1 cM. QTL analysis revealed three loci related with root thickening. One locus with the highest LOD score (2.92) showed additive effect of 1.26 in 'Aokubi' allele and 19.6% phenotypic variance. We are producing more dot-blot-SNP markers to construct a high-density genetic map."
(a) Graduate School of Agricultural Science, Tohoku University (b) National Institute of Fruit Tree Science, NARO

P54007 Reducing the list of candidate genes in the QTL interval
Ranjan, Priya-presenter ranjanp@ornl.gov(a) Yin, Tongming (a) Zhang, Xinye (a) Kalluri, Udaya C (a) Yang, Xiaohan (a) Tuskan, Gerald A (a)
" Nineteen loci distributed on six linkage groups in the *Populus* genome were associated with cell wall phenotypes of lignin content and lignin S/G ratio. A comprehensive list of genes in these QTL intervals was identified based on SSR marker intervals. More than three thousand putative candidate genes were present in these intervals. Experimental analysis and validation of each candidate gene would be a very laborious task, and ultimately, the proof of gene function would require transgenesis and restoration of lost function. Hence, *in silico* approaches that reduce the initial candidate gene set, or that establishes preference criteria for a subset of genes, could save time and investment. The *Populus* genome has experienced a whole-genome duplication event that is conserved and shared among all modern *Populus* genera. Moreover, the molecular clock in *Populus* is ticking at a rate that is slower than herbaceous annuals such as rice and *Arabidopsis*. Here, we present a computational approach based on intragenomic comparisons of gene synteny and differential microarray analysis to partition the list of candidate genes based on the whole-genome duplication in the *Populus* genome and the expression analyses of the genes and its paralogs in the duplicated interval."
(a) oak ridge national laboratory

P54008 Genetic and Functional Analysis of Soybean Seeds' Phytate Content
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"Phosphorous, a component of adenosine triphosphate, is a source of energy. In plants, inorganic phosphorous is stored as phytic acid, a myo-inositol derivative. Low phytase activity in monogastric livestocks lead to inadequate phosphorus uptake and the need to boost animal feed with costly and non-renewable inorganic phosphorus. Furthermore, phytic acid strongly binds to essential minerals diminishing their bioavailability. With focus on nutritional quality of seeds, we are seeking variable Pi levels in soybean following an integrated approach that combines Functional and QTL mapping, TILLING and Eco-TILLING. We targeted different enzymes in phytate biosynthetic pathway using Tilling. One of them is D-myo-inositol-3-phosphate synthase (MIPS, EC 5.5.1.4), the first enzyme in the cascade. MIPS 1, highly expressed in developing seeds, possesses two variants MIPS 1a and MIPS 1b. The other targeted enzyme D-myo-inositol polyphosphatase 5 kinase (IPK EC 2.7.1.37) is at the end of the pathway. Induced mutants for these enzymes were identified by TILLING using two mutagenized soybean populations (Forrest and Williams 82) and spontaneous mutations in the Glycine max wild type core collection were identified using Eco-TILLING. The analyses of other enzymes involved in phytate content is undergoing. The screening of RILs from two soybean populations, the Essex x Forrest and Hamilton x PI 438489B showed 2 lines with statistically significant high Pi levels in each population, QTLs were identified in five genomics regions and the molecular mapping of candidate genes in these populations is undergoing. The molecular and genetic dissection of phytate seed content will allow the development and release of soybean germplasm and cultivars with a desirable phytate content. "
(a) Department of Plant Soil and Agricultural Systems, Room 176; MC-4415. Southern Illinois University Carbondale, Carbondale, IL 62901

SESSION P55 – REACTIVE OXYGEN, NITRIC OXIDE & REDOX REGULATION

P55001 Sensing cellular environment through molecular switches in thiol metabolism
Jez, Joseph M.-presenter jjez@biology2.wustl.edu(a,b) Hicks, Leslie M. (b) Cahoon, Rebecca E. (b) Preuss, Mary L. (b)
"Regulation of metabolic pathways requires the integration of cellular signals at multiple levels. Our work on thiol metabolism in plants reveals different strategies for how supramolecular processes control cellular biochemistry. In glutathione biosynthesis, which acts to prevent oxidative damage and maintain intracellular redox environment, changes in redox-state directly modulate the rate-limiting enzyme of the pathway. Examination of glutamate-cysteine ligase (GCL) demonstrates that multiple oxidative stresses alter the distribution of oxidized (active) and reduced (inactive) enzyme and, thereby, glutathione production. A thiol-based sensing system provides a mechanism for modulating GCL activity in response to cellular oxidation state and implies a general role for oxidative signaling in the maintenance of glutathione homeostasis in plants. Ultimately, supramolecular regulation of primary metabolic pathways by direct sensing of cellular conditions may be more common and important than previously believed."
(a) Dept. of Biology, Washington University, St. Louis, MO (b) Donald Danforth Plant Science Center, St. Louis, MO

P55003 Deficiency in Fibrillin 6 decreases resistance to oxidative stress and plastoglobule osmiophilicity in apple trees
Singh, Dharmendra K.-presenter dks156@psu.edu(a,b) Maximova, Siela N. (a,c) McNellis, Timothy W. (c)
"Reactive oxygen species (ROS) are important signaling and defense molecules whose production is increased in plants during oxidative stress. Plastoglobules, which store antioxidants like tocopherols and carotenoids, are attached to the thylakoids and may have a function during oxidative stress. Here we studied the role of Fibrillin 6 (Fbr6), a plastoglobule-localized protein, in oxidative stress resistance. *Fbr6* RNAi knockdown apple trees were more susceptible to infection by *Erwinia amylovora*, the causal agent of fire blight disease. RNAi-*Fbr6* apple trees also had increased sensitivity to high light and ozone as measured by anthocyanins accumulation and ion leakage from the leaves. Similarly, *Arabidopsis thaliana fbr6* T-DNA knockout mutant plants had increased sensitivity to ozone and increased susceptibility to *Pseudomonas syringae*, the causal agent of bacterial speck disease. In addition, RNAi-*Fbr6* apple trees accumulated more peroxides during ozone treatment, as measured by the ferrous xylenol orange assay. Finally, RNAi-*Fbr6* apple trees were more sensitive to externally applied hydrogen peroxide and to the ROS generator paraquat, as measured by electrolyte leakage from treated leaves. Using transmission electron microscopy, we observed reduced numbers of osmiophilic plastoglobules in RNAi-*Fbr6* apple chloroplasts compared to wild-type apple chloroplasts. This suggests that Fbr6 is important for the accumulation of high reducing power, osmiophilic molecules in the plastoglobules. Fbr6 has a conserved lipocalin domain that may be involved in transport of small, osmiophilic molecules such as tocopherols. We propose that disruption of Fbr6 alters plastoglobule antioxidant content, thereby affecting ROS homeostasis during oxidative stress in plants."
(a) The Huck Institute of Life Sciences, Pennsylvania State University (b) The Department of Plant Pathology, Pennsylvania State University (c) The Department of Horticulture, Pennsylvania State University

P55004 Reactive oxygen species mediate a rapid systemic signal in Arabidopsis thaliana.
Miller, Gad-presenter gadmiller@gmail.com(a) Schlauch, Karen (a) Tam, Rachel (a) Cortes, Diego (b) Torres, Miguel A (c) Shulaev, Vladimir (b) Dangl, Jeffery L (d) Mittler, Ron (a,e)
"Cell-to-cell communication and long distance signaling play an important role in the response of plants to pathogens, pests, mechanical wounding and extreme environmental conditions. Here, we uncover a rapid systemic signal that travels at a rate of ~8 cm min-1 and is dependent on the presence of the respiratory burst oxidase homolog D (RbohD) gene. Signal propagation is accompanied by the accumulation of reactive oxygen species (ROS) in the extracellular spaces between cells, and can be inhibited by the suppression of ROS accumulation at locations distant from the initiation site. The systemic signal can be triggered by wounding, heat, cold, high light and salinity stresses. Our results reveal a profound and general role played by ROS in mediating rapid, self-propagating systemic signals in plants. "
(a) Department of Biochemistry University of Nevada, Reno (b) Virginia Bioinformatics Institute, Virginia Tech (c) Department of Biotechnology, Madrid Technical University (d) Department of Biology, University of North Carolina (e) Department of Plant Sciences, Hebrew University of Jerusalem

P55005 Leaf senescence signaling: Ca^{2+} accumulation mediated by Arabidopsis cyclic nucleotide gated channel2 acts through nitric oxide to repress senescence programming
Ma, Wei (a) Smigel, Andries (a) Walker, Robin K (a) Moeder, Wolfgang (b,c) Qi, Zhi (a) Yoshioka, Keiko (b,c) Berkowitz, Gerald A-presenter gerald.berkowitz@uconn.edu(a)
"Ca^{2+} and nitric oxide (NO) are essential molecules involved in plant senescence signaling cascades. Previous studies suggest that Ca^{2+} and NO may act as negative regulators deferring senescence. In some signaling pathways, NO generation can be dependent on cytosolic Ca^{2+}. The Arabidopsis mutant *dnd1* lacks a functional plasma membrane-localized cation channel (CNGC2). Using this mutant, we recently demonstrated this channel affects plant response to pathogens through a signaling cascade involving Ca^{2+} modulation of NO generation; the pathogen response phenotype of *dnd1* can be complemented by application of an NO donor. At present, the interrelationship between Ca^{2+} and NO generation in plant cells during leaf senescence remains unclear. Here, we use *dnd1* plant to present genetic evidence indicating that Ca^{2+} uptake and NO production play pivotal roles in plant leaf senescence. Leaf Ca^{2+} accumulation is reduced in *dnd1* leaves compared to wild type. Importantly, many early senescence-associated phenotypes (such as loss of chlorophyll, expression level of senescence associated genes, H_2O_2 generation, lipid peroxidation, tissue necrosis, and salicylic acid levels) were more prominent in *dnd1* leaves compared to wild type. Application of an NO donor effectively rescues many *dnd1* senescence related phenotypes. We also identify a new phenotype associated with CNGC2 loss-of-function; *dnd1* plants are less sensitive than wild type to (exogenous) NO. Our work demonstrates that the CNGC2 channel is involved in Ca^{2+} uptake during plant development. Work presented here suggests that this function of CNGC2 may mediate downstream 'basal' NO production during the course of plant development, and that this NO generation acts as a negative regulator during plant leaf senescence signaling."
(a) Agricultural Biotechnology Laboratory,Department of Plant Science, University of Connecticut , 1390 Storrs Rd., Storrs, CT 06269-4163, USA (b) Department of Cell and Systems Biology, University of Toronto, 25 Willcocks Street, Toronto, Ontario M5S 3B2, Canada (c) Center for the Analysis of Genome Evolution and Function, University of Toronto, 25 Willcocks Street, Toronto, Ontario M5S 3B2, Canada

P55006 Using synthetic biology to alter ROS signaling and reduce superoxide in Arabidopsis enhances heat tolerance
Im, Yang Ju-presenter yim2@ncsu.edu(a) Ji, Mikyoung (a) Lee, Alice (a) Grunden , Amy M (a) Boss, Wendy F (a)
"Reactive oxygen species (ROS) are important signal molecules in plant responses to stress. However, studying the short-lived ROS signals *in vivo* is difficult and the downstream effectors of ROS such as cytosolic superoxide (O_2^-) are not well characterized. Our approach was to selectively remove O_2^- and to use comparative biology to reveal downstream events normally mediated by the ROS signal. Although plants, like other aerobic organisms, have superoxide dismutase (SOD) to remove O_2^- and prevent the production and buildup of toxic free radicals, the dismutase reaction produces

oxygen, which can serve as an additional source of ROS. Unlike SOD, superoxide reductase (SOR), an enzyme present in the extremophilic Archaea has a lower Km for O_2^- than SOD and reduces O_2^- without producing oxygen. Therefore, we chose a synthetic approach using *Pyrococcus furiosus* SOR to reduce cytosolic ROS. Our hypothesis was that expressing the *P. furiosus* SOR in planta would provide a more effective ROS reducing system. We found that *P. furiosus* SOR can be produced as a soluble enzyme in planta and that plants producing SOR have enhanced tolerance to heat, light and chemically-induced ROS. Stress tolerance in the SOR-producing plants correlates positively with a delayed increase in ROS-sensitive transcripts and a decrease in ascorbate peroxidase activity. The SOR plants provide a good model system to study the impact of cytosolic O_2^- on downstream signaling events in plant growth and development as well as to promise as a means for improving stress tolerance in crop plants."
(a) North Carolina State University

P55007 "Nuclear activity of ROXY1, a glutaredoxin interacting with TGA factors, promotes petal development in Arabidopsis "
Li, Shutian-presenter shutian.li@biologie.uni-osnabrueck.de(a,b) Lauri, Andrea (a) Ziemann, Mark (a,c) Busch , Andrea (a,b) Bhave, Mrinal (c) Zachgo, sabine (a,b)
"Glutaredoxins (GRXs) are ubiquitous glutathione-dependent oxidoreductases that catalyze reversible reduction of disulfide bonds and regulate protein activities in a variety of cellular processes. In plants, three subclasses of GRXs are defined according to their respective active sites. GRXs with the CPYC and CGFS active sites are common to pro- and eukaryotes, while GRXs with the CC-type motif have so far only been identified in land plants. *ROXY1*, encoding a CC-type GRX, is known to regulate petal primorida initiation and further petal morphogenesis in Arabidopsis. Intracellular localization studies revealed a nucleocytoplasmic expression of ROXY1. However, exclusively redirecting ROXY1 either to the cytoplasm or the nucleus proved that nuclear localization of ROXY1 is indispensable and thus crucial for its activity in flower development. Yeast two-hybrid screens identified TGA factors as ROXY1-interacting proteins and their nuclear interactions in planta were further confirmed using BiFC assays. Overlapping expression patterns of *ROXY1* and TGA genes during flower development support their biological relevance in petal development. Deletion analysis demonstrates the importance of the C-terminus for its functionality and for mediating ROXY1/TGA protein interactions. Phenotypic analysis of the *roxy1 pan* double mutant and an engineered chimeric repressor mutant from *PERIANTHIA* indicates a dual role of ROXY1 in petal development. Collectively, our data show that nuclear activity of ROXY1 controls petal primordial formation likely by modifying PAN posttranslationally. Additionally, ROXY1 affects later petal morphogenesis probably by modulating other TGA factors that act redundantly during differentiation of second whorl organs. "
(a) Max-Planck Institute for Plant Breeding Research (b) Department of Botany, University of Osnabrueck (c) Environment and Biotechnology Centre, Faculty of Life and Social Sciences, Swinburne University of Technology

P55008 Cytosolic alkalinization is an early signaling component essential for the elevation of H_2O_2 and NO in stomatal guard cells by abscisic acid during stomatal closure in epidermis of pea and *Arabidopsis*
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"Guard cells are popular model systems to study the sequence of events during the stomatal closure caused by abscisic acid (ABA). Reactive oxygen species (ROS, mainly H_2O_2), nitric oxide (NO) and cytosolic calcium are known to rise during such ABA-induced stomatal closure in epidermis of *Pisum sativum* and *Arabidopsis thaliana*. We studied the role and importance of cytosolic pH, H_2O_2, and NO during stomatal responses to ABA as well as other signals, such as bicarbonate and chitosan. Exposure to ABA raised the levels of not only reactive oxygen species and nitric oxide (NO) but also the cytosolic pH in guard cells, while inducing stomatal closure. Modulation of cytosolic pH changed the patterns of H_2O_2 or NO production. Acidification of cytosol by butyrate reduced H_2O_2/NO production and restricted stomatal closure by ABA. Similarly, alkalinization by methylamine enhanced further the cytosolic alkalinization and promoted stomatal closure by ABA. EGTA (calcium chelator) prevented ABA-induced stomatal closure and also restricted partially the production of H_2O_2/NO. Real time monitoring by fluorescent dyes of pH (by BCECF-AM), H_2O_2 (H_2DCF-DA) and NO (DAF-2DA) revealed that the rise in cytosolic pH is the earliest event followed by the increase in the levels of H_2O_2 and NO. Thus, our results demonstrate that the cytosolic pH in guard cells is an early and essential secondary messenger during stomatal closure by ABA. A rise in internal Ca^{2+} appears to facilitate the rise in cytosolic pH and H_2O_2/NO, suggesting an important role for Ca^{2+} upstream of cytosolic alkalinization. The interrelationship and interaction of cytosolic calcium, cytosolic pH, H_2O_2 and NO during stomatal closure by ABA are intriguing and warrant further studies."
(a) University Of Hyderabad

P55009 The Role of Redox Reactions in Plant Resistance against Pathogen infection
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http://briggs.ucsd.edu/people.html
"Plants use different mechanisms to fight pathogen infection including pathogen recognition which elicits a series of physiological reactions to confer resistance. Basal defense and Resistance (R)-gene mediated response are two examples of defense mounted by plants to fight pathogen infection. Recognition of pathogens by either R-proteins or basal defense leads to systemic acquired resistance (SAR), which requires Salicylic acid (SA) and positive regulatory protein, NPR1. Previous studies show that upon infection there is an increase in SA which causes an important reduction in NPR1 changing it from its oxidized oligomeric form to a reduced nuclear localized monomeric form; this regulates the expression of Pathogenesis related (PR) genes. Our research involves the study of proteins that undergo redox change during pathogen infection to regulate defense and confer resistance. Proteins extracted from Arabidopsis thaliana plants infected with virulent or avirulent pathogens, and defense elicitors such as BTH were subjected to thioredoxin affinity chromatography, which takes advantage of thioredoxins natural ability to reduce disulfide bonds as a means of trapping redox reducible proteins. The eluted proteins were analyzed by mass spectrometry. We have observed from our column and mass spectrometry data that there is differential binding of redox regulated proteins in treated versus untreated plants. Several of the proteins observed on the treated column are known redox regulated proteins that play a role in stress tolerance, hypersensitive response, reactive oxygen species (ROS) signaling and cell death. "
(a) University of Californai, San Diego

P55010 GO plants as an ideal non-invasive model system to study the effects of plastidic generated H_2O_2
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"Reactive oxygen species (ROS) are both toxic by-products of aerobic metabolism as well as signaling molecules. Some studies indicated that a flow of information may exist from the site of ROS action to the nucleus. At high concentrations, hydrogen peroxide (H_2O_2) induces cell death, while at low concentrations it can act as a messenger molecule. To assess the effects of metabolically generated H_2O_2 in chloroplasts, transgenic Arabidopsis plants were generated in which the peroxisomal glycolate oxidase (GO) was targeted to the chloroplasts. GO overexpressing lines (GO plants) grown at moderate light intensities show retarded development, yellowish rosettes, impaired photosynthetic performance and accumulate both H_2O_2 and glyoxylate, while at low light intensities this phenotype virtually disappears. The GO plants develop oxidative stress lesions under photorespiratory conditions but grow like the wild-type under non-photorespiratory conditions. GO plants co-expressing enzymes, which further metabolize glyoxylate still accumulate H_2O_2 and show all features of the GO phenotype indicating that H_2O_2 is responsible for the GO phenotype. A response to oxidative

stress is installed, with increased expression and/or activity of known oxidative stress responsive components. Metabolic profiling of GO plants indicated a metabolic scenario consistent with decreased carbon assimilation, which results in lower abundance of glycolytic and tricarboxylic acid cycle intermediates, while simultaneously amino acid metabolism routes are specifically modulated. The amount of H_2O_2 produced in the GO plants could be controlled by changing the conditions of growth, thus making the GO plants a challenging non-invasive model to study the action of plastid-produced H_2O_2 as a signal molecule."
(a) Institute of Botany, University of Cologne

P55011 Arabidopsis Calmodulin Like Protein 24 (CML24) is Required for Lipoploysaccharide and ABA-Induced Stomata Closure and Nitric Oxide/Reactive Oxygen Species Production in Guard Cells
Walker, Robin K-presenter robin.walker@uconn.edu(a) Zhi, Qi (a) Braam, Janet (b) Berkowitz, Gerald A (a)
"Cytosolic Ca^{2+} elevation is known to be upstream from reactive oxygen species(ROS) and nitric oxide(NO) generation during pathogen response signaling cascades. Ca^{2+} as well as ABA and NO are also involved in guard cell responses to ABA. CML24 is one of the fifty calmodulin-like Ca^{2+}-binding proteins in Arabidopsis. Prior work from this lab demonstrated that the hypersensitive response to pathogens in Arabidopsis and NO generation in response to Pathogen Associated Molecular Pattern(PAMP) molecules such as lipoploysaccharide(LPS) in guard cells requires CML24(Ma *et al.*, 2008). LPS-induced stomatal closure is dependent on the ABA signaling pathway(Melotto *et al.*, 2006). Ca^{2+} influx is required for both ABA and pathogen response signal transduction. ROS generation induced by NADPH oxidase is required for ABA-induced stomatal closure(Kwak *et al.*, 2003). Here we examine the overlap and differences in Ca^{2+}-dependent signaling in guard cells responding to ABA and the PAMP LPS. LPS and ABA application to epidermal peels of Arabidopsis leaves evoked transient production of NO and ROS. LPS-induced NO(Ali *et al.*, 2007) and ROS was inhibited by chelation of external Ca^{2+} with EGTA and by the CaM antagonist W7. LPS and ABA-induced NO and ROS production were impaired in guard cells of a CML24 loss function mutant(*cml24*). These results demonstrate that CML24 functions upstream of NO and ROS generation and requires Ca^{2+} for ABA and pathogen response signaling. H_2O_2 induced stomatal closure of both wild type and *cml24* tissue; however, LPS- and ABA-dependent stomatal closure was impaired in *cml24* tissue. Taken together, work in these studies provides evidence that CML24 is a component upstream from ROS and NO generation in both LPS and ABA signaling pathways in Arabidopsis guard cells. "
(a) University of Connecticut (b) Rice University

P55012 Quantitative trait loci associated with ozone tolerance in different accessions of *Arabidopsis*
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"Ozone is an air pollutant causes damage to natural and cultivated plants, resulting in forest decline and loss of crop yield. Identifying the genomic regions associated with the ozone sensitivity in plants will aid in our understanding of the genetics of ozone tolerance and development of more ozone tolerant plants. To detect the quantitative trait loci (QTL) associated with the different sensitivity of two Arabidopsis accessions, Col (ozone-tolerant) and Ws (ozone-sensitive), we investigated recombinant inbred lines derived from a cross between Col and Ws. As a result, two significant QTLs were identified; one was located on chromosome 1 and the other was on chromosome 4. The latter locus exhibited stronger linkage with the ozone-sensitive phenotype, and located near the *ACS6* gene, which encodes a key enzyme in ozone-induced ethylene biosynthesis. The *ACS6* expression and ethylene evolution in Ws was higher than in Col under ozone exposure. The *ACS6* promoter was dissected via deletion and gain-of-function analysis using luciferase reporter gene in transgenic Arabidopsis. The results indicated the presence of an ozone-responsive *cis*-acting element between -941 and -764 of the *ACS6* promoter. Comparison of the *ACS6* promoter sequences revealed that Ws has a 13-bp-long extra sequence (from -822 to -810). We named this sequence an ozone-responsive element (ORE). To test the function of ORE, we made two transgenic plants; one expressing luciferase under the control of the Ws promoter with the ORE deleted and the other harboring the Col promoter with an ORE inserted. Our results showed that the ORE was essential for ozone-induced expression of *ACS6*, suggesting that ORE is involved in ozone-induced ethylene production and at least partly determines the ozone sensitivity in Ws."
(a) National Institute for Environmental Studies

P55013 $^1O_2$-mediated retrograde signaling during late embryogenesis predetermines plastid differentiation in seedlings by recruiting abscisic acid.
Kim, Chanhong-presenter ck438@cornell.edu(a) Lee, Keun-Pyo (b) Baruah, Aiswarya (a) Goebel, Cornelia (c) Feussner, Ivo (c) Apel, Klaus (a)
Plastid development in seedlings of Arabidopsis thaliana is affected by the transfer of $^1O_2$-mediated retrograde signals during late embryogenesis from the plastid to the nucleus that changes nuclear gene expression. Their potential impact on plastid differentiation is maintained throughout seed dormancy and becomes effective only after seed germination. Inactivation of the two nuclear-encoded plastid proteins EXECUTER1 and EXECUTER2 blocks $^1O_2$-mediated retrograde signaling and impairs normal plastid formation in germinating seeds. This long-term effect of $^1O_2$ retrograde signaling depends on the recruitment of abscisic acid (ABA) that acts as a positive regulator of plastid formation in etiolated and light-grown seedlings.
(a) Boyce Thompson Institute (b) Swiss Federal Institute of Technology (ETH) Zurich (c) Albrecht-von-Haller-Institute for Plant Sciences

P55014 "Dehydration-responsive element binding factor, *DREB2C*, confers an oxidative stress tolerance in *Arabidopsis*"
Kim, Yujung-presenter aimeyj@naver.com(a) Lee, Sang Yeol (a) Lim, Chae Oh (a)
"A subfamily of dehydration-responsive element binding factor2 (*DREB2*) belong to the plant-specific AP2/ERF family of transcription factors. A *DREB2* homolog gene, *DREB2C*, functions as a key regulator in heat stress (HS) response, and activates expression of many abiotic stress-responsive-genes involved in HS tolerance. In this study, the *DREB2C* showed that the transcripts were elevated in response to H_2O_2 and methyl viologen (MV) and the *DREB2C* overexpressors led to enhanced oxidative stress tolerance. In addition, the transcription of ascorbate perosidase2 (*APX2*) was induced and APX^s and APX activity was detectable at normal growth conditions and persisted after treatment with 0.5 μ;M MV in the overexpressors, whereas the activity was strongly decreased in wild-type plants (WT) under MV stress. Moreover, *DREB2C* overexpressors were found to be lower than that of WT as measured by intracellular accumulation of H_2O_2. In spite of these data, an electrophoretic mobility shift assay (EMSA) revealed that $DREB2C^{145-528}$ was unable to form a complex with the putative dehydration responsive element (DRE; A/GCCGAC) in *APX2* promoter sequences. By contrast, $DREB2C^{145-528}$ was able to bind with the *Arabidopsis* heat-shock transcription factor 3 (*HsfA3*) promoter, which is a downstream gene directly regulated by DREB2C under HS, and this HsfA3 was able to form a complex with the *APX2* promoter. Based on these results, we suggest that DREB2C activates *HsfA3* and the activated HsfA3 regulates expression of many oxidative stress-inducible genes including *APX2* and functions in acquisition of oxidative stress tolerance under the control of the DREB2C cascade."
(a) Division of Applied Life Science (BK21), Environmental Biotechnology National Core Research Center, Graduate School of Gyeongsang National University

P55015 Arabidopsis null mutant for ATM protein kinase shows high sensitivity to gamma radiation and fails to elevate superoxide levels in roots

Einset, John W-presenter john.einset@umb.no(a)

"Development of effective radiation protection strategies for the environment will depend on methods for measuring impacts not just in humans but also in non-human components. An advantage of Arabidopsis as a reference plant is the availability of mutants. In this respect, the ATM (At3g48190) gene is of special interest as it codes for a protein kinase similar to the ATM protein kinase in animals whose function is to signal cellular responses to ionizing radiation. The null mutant for Arabidopsis ATM was studied earlier but only after exposure to high radiation exposures corresponding to 100 Gy. End points based on petiole elongation and root hair development were used to compare sensitivities of the genotypes to gamma exposures 0.125-20 mGy/h from a Co-60 source. Wild type Arabidopsis responded significantly in the root hair bioassay only at the highest 20 mGy/h exposure while the ATM null mutant responded as low as 0.125 mGy/h. When levels of superoxide were measured, as little as 0.125 mGy/h affected wild type while 0.125-20 mGy/h did not elevate superoxide levels in the mutant. These results provide improved data and end points for describing plant reactions to radiation and show that loss of only a single ATM gene can dramatically affect sensitivity. The results also show that the ATM null mutant does not show the ROS accumulation response that wild type shows. This interesting finding raises the possibility that the ATM protein kinase is required for ROS increases which in turn signal some aspects of the radiation protection response."

(a) Norwegian University of Life Sciences

SESSION P56 – REPRODUCTIVE DEVELOPMENT

P56001 Pollen tube guidance is severely affected in the Solanum chacoense MAPKKK ScFRK1 mutant

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"The Solanum chacoense Fertilization-Related Kinase 1 is a member of the MEKK subfamily of plant MAPKKK that is predominantly expressed in ovules. ScFRK1 mRNAs accumulate predominantly in the egg apparatus cells of the embryo sac and ScFRK1 mRNA levels decrease rapidly following pollination. Transgenic plants down-regulated in ScFRK1 mRNA expression showed no abnormal phenotypes in vegetative tissues but produced parthenocarpic seedless fruits upon pollination. This phenotype could be linked to formation of aberrant embryo sacs, which did not progress further than the functional megaspore stage in affected transgenic lines. Since embryo sac integrity is a prerequisite for pollen tube guidance, we devised a semi in vivo pollen tube growth system to assess the ability of ScFRK1 mutant ovules to attract pollen tubes. As expected, guidance was severely affected, confirming the involvement of the egg apparatus cells as the source of attracting molecules. In WT plants, acquisition of attraction competence was shown to be developmentally regulated and not pollination-induced. Species-specificity of the attraction signal was tested and was robust even with very close S. chacoense relatives from the Solanum Petota section, where natural interspecific hybridization is quite common among many species. This suggests a high discrimination level for the micropylar guidance phase. This also suggests that the attractant must be highly polymorphic, hinting towards a proteinaceous nature. This was further demonstrated with the use of crude and purified protein extracts. Proteomic as well as genomic strategies are currently used to isolate the Solanaceous pollen tube guidance protein chemoattractant. "

(a) Universite de Montreal

P56002 "ScRALF3, a secreted RALF-like peptide implicated in the establishment of embryo sac polarity during ovule development"

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"Major advances in the understanding of RALF, Rapid Alkalinization Factor, are coming from studies of root-specific *RALF* genes. NtRALF (*N. tabacum*) induce a fast, dramatic change of the apoplastic pH and arrest root growth; the root specific AtRALF1 *(A. thaliana*) induce release of cytoplasmic Ca^{2+} and silencing the *NaRALF* (*N. attenuata*) transcript causes loss of root hair elongation. However, nothing is known about *RALF-like* genes in other tissues; more than 34 *RALF-like* genes were found in the genome of *A. thaliana*. *ScRALF3* was isolated in a cDNA library of genes expressed in the ovaries of *Solanum chacoense* after pollination. It was shown that this gene has a peak of expression eight days after pollination. To investigate a potential role in embryo development, twelve lines with a silencing *ScRALF3* transcript were analyzed. In seven independent lines, we observed a small fruit phenotype with a low number of seeds. Surprisingly, this phenotype can be explained by immature ovules: the ovules at anthesis have under-developed embryo sacs. Approximately 66% of the phenotype can be explained by arrest during mitosis I, II or III. During megagametogenesis, the highest expression appears during meiosis and is slowly downregulated during mitosis. Also, ScRALF3 seems to control the polarity during the migration of nuclei at mitosis, asymmetrical distribution of nuclei occur in mutants. Specific *ScRALF3* expression in the cells surrounding the embryo sac is under evaluation by in situ analysis to assess if the expression is polarized. This hypothesis is supported by data obtained with the closest ScRALF3 homolog, AtRALFL34. *AtRALFL34* expression is distributed asymetrically in a growing tissue."

(a) University of Montreal, IRBV

P56003 "*GASA5*, a regulator of flowering time and stem growth in *Arabidopsis thaliana*"

Zhang, Shengchun (a) Yang, Chengwei (a) Peng, Jianzong (a) Sun, Shulan (a) Wang, Xiaojing-presenter wangxj@scnu.edu.cn(a)

"We provide clear genetic evidence that *GASA5*, a *GASA (Gibberellic Acid-Stimulated Arabidopsis)* family gene in Arabidopsis is involved in controlling flowering time and stem growth. *GASA5* expressed in all tissues of plants, as detected by RT-PCR, and robust GUS staining was observed in the shoot apex of 8-old seedlings and inflorescence meristems during reproductive development. Phenotypic analysis showed that a *GASA5* null mutant (*gasa5-1*) flowered earlier than wild type with a faster stem growth rate under both LD and SD photoperiods. In contrast, transgenic plants overexpressing GASA5 (*35S::GASA5*) demonstrated delayed flowering with a slower stem growth rate. However, neither the *gasa5-1* nor the *35S::GASA5* plants revealed obvious differences in flowering time upon treatment with GA_3, indicating that GASA5 is involved in GA-promoted flowering. *GAI (GA INSENSITIVE)*, one of the five DELLAs in Arabidopsis, was more highly expressed in *35S::GASA5* plants, but it was lower in *gasa5-1*. The crossing of the different GASA5 genotypes into the mutant *gai-t6* background showed a smaller difference in flowering times under both LD and SD conditions in homozygous hybrid *gai-t6* /Col,*gai-t6/gasa5-1*, and *gai-t6/35S::GASA5* plants, implying GASA5 affected flowering in part through GAI function. Further transcript profiling analysis suggested that GASA5 delayed flowering by enhancing *FLOWERING LOCUS C (FLC)* expression and repressing the expression of key flowering-time genes, *FLOWERING LOCUS T (FT)* and *LEAFY (LFY)*. Our results suggest that GASA5 is a negative regulator of GA-induced flowering and stem growth. This research was supported by grants from the Nature Science Foundation of China (NO 30570165,U0731006) and the National Key Technology R & D Program in China (No.2007BAD59B06)."

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P56004 The overlapping roles of NF-Y transcription factors in flowering

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"The heterotrimeric NUCLEAR FACTOR Y (NF-Y) transcription factor has undergone extensive duplication in the plant lineage. For example, while

mammals have only one copy of each gene, Arabidopsis has 36 *NF-Y* in the three distinct families (10 *NF-YA*, 13 *NF-YB*, and 13 *NF-YC*). Individual subunits have functions in drought resistance, maintenance of nitrogen-fixing nodule meristems, seed germination, and flowering time. Currently, there is no plant process for which the complete NF-Y complex has been described (from animal systems, there is an expectation that functional NF-Y transcription factors will include one subunit from each family). To help identify complete NF-Y complexes, we recently developed stable promoter:GUS fusions for all 36 Arabidopsis *NF-Y* (Siefers et al., 2008. PPhys 149:625-641). We are currently utilizing these lines to identify complete, floral promoting NF-Y complexes. Because it is already known that *NF-YB2* and *NF-YB3* are redundantly necessary for photoperiod dependent flowering, we are focusing our efforts on the *NF-YC* and *NF-YA* components. Using the promoter:GUS lines, we have identified three *NF-YC* genes that are 1) simultaneously expressed in the leaf vasculature with *NF-YB2*, *NF-YB3*, *CONSTANS* (*CO*), and *FLOWERING LOCUS T* (*FT*), and 2) are redundantly required for photoperiod dependent flowering, and 3) are genetically required for the promotion of early flowering by constitutively expressed *CO*. Further, these NF-YC interact in vivo with NF-YB2 and NF-YB3. We currently estimate that at least 12 unique NF-Y complexes are involved in flowering. At the ASPB meeting, we will discuss these results and progress towards further characterizing the functions of *NF-YA* in flowering, as well as current theories on the roles of NF-Y in the plant lineage."
(a) University Of Oklahoma

P56005 Dynamic behavior of MADS domain proteins in flowers
Urbanus, Susan L. (b) Martinelli, Adriana P.-presenter adriana@cena.usp.br(a) Dinh, Q.D. (Peter) (b) Angenent, Gerco C. (b) Immink, Richard G.H. (b)
"Members of the MADS box transcription factor family are among others involved in the initiation and differentiation of floral organs in plants. To investigate the dynamic behavior of selected MADS domain transcription factors in living plants, C-terminal *GFP* fusions were made and introduced into Arabidopsis thaliana (Columbia). Genomic clones of *AGAMOUS (AG)*, *SEPALLATA3 (SEP3*), *FRUITFULL (FUL)* and *APETALA1 (AP1)* including the upstream regulatory regions were used to generate the GFP protein fusion constructs. Using confocal microscopy the temporal and spatial localization patterns of these GFP-tagged transcription factors were analyzed during flower development. This work revealed differences between localizations of mRNA and protein that in some cases could be due to intercellular transport of the proteins (Urbanus et al., 2009). To further investigate this possibility of intercellular transport of MADS domain proteins we made *GFP* tagged cDNA clones of *AG*, *SEP3*, *AP1*, *PISTILLATA (PI)* and *APETALA3 (AP3)* under the control of the epidermis specific AtML1 promoter. We studied the ability of the selected MADS domain proteins to move between different clonal cell layers and within the epidermal cell layer with confocal microscopy. In addition to this, the effect of the epidermal (over)expression of these MADS box transcription factors on plant morphology and flowering time was studied in wild type and in mutant background. Urbanus SL, de Folter S, Shchennikova AV, Kaufmann K, Immink RGH, and Angenent GC: *In planta* localization patterns of MADS domain proteins during floral development in Arabidopsis thaliana. BMC Plant Biology 2009, 9:5 doi:10.1186/1471-2229-9-5. (Acknowledgements: Centre for Biosystems Genomics (CBSG) in Wageningen, the Netherlands; CAPES and CNPq, Brazil)"
(a) University of Sao Paulo, CENA (b) Plant Research International

P56006 Flower reversion in the natural allopolyploid *Arabidopsis suecica*
Madlung, Andreas-presenter amadlung@ups.edu(a) McCullough, Erin (a) Wright, Kirstin (a) Alvarez, Aurelia (a) Parker, Chanel (a) Rickoll, Wayne (a)
"While studying genomic changes in response to allopolpyloidy in the genus *Arabidopsis* we noticed frequent peculiar floral abnormalities in wild type accessions of the natural allopolyploid *A. suecica*. Upon further investigation we were able to distinguish several rare phenotypes that resembled floral homeotic gene mutations as well as a more frequent floral reversion phenotype. These phenotypes only affected a relatively small portion of flowers on each individual, while the majority of flowers appeared normal. Floral reversion was not day length dependent, nor was there a clear difference in the position of normal versus revertant flowers along the maturing inflorescence. To investigate the underlying molecular cause of the floral reversion phenotype, we compared the transcriptional activity of eight genes involved in meristem development and maintenance, and floral patterning between normal and revertant flowers. Using quantitative PCR, we found that revertant flowers showed a significant decrease in the expression of *AGAMOUS-LIKE-24 (AGL-24), APETALA1 (AP1), and SHORT VEGETATIVE PHASE (SVP)*. At the same time we observed a significant increase in the expression of *SUPRESSOR OF CONSTANS1 (SOC1)* in reverting flowers and smaller yet significant variations in the expression of *AGAMOUS (AG), FLOWERING LOCUS C (FLC), and LEAFY (LFY)*. It is possible that allopolyploidy has lead to a change in dosage or an imbalance in signaling interactions of proteins involved in flower development. We propose a model, in which floral reversion is aided by decreased maintenance of the floral meristem. "
(a) University of Puget Sound

P56007 Studying the transcriptome of rice pollen mother cell with laser microdissection and microarray
Tang, Wei-Hua-presenter whtang@sibs.ac.cn(a) Tang, Xiang (a,b) Zhao, Xing-Ming (c) Zhang, Dong (a)
"The development of pollen mother cells (PMCs) is a key phase in the transition from sporophyte to gametophyte in the plant sexual reproduction. It represents the last stage before meiosis in plant life-cycle. So far only a few genes are known to be expressed in pollen mother cell. We isolated specific pollen mother cells of rice using laser microdissection, and did microarray analysis with rice whole genome microarray. We validated some gene expression by real-time PCR. Our results show that at least 70% of genes presented on microaray are expressed in rice pollen mother cell. The results also show that some pathways, including nucleotide metabolism, DNA replication and repair, etc. are enriched in pollen mother cells, while some other pathways such as biosynthesis of secondary metabolites are missing. Our results also provide more information which is helpful for understanding mitosis-meiosis switch and/or meiosis preparation."
(a) Shanghai Institute of Plant Physiology and Ecology, SIBS, CAS, China (b) Laboratory of Plant Functional Genomics, Yangzhou University, China (c) Institute of Systems Biology, Shanghai University, China

P56008 Development of a microgenomics approach to identify genes involved in a key step of sexual plant reproduction
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"Reproduction is a crucial step in the life cycle of all species. In plants, reproduction through seeds involves the progression of well-defined developmental steps and many discrete differentiation events controlled by a large number of genes. Key events during female sexual reproduction are: (i) megasporogenesis involving meiosis of a megaspore mother cell (MMC) to give rise to the functional megaspore, (ii) megagametogenesis, the formation of the female gametophyte (embryo sac) harboring the two gametes (the egg cell and the bi-nucleate central cell) from the functional megaspore, and (iii) double fertilization during which the female and male gametes fuse. Although sexual reproduction attracted the attention of scientists for over a century, the molecular basis is currently not well understood. One reason for this is that the identification of genes involved in the differentiation and specification of single cells within the ovule has been difficult due to the inaccessibility of these cells. Our goal is to identify the genetic basis and molecular mechanism underlying megasporogenesis in *Arabidopsis thaliana*. To this aim we have used a microgenomics approach to define the transcriptomes of the MMC. This involved the isolation of the cell from the surrounding tissue by Laser Assisted Microdissection (LAM),

the isolation and linear amplification of mRNA, and subsequent GeneCHIP hybridization. Expression profiles of selected genes are being experimentally validated. Our work provides important new insights in the molecular basis underlying the first step of female reproductive development. "
(a) University of Zurich, Switzerland (b) Keygene, Wageningen, The Netherlands (c) Trinity College Dublin, Ireland

P56009 Map based cloning of the S-locus in a self incompatible Asteraceae Species (*Cichorium intybus* L.)
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"Self incompatibility (SI) is one of the most important mechanisms to prevent selfing in hermaphrodite plants. This mechanism is generally under the control of a single multiallelic locus, the S-locus. Pollen and pistil determinants have been identified in some plant families (Brassicaceae, Solanaceae, Convolvulaceae) and are different in each family, suggesting that different molecular mechanisms of SI have evolved independently. Chicory (*Cichorium intybus* L.) is a sporophytic self incompatible species belonging to the Asteraceae family for which these determinants are different and still unknown. Our goal is the positional cloning of male and female determinants of SI in chicory. We assigned the S-locus to one end of one of the 9 linkage groups of the chicory map in an interval spanning 0.1cM. In order to obtain a high resolution and high density map we have genotyped 2500 individuals to increase the number of specific markers in the S-locus region. In parallel, two 6X BAC libraries were constructed from two genotypes with different S-alleles. The most tightly linked markers to the S-locus are being used to screen both libraries to obtain a physical map. Since a high haplotypic diversity between S-alleles is expected, the comparison of the genomic sequences from the two genotypes used for BAC libraries construction will reveal the features of the S-locus in chicory. "
(a) SADV Universite de Lille 1 (b) CNRGV INRA de Toulouse

P56010 A common genetic module mediates both female floral organ development and shade avoidance
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"The female reproductive structure or gynoecium of the Arabidopsis thaliana flower consists of an ovary, divided longitudinally by a septum, and topped by a stigma and short style. The formation of the stigma, style and septum is controlled by SPATULA(SPT), which encodes a basic helix-loop-helix (bHLH) transcription factor. However,nothing was previously known of the downstream pathway through which SPT controls the development of these tissues. We demonstrate SPT to positively regulate genes known to respond to changes in light quality, revealing an unexpected link between the development of the gynoecium and shade avoidance responses in vegetative parts of the plant. The promoter regions of these genes contain extended G-box motifs to which SPT is able to bind. The link between SPT and shade avoidance was confirmed by the complete restoration of wild-type gynoecium development to spt mutants, either by growing these under simulated vegetation shade conditions, or by inactivating the phytochrome B (phyB) photoreceptor, which is a negative regulator of shade avoidance responses. These data indicate the target genes of SPT to participate in a developmental module that mediates both gynoecium development and shade avoidance. The dual functions of this module may reflect a mode of evolution in which a pre-existing developmental pathway was recruited to a novel role through an evolutionary change in an upstream regulatory component."
(a) ENS LYON laboratoire RDP

P56011 Identification of Pollen Tube Attractants derived from the Synergid Cell
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"For more than 140 years, pollen tube guidance in flowering plants has been thought to be mediated by chemo-attractants derived from target ovules. However, there has been no convincing evidence of any particular molecule being the true attractant that actually controls the navigation of pollen tubes towards ovules. Here we report first identification of pollen tube attractants derived from the synergid cell on the side of the egg cell (Okuda et al., Nature, in press). Our group developed the in vitro Torenia system, whereby pollen tubes growing through a cut style were attracted to a protruding embryo sac (Higashiyama et al., Plant Cell, 1998). By using this system and laser cell ablation technique, the synergid cell was shown to emit some diffusible attractant(s) (Higashiyama et al., Science, 2001). The attractant molecule was species preferential even in closely relating species, implying that the molecule had rapidly evolved (Higashiyama et al., Plant Physiol., 2006). Thus, we investigated genes expressed in the synergid cell of Torenia, by collecting isolated synergid cells. We finally identified attractant proteins derived from the synergid cell. We will present in this talk detailed properties of attractants molecules."
(a) Graduate School of Science, Nagoya University (b) Presto, JST (c) Cell Biology/Plant Physiology, University of Regensburg

P56012 "Temporal and spatial mapping of reproductive barriers in the developing pistils of *Solanum pennellii*, a tomato wild species"
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"Many wild species in the tomato clade show both sympatric and allopatric distributions and possess both self-incompatibility (SI) & unilateral incongruity (UI) barriers. Thus, this group offers a useful model system to analyze the molecular mechanisms & evolution of mating barriers in plants. Using a semi-*in vitro* system to track pollen tube growth in styles, we have successfully determined the developmental timing and stylar location of both SI & UI barriers in three accessions of *S. pennellii*. Two of the accessions, LA2560 & LA1340 are self-incompatible, whereas LA0716 is self-compatible. However, all the three accessions reject pollen unilaterally from *Solanum lycopersicum*, the domesticated tomato. Our studies demonstrate that UI is developmentally regulated. Further, early stages of developing pistils (i.e., 5 days before bud break) in all three accessions lack these barriers and allow the growth of pollen tubes that are normally rejected by fully-developed pistils, thus indicating that UI machinery is fully activate only in the later stages of development. Further, SI barriers appear on day -4 while UI seems to be operative a day later (day -3). As found in our earlier in vivo analysis, UI barriers seem to function in or near the stigma (i.e., within the top 20% of the style), where as SI barriers act deeper in the style (~below 40% of the style). We are also tracking the appearance of proteins known to be involved in SI, particularly the S-RNases and HT-proteins. Early results indicate that the appearance of HT proteins coincides with the activation of mating barriers in the developing pistil. We are further leveraging the information by proteome and transcriptome profiling to identify the novel components of molecular machinery associated with SI & UI. "
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P56013 Molecular genetics of floral symmetry in *Dendrobium* orchids
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"Orchid floral symmetry is determined by the presence of a highly modified dorsal petal called the lip (labellum) and a column, a structure made by the fusion of the stamen, style and the stigma. The main goal of our research is to isolate and characterize the key genes involved in determining the floral symmetry of orchids, one of the most species-rich plant families displaying obligatory zygomorphy (bilateral symmetry). Our research is focused on a peloric mutant, *Dendrobium* Ethel Kamemoto hybrid, in which the lip is replaced by a normal petal which changes the perianth symmetry from bilateral to radial. Classical breeding data showed that this peloric trait is due to a single, recessive, loss of function, mutation. The floral symmetry in *Phalaenopsis* orchid is suggested to be regulated by the differential expression of *DEFICIENCE*-like (*DEF*-like) MADS box genes while that of several eudicot plant families was shown to be regulated by TCP transcription factors. We have isolated three *DEF*-like MADS box genes from *D*. Ethel Kamemoto as opposed to the four genes isolated from *Phalaenopsis*. The expression profile of one key gene, *DkMADS4*, contradicts that of Phalaenopsis *PeMADS4* gene, the suggested regulatory gene of lip formation. Therefore, the simple model for orchid floral symmetry based on *Phalaenopsis* MADS box gene expression might not be universally applicable to all orchids. We also have identified two TCP genes, *Den-TCP-1* and *Den-TCP-2*, differentially expressed between the normal and peloric sibling lines. Expression profile of *Den-TCP-1* suggests it is preferentially expressed in the dorsal region of the young floral buds. We will discuss the possible role of TCP genes and/or MADS box genes in the evolution of orchid floral symmetry."
(a) University of Dubuque (b) University of Khon Kaen (c) University of Hawaii at Manoa

P56014 The tomato early fruit specific small MYB/SANT protein SlFSM1 restricts excess cell expansion: A possible role in coordination between dividing and expanding cells via interaction with SlFSB1 a novel nuclear protein
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"The mechanisms dictating early tomato development post fertilization to be driven concurrently by cell-division and cell expansion, before transition to the cell-expansion growth phase is poorly understood. We have isolated and characterized the gene *SlFSM1* (*Fruit SANT/MYB-like1*) harboring a single SANT/MYB-like domain, which is expressed specifically during the very early stages of tomato fruit development (Planta 221:197). Histological analyses of tomato plants with low ectopic over-expression of *SlFSM1* demonstrated that it leads to significant reduction in the size of the cells in all the determinate organs tested including the pericarp of mature fruits, compared to WT fruits of similar size. The Arabidopsis gene *At2g21650* encodes for an ortholog of *SlFSM1*. Yeast-2-Hybrid analysis, supported by pull-down of in vitro transcribed and translated proteins, indicated that its partners are two homologous proteins, At1g10820 and At1g68160, belonging to a novel small plant specific gene family, of unknown function, containing a MYB/homeodomain-like domain. We have cloned their tomato ortholog and designated it *SlFSB1* (*Fruit SANT/MYB1 Binding protein1*). FSB1-GFP fusion protein is strictly nuclear localized when agroinfiltrated on its own, yet when co-agroinfiltrated with its partner SlFSM1, fluorescence is observed also in the cytoplasm, indicating that FSM1 inhibits its shuttle to the nucleus. Based on SlFSM1 functioning as a suppressor of cell expansion, its interaction with the nuclear protein SlFSB1, and the stage specificity of its expression, the role of these two proteins in the coordination between dividing and neighboring expanding cells during early fruit development, and possibly in the transition to the cell expansion growth stage will be discussed. "
(a) The Volcani center, ARO (b) Ohio State University,Columbus, OH

P56015 "Arabidopsis *CELL WALL INVERTASE 4* (*AtCWINV4*, At2g36190) is required for nectar production"
Ruhlmannn, Jeffrey M (a,b) Kram, Brian W-presenter bkram@d.umn.edu(a) Carter, Clay J (a)
http://www.d.umn.edu/~cjcarter/carterlab.html
"To date, no genes have been reported to directly affect the de novo production of floral nectar. To identify genes involved in nectar production, we previously used the Affymetrix ATH1 microarray to examine global expression patterns in Arabidopsis nectaries. One of the genes displaying highly enriched expression in nectaries was *CELL WALL INVERTASE 4* (*AtCWINV4*, At2g36190), which catalyzes the hydrolysis of sucrose into glucose and fructose. RT-PCR was used to verify the nectary-enriched expression of *AtCWINV4*, as well as an ortholog from *Brassica rapa*. To probe biological function, two independent Arabidopsis T-DNA *cwinv4* null mutants were isolated. Unlike wild-type plants, *cwinv4* lines did not produce nectar. While overall nectary morphology appeared to be normal, *cwinv4* lines accumulated higher than normal levels of starch in the receptacle, but not within the nectaries themselves. Cell wall extracts were prepared from mutant flowers and displayed greatly reduced invertase activities when compared to wild-type plants. It is proposed that AtCWINV4 is responsible for maintaining a high intracellular:extracellular sucrose gradient, and is required for the generation of the hexose-rich nectar observed in Arabidopsis and related *Brassica* flowers."
(a) University Of Minnesota Duluth (b) University of Minnesota-Twin Cities

P56016 Arabidopsis inositol polyphosphate 6-/3-kinase gene (*AtIpk2β*) is involved in flowering regulation through the photoperiod pathway
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"Flowering is the developmental turning point from the vegetative to the reproductive phase and is regulated by a combination of endogenous controls and environmental cues. Phosphatidylinositol (PI) signaling pathway and the relevant metabolites are crucial for the modulation of plant growth, development and stress responses. Although the importance of phosphoinositides as lipid signaling molecules in eukaryotic cells has long been explored, their function in flowering regulation has never been reported. In this study, we firstly reported a novel role for Arabidopsis inositol polyphosphate 6-/3-kinase gene (*AtIpk2β*), which encodes a key enzyme in PI metabolism, in flowering regulation. We provided evidence that *AtIpk2β* modulates flowering through the photopheriod pathway. The T-DNA insertional knockout mutant, *ipk2β*, showed earlier flowering phenotype than wild type under both long-day (LD) and short-day (SD) conditions. In contrast, transgenic plants overexpressing *AtIpk2β* delayed flowering. Consistent with this finding, the expression of some key genes in flowering pathways was changed in *ipk2β* mutant. However, no transcript level alteration of some circadian-related gene was observed. In addition, our further experiments also demonstrated that *AtIpk2β* is not involved in flowering promoted by vernalization or GA. Taken together, our results suggest an important role for *AtIpk2β* in flowering regulation most likely through the photoperiod pathway."
(a) Wuhan University

P56017 NtGNL1 plays a essential role in pollen rube growth and orientation via the regulation of post-Golgi trafficking
Sun, Mengxiang-presenter mxsun@whu.edu.cn(a) liao, fanglei (a)
"Tobacco GNOM LIKE 1 (NtGNL1) is a new member of Big /GBF family,which contain a sec 7 domain. We proposed it might have a function in regulating pollen tube growth concerning vesicle trafficking. To elucidate this, we applied RNAi combined with antisense oligodeoxynucleotide inhibition techniques to down regulate NtGNL1 expression and found that pollen tube growth and orientation were obviously inhibited. Cytological observation revealed that both timing and behavior of endosytosis was disrupted and endosome trafficking to Prevacuolar Compartments and Mutivesicular Bodies was greatly altered in pollen tube tips. We further demonstrated that in such pollen tubes Golgi apparatus disassembled and fused with endoplasmic reticulum. During this process, ARA7 and other early endosome trafficking-related genes were also down regulated. Thus, we

revealed that NtGNL1 is essential for pollen tube growth and orientation via stabilizing the structure of Golgi apparatus and ensuring endocytosis and secretary pathway, which is defined as post-Golgi trafficking. The result present here also illustrated that naked antisense ODN is a reliable and convenient assay for gene function analysis in pollen tube."
(a) Wuhan University

P56018 Identification of TARGET GENES of the OVULE IDENTITY COMPLEX in ARABIDOPSIS
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"In Arabidopsis, ovule identity is controlled by the action of the MADS-box genes SEEDSTICK (STK), SHATTERPROOF1 (SHP1), SHP2 and AGAMOUS (AG) .Among these genes, STK is specifically expressed in the ovule while the SHP and AG genes are also expressed in the developing carpel. Protein interaction experiments demonstrated that the ovule identity factors assemble in protein complexes in the presence of SEPALLATA (SEP) MADS-box proteins. In order to better elucidate the molecular mechanisms controlling ovule development we are interested in the identification and characterization of genes regulated by the ovule identity MADS-box protein complex. Arabidopsis ovule primordia were isolated using the Laser Microdissection System, .RNA was extracted, amplified and used to hybridise Affymetrix microarrays. This analysis led to the identification of a set of transcription factors that are expressed during early stages of ovule development. This gene set was analysed for the presence of multiple MADS-box binding sites in their putative regulatory regions. A subset of genes that were positive in this bioinformatics screen were analysed by Chromatin Immunoprecipitation (ChIP) experiments using an antibody for STK. This allowed to identify two REM genes as direct targets of the ovule identity factor STK. Expression analysis showed that these genes are developmentally regulated and broadly expressed. However in situ hybridisation using developing flowers revealed that in the stk shp1 shp2 triple mutant REM 37 is not expressed in the developing ovules. We will present the characterization of the rem37 mutant. Furthermore the identification of a genome wide set of STK targets using a ChIP-sequencing approach will be discussed. "
(a) Universita' di Milano Dipartimento di Biologia (b) Universita' di Milano Dipartimento di Scienze Biomolecolari (c) University of Zurich

P56020 The role of gibberellins on ovary expansion in fertilized and unfertilized grape berries
Dauelsberg, Patricia-presenter pvdauels@puc.cl(a,b) Medina, Consuelo (a) Matus, Tomas (a) Arce-Johnson, Patricio (a)
" Grapevine cultivation represents 13% of fruit production worldwide. The fruit market is increasingly demanding specific features in berry morphology and composition, such as berry size, absence of seeds and color uniformity, among others. Gibberellins participate in many of these processes. In this work, the expansion of 'Red Globe' ovaries and the initial stages of berry development were studied in relation to the fertilization event. Ten grape flower bunches were emasculated before cap-fall (fused petal dehiscence). Five were pollinated manually while the others were left unpollinated. After the emasculation event (0DAE), samples were taken every day until 29DAE. Ovary size and the expression of fruit set-related genes, which included auxin, gibberellin and cytokinin synthesis and signal transduction genes, were analyzed. Ovary lengths were significantly higher at 13 and 29DAE when compared to unpollinized/unfertilized ovaries. At 13DAE, the expression of VvGA20ox, VvIPT and VvTRIP were higher in fertilized berries, while the expression of putative repression targets of gibberellin action (e.g. MADboxTM6) was higher in unfertilized fruits. We suggest that upon grape berry fertilization, gibberellins could repress genes which negatively regulate the size of the ovary. Acknowledgements: This work was supported by Chilean Fruit Consortium and 07 Genoma 01 Proyect. "
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P56021 Fleshy fruit expansion and ripening are regulated by the tomato MADS-box transcription factor TAGL1
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"The maturation and ripening of fleshy fruits is a coordinated developmental program designed to synchronize seed maturation with the metabolic changes necessary to render fruit tissues desirable to seed dispersing organisms. Many fruits employ ethylene as a signal to regulate this process though the molecular basis of ethylene regulation during ripening is not fully understood. In an effort to identify novel regulators acting prior to ethylene during ripening we have mined expression profiling data to identify transcription factors associated with ripening. *TAGL1* (tomato *AGAMOUS-LIKE1*) is a tomato MADS-box gene expressed throughout carpel development and up-regulated in association with fruit maturation. Using RNAi repression we show that *TAGL1* is necessary for the onset and completion of ripening. Tomato plants with reduced *TAGL1* mRNA produced yellow-orange fruit with reduced carotenoid content. These fruit also evolved less than 20% of normal ethylene levels during maturation indicating a comprehensive inhibition of ripening mediated through *ACS2*. In addition to ripening inhibition, *TAGL1* RNAi fruit were characterized by a thin pericarp suggesting an additional function in carpel expansion and fruit fleshiness, likely through promotion of cell division during early development. Analysis of tomato lines harboring wild-species alleles of *TAGL1* further supports a role in both fleshy fruit development and ripening."
(a) Boyce Thompson Institute/ USDA-ARS

P56022 Floral Initiation Process in Soybean (*Glycine max*)
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"The transition to flowering is characterized by a shift of the shoot apical meristem (SAM) from leaf production to the initiation of a floral meristem. This is a major event in a plant's life that has to be precisely timed to ensure reproductive success. Though this process is of extreme importance for agriculture and breeding, there have only been limited studies on floral initiation in legume species, especially so for the agriculturally and economically important soybean. This study aims to characterize the molecular events leading to floral transition in soybean SAM. Our approach involves the use of Affymetrix GeneChip Soybean Array containing probe sets for 37,500 sequences to obtain the transcript profiles of SAM during floral transition. To this end, we have isolated RNA from dissected soybean SAM at various time points after plants were shifted from non-flowering to flowering-inducing growth conditions. Analysis of the resulting microarray data revealed a total of 331 transcripts that have differential expression profiles during the time points investigated. In silico and RT-PCR analysis on differentially regulated transcripts implies the intriguing involvement of hormones including abscisic acid in events prior to the induction of floral homeotic transcripts. Our finding suggests that molecular events mediated by multiple hormonal pathways are part of the mechanism employed by soybean to regulate the floral transition process."
(a) University of Melbourne

P56023 Functional analysis of genes that control rice panicle branching
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" Transition of meristem identity is a major determinant of the inflorescence architecture. We identified a dominant mutant allele of *ABERRANT PANICLE ORGANIZATION 1* (*APO1*), *apo1-D3*. *apo1-D3* contains an increased number of rachis branches and spikelets, suggesting that the transition

to spikelet meristem identity is delayed in *apo1-D3*. *APO1* expression level is increased in *apo1-D3* due to the insertion of an active transposon in the promoter region of *APO1*. The negative effect of *APO1* on the transition to spikelet meristem identity was further confirmed by the *APO1* over-expression experiment. The inflorescence meristem of *apo1-D3* was enlarged compared with that of wild type while the inflorescence meristem in *apo1* is smaller than that of wild type. These results indicate a possibility that the expression level of *APO1* determines the panicle size through controlling cell proliferation in the inflorescence meristem. *APO1* is an ortholog of *Arabidopsis UNUSUAL FLORAL ORGANS* (*UFO*) that controls floral meristem identity together with *LEAFY* (*LFY*) and *TERMINAL FLOWER 1* (*TFL1*). Yeast two-hybrid assay showed that APO1 directly interacts with RFL, a rice ortholog of LFY. Over-expression of *RCN1*, a rice *TFL1* ortholog, also conferred the increase in panicle branching, however, the effect of *RCN* over-expression was abolished in the absence of *APO1* function. This indicates that *APO1* works downstream of *RCN2*. Based on these results, we propose a genetic pathway controlling branching in rice panicle. "
(a) Graduate School of Agriculture and Life Sciences, The University of Tokyo (b) Research Institute for Bioresources, Okayama University (c) National Institute for Basic Biology

P56024 "*RES1*, a member of SEP family MADS-box gene, determines spikelet identity in rice"
Kobayashi, Kaoru-presenter aa087003@mail.ecc.u-tokyo.ac.jp(a) Maekawa, Masahiko (b) Kyozuka, Junko (a)
"Grass species have a unique inflorescence structure called a spikelet, a unit that contains a various number of florets. The SAM initiates several axillary meristems that grow as primary branches, and the primary branch meristem generates secondary branches and spikelets. The timing when meristems acquire the spikelet identity determines the basic structure of a rice panicle. To reveal the mechanism of spikelet identity determination, we analyzed *retarded spikelet 1* (*res1*) mutant. The *res1* mutant has highly branched panicles. The number of axillary meristems generated on each branch in *res1* is comparable to wild type, while meristems produced in *res1* tend to grow as next order branches rather than spikelets. Abnormal spikelet development indicating partial conversion to the spikelet meristem identity was also observed in *res1*. Empty glumes and rudimentary glumes are longer than that of wild type and an ectopic organ is generated on the axil of a rudimentary glume where organs are not generated in wild type. These observations suggest that *RES1* is a positive regulator of the spikelet identity in rice. Map based cloning of *RES1* and function of *RES1* related gene will be discussed."
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P56025 Auxin reduction is a primary cause of high temperature injury to male reproductive development
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http://www.lifesci.tohoku.ac.jp/english/environmental/laboratory_genomic.html
"Plant high-temperature injury has become a serious problem associated with global warming. The early phase of anther development is especially susceptible to high temperature injury in wheat, barley and other crops. Here, we have found that high temperature conditions showed a high correlation coefficiency with transcriptional repression of replication-related and auxin-induced genes, and resulted in decreased endogenous auxin levels in developing barley anthers. Consistent with this observation, the application of auxin to barley completely reversed high-temperature-induced male sterility. Similar reductions in auxin levels occurred in the developing anther cells of Arabidopsis exposed to increased temperatures. These data suggest that tissue-specific auxin reduction is a primary cause of high temperature injury, which leads to the abortion of pollen development and moreover suggests a novel use for auxin treatment to sustain steady yields of crops under conditions of climate change."
(a) Graduate School of Life Sciences, Tohoku University (b) Meiji University

P56026 Deletion analysis of *VERNALIZATION INSENSITIVE 3* promoter
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"Vernalization, a long term cold induced acceleration of flowering, is one of the major environmental factors to determine the timing of flowering in plants. In Arabidopsis, mitotically stable repression of floral repressor *FLOWERING LOCUS C (FLC)* is a crucial mechanism for the vernalization-induced flowering. Transcriptionally activated PHD finger domain protein VERNALIZATION INSENSITIVE 3 (VIN3) is known to induce the repression of *FLC* chromatin. Different to cold-induced genes, whose expressions are increased in a short period of cold, a transcription of *VIN3* is slowly activated according to the length of cold period. Despite of intensive research, the question how plants can distinguish winter signal from cold signal are still elusive. To elucidate this, we have generated transgenic lines of series of *VIN3* promoter with GUS fusion. Promoter includes 5' UTR and 1st intron. Our results show that the length of promoter is loosely related to the strength of signal, and -150bp of promoter and 1st intron sequences are sufficient to induce *VIN3* by vernalization treatment. Interestingly, in the absence of intron, GUS expression is greatly reduced in both vernalization and non-vernalization condition. But 1st intron itself has not shown vernalization response. In conclusion, we found that 150 bp promoter is sufficient for vernalization-induced *VIN3* expression and 1st intron does not have vernalization-responsive *cis*-elements, but instead, has a role for transcriptional enhancement in *VIN3* expression."
(a) National Research Laboratory of Plant developmental genetics

P56027 Genetic characterization of self-compatible mutants in *Brassica rapa*.
Suwabe, Keita-presenter suwabe@bio.mie-u.ac.jp(a,b) Sachiyo, Isokawa (b) Akira, Shirasawa (b) Yoshinobu, Takada (b) Go, Suzuki (c) Akira, Isogai (d) Seiji, Takayama (d) Masao, Watanabe (b,e)
" Most flowering plants have hermaphrodite flowers and male and female reproductive organs co-exist in the same flower. Because of such structural morphology, self-pollination which leads a loss of genetic diversity can be accelerated. To prevent this, *Brassica* species have self-incompatibility (SI) system to reject self-pollen, which consequently promote an outcrossing. The SI system in *Brassica* is primarily controlled by a multi-allelic *S* locus which includes two tightly linked genes, *SRK* (the female determinant) and *SP11/SCR* (the male determinant). However, little is known about downstream factors of signal transduction and a mechanism of self-pollen rejection. The objective of this study is to identify novel factors related to SI, by genetic analysis of self-compatibility (SC) mutants in *Brassica rapa*. We isolated five SC mutant lines, LVC28, LVC17, K28, K4 and K2. In the SC mutants, *SRK* and three known downstream factors, *MLPK, ARC1* and *THL*, expressed normally, and *SP11* also expressed in all lines except for LVC17, suggesting that all known SI factors are functional in four SC lines, at least. In genetic analysis of F_2 population between LVC28 and SI line, the segregation ratio of SI to SC was 5 to 1. In this population, genotype of *S* locus did not co-segregate with the SC phenotype. This result suggests that more than one SI-related gene have been mutated and are independent from *S* locus in LVC28. "
(a) Mie University (b) Graduate School of Life Scinces, Tohoku University (c) Osaka Kyoiku University (d) Nara Institute of Science and Technology (e) Faculty of Science, Tohoku University

P56028 *SNB* and *OSIDS1* are required for floral meristem identity and inflorescence meristem cell fate in rice.
Lee, Dong-Yeon-presenter noey76@postech.ac.kr(a) An, Gynheung (a)

"Grasses have complex inflorescence architecture with specific types of secondary meristem. Inflorescence architecture is largely dependent on activity of secondary meristem. We previously reported that the *SUPERNUMERARY BRACT (SNB)* regulated transition of spikelet meristem into floral meristem. Here we show that *SNB* and *OSINDETERMINATE SPIKELET1(OsIDS1)*, *APETALA2*-like gene, play a multiple roles in inflorescence architecture and floral meristem establishment. In double mutants between *snb* and *osids1*, transition of floral meristem was more delayed than *snb* and number of branches and spikelets were significantly reduced. These phenotypes were more prominent in long-day conditions than short-day conditions. Phenotypic analyses and expression analyses identified that *SNB* and *OSIDS1* positively regulate Class B, C, and E function floral organ identity genes and negatively regulate *FRIZZY PANICLE (FZP)* gene. These results suggest that *SNB* and *OsIDS1* are required in the spikelet meristem to both promote floral meristem development and inflorescence meristem to suppress the precocious determination of inflorescence meristems to spikelelt meristem. Furthermore, we show that overexpression of rice *microRNA172*s caused early flowering and *snb osids1* phenotypes in panicle and spikelets. *miR172* overexpression caused the reduction in level of *SNB* and *OSIDS1* transcipts, suggesting that *miR172*s downregulate these target genes by transcriptional degradation in rice"
(a) Pohang university of science and technology

P56029 Anther-specific and nonspecific transcriptional regulation of male reproductive organ from transcriptome analysis of *Arabidopsis* anther mutants
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"In flowering plants, the development of male reproductive organs is controlled precisely to achieve successful fertilization. Despite the increasing knowledge of genes that contribute to anther development, the regulatory mechanisms controlling this process is still unclear. In this study, we analyzed the transcriptome profiles from early anthers of two male sterile mutants, *dysfunctional tapetum1* (*dyt1*) and *aborted microspores* (*ams*) using ATH1 array. 2879 genes were differentially expressed in *dyt1* and/or *ams*, especially those involved in transport, catabolism and biosynthesis. Combining transcriptome profiles from *dyt1* and *ams* with those of two other sterile mutants, *spl/ nzz* and *ems1/ exs*, expression of about 4000 genes were changed in at least one mutant. In addition, co-expression patterns were revealed by hierarchical heat-map and K-mean clustering. Further investigation of expression pattern of major transcription factor families, such as *bHLH*, *MYB* and *MADS*, suggested that closely related homologs might have either redundant or divergent functions. Additionally, comparison of expression levels of genes encoding transcription factors between different organs showed that both anther-specific and nonspecific transcription factors could play important roles in anther development. Moreover, consensus of known binding sites found in the promoters of differentially expressed transcription factors implied a more complex regulatory system controlling anther development and function. We proposed an expanded regulatory network for early anther development, providing a series of hypotheses for future experimentation. "
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P56030 *VRN-D4* is a vernalization gene located in the centromeric region of wheat chromosome 5D
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"Wheat growth habit is mainly controlled by four loci, *VRN1*, *VRN2*, *VRN3*, and *VRN4*. The first three loci have already been cloned, and the fourth one has been assigned to wheat chromosome 5D. Triple Dirk F (TDF) is an isogenic stock that carries the *Vrn-D4* allele for dominant spring growth and has recessive *vrn-A1*, *vrn-B1*, and *vrn-D1* alleles. Here we report the genetic mapping of the *VRN- D4* locus using 159 F2 plants from the cross TDF x *Ae. tauschii* chromosome 5D (Synthetic 5402) substitution line in Chinese Spring. *VRN-D4* was mapped to a 9 cM centromeric region on chromosome 5D between markers *CFD81* and *BARC143* and completely linked to markers *CFD67, WMC318, GDM68, GDM3,* and *BARC205*. Using an additional mapping population from the cross TDF x Hayakomugi *VRN-D4* was mapped to a shorter 1.8 cM interval between *CFD78* and *BARC205*. This last population was less polymorphic than the Synthetic 5402 population but showed higher recombination. The *VRN4* locus is completely linked to the short arm marker *BG313707* and the long arm marker *CFD67*, so the arm location of *VRN-D4* is still unknown. To test the interaction between the *VRN-D4* alleles and vernalization we selected 20 plants homozygous for the *Vrn-D4* allele from TDF and 20 homozygous for the *vrn-D4* allele from Hayakomugi, and vernalized half of the plants. A 2x2 factorial ANOVA showed significant differences in heading time between vernalization treatments (P= 0.004) and between *VRN-D4* alleles (P<0.0001), and more importantly, a significant interaction (P<0.0001) between vernalization and *VRN-D4* alleles. Unvernalized plants showed significantly larger differences in heading time between *VRN-D4* alleles (35 days) than vernalized plants (10 days), confirming that *VRN-D4* interacts with the vernalization pathway."
(a) University of California, Davis. Dept. Plant Sciences (b) China Agricultural University. Dept. of Plant Genetics and Breeding, (c) Okayama University. Graduate School of Natural Science & Tech.

P56031 The diversity of SRK and SCR/SP11 self-incompatibility genes in Australian populations of *Raphanus raphanistrum* (Brassicaceae)
Koh, Joshua-presenter j.koh8@pgrad.unimelb.edu.au(a) Hoebee, Susan E. (b) Young, Andrew G. (c) Griffin, Pip (a) Newbigin, Ed (a)
"*Raphanus raphanistrum*(wild radish) is a self-incompatible species and a major weed across Australia's southern cropping zone, costing an estimated $210 million per year in yield loss and control measures. Rapid and recurrent evolution of multiple herbicide resistance is making control of this aggressive weed difficult. Because this annual plant grows from seed each year, one suggested control approach is to block seed production by activating the self-incompatibility response. As a first step to examine the feasibility of this approach, we estimated the number and distribution of S-alleles in natural populations of *R. raphanistrum* in Australia using a combination of diallel crosses and PCR amplification of the kinase domain of the female determinant, S-locus receptor kinase (SRK). We identified and sequenced 30 putative S-alleles from 118 individuals sampled from across southern Australia. In diallels, approximately 30 ~ 40% of crosses within a population were incompatible, suggesting that shared S-alleles are limiting mate opportunities. In addition, the S-haplotypes of individuals from one Western Australian population (ML-8) were determined by sequencing the extracellular domain (S-domain) of SRK and the entire region of the pollen determinant, SP11/SCR. Further characterization of these genes is presented and the results are discussed in the context of approaches to wild radish control. "
(a) School of Botany, University of Melbourne (b) Department of Botany, LaTrobe University (c) Biodiversity and Sustainable Production, CSIRO Plant Industry

P56032 Unilateral Incompatibility in *Brassica rapa* lead to a novel pollen-stigma recognition mechanism.
Takada, Yoshinobu-presenter ytakada@ige.tohoku.ac.jp(a) Suzuki , Go (b) Shiba, Hiroshi (c) Takayama, Seiji (c) Isogai, Akira (c) Watanabe, Masao (a)
"Plants have evolved many systems to prevent irrelevant fertilization. Among them, incompatibility is well-organized system, in which pollen

germination or pollen tube growth is inhibited in pistils. Self-incompatibility (SI), rejecting self-pollen, promotes outbreeding in flowering plants. On the contrary, inter-species incompatibility, preventing gene flow among species to restrict outbreeding, usually occurs unilaterally, and is known as unilateral incompatibility (UI). In Brassicaceae, little is known molecular mechanism of UI, although the *S*-locus genes have been characterized to know how to recognize the self-pollen in the SI system. In the present study, we characterized novel UI observed between the same species, *Brassica rapa*; pollen of Turkish SI lines was specifically rejected in pistil of Japanese commercial SI variety Osome. Incompatible phenotype of this intra-species UI was closely resembled with those of SI. The segregation analysis revealed that this UI recognition is regulated by single pollen and stigma factor each other, furthermore, both of this UI factors were not linked to the *S*-locus. "
(a) Tohoku University (b) Osaka Kyoiku University (c) Nara Institute of Science and Technology

P56033 Molecular genetics studies on the development of the spikelet in rice.
Yoshida, Akiko -presenter ayoshida@biol.s.u-tokyo.ac.jp(a) Suzaki, Takuya (a) Takamure, Itsuro (b) Hirano, Hiro-Yuki (a)
"Morphologies of flowers and inflorescences in monocots are distinct from those of flowers and inflorescences in eudicots. Especially in grasses, the inflorescences comprise of unique structural units, the spikelet and the floret. The rice spikelet has one floret, which is subtended by two pairs of bracts called sterile lemma and rudimentary glume. Floral organs such as stamens and carpels are formed in the floret. By contrast, it remains unclear what genes are involved in the development of the organs specific to the spikelet. In this study, we are focusing on a rice mutant which has longer sterile lemma. Phenotypic analysis suggests that the long sterile lemma in this mutant has the identity of the lemma. The sterile lemma is thought to be a rudimentary organ of two lateral florets, which have been degenerated during rice evolution. Here, we report the developmental mechanism of the sterile lemma to elucidate morphological evolution of rice spikelet."
(a) The University of Tokyo (b) Hokkaido University

P56034 Proteomic Study on ovules of female-sterile clone of Chinese Pine (*Pinus tabulaeformis*Carr.)
zheng, caixia-presenter zhengcx@bjfu.edu.cn(a) bao, renyan (a)
"Pervious study indicated that the female gametophyte stop forming due to the failure of the mitosis of free nuclei after the winter dormancy, causing ovule abortion in Pinus tabulaeformis Carr. clone 28. Ovule proteins during the key period of the mitosis of free nuclei were analyzed with 2-D electrophoresis. The result showed that in the early stage of failure there were about 800 protein spots in normal clone and 700 spots in female-sterile clone, while in the late stage there were about 1000 and 900 protein spots respectively. There were 20 proteins expressed only in normal clone and 13 proteins expressed only in female-sterile clone. 25 proteins were expressed but with different levels in normal clone and female-sterile clone. These differently expressed proteins maybe involved in the female gametophyte development, and the female-sterile clone may lost some proteins which are necessary for the gametophyte development . Nine special proteins were analyzed by peptide mass fingerprinting. Five of them were identified and they are H+ -transporting ATPase beta-1 chain, chaperonin, MADS protein and BEL1-like homeodomain protein respectively. MADS protein and BEL1-like homeodomain protein are transcription factors important for the regulation of ovule development in angiosperm. Our result suggested that they were also required for the ovule development in gymnosperm. The level of chaperonin affects plant growth and development. Plant HSP 60 was found mostly in chloroplasts and mitochondria. In this study, HSP 60 was found to be present only in normal clone and absent in female-sterile clone. Thus, the abortion of ovule in Pinus tabulaeformis Carr. clone 28 could be due to the absence of HSP60 and malfunction of mitochondria. "
(a) department of plant science,college of biological sciences and biotechnology,beijing forestry university

P56035 A tale of two orchids: comparative reproductive development in *Vanilla* and *Phalaenopsis*
O'Neill, Sharman D-presenter sdoneill@ucdavis.edu(a) Wang, Yin-Tung (b,c) Gonsalves, Dennis (d,e) Matsumoto, Tracie (d,e)
"The orchid family of flowering plants (Orchidaceae) represents the largest, most diverse and successful family of flowering plants yet they are an understudied group from a molecular and genomic perspective. To further our longer-term goal of developing enabling genomic resources for key phylogenetic taxa within the Orchidaceae, we initiated fundamental and foundational studies of flower and fruit development in two phylogenetically distant orchid taxa: *Vanilla planifolia* Jackson (Vanilloideae) and *Phalaenopsis* Sogo Yokidian V3 (Epidendroideae), both economically important horticultural crop plants. *Vanilla planifolia* is an emerging tropical fruit crop for Hawaiian agriculture. Although *V. planifolia* produces a fruit of major economic importance as a spice crop and is of critical importance for generating basic subsistence income in many food-deficient, developing nations, there have been no recent studies of its reproductive development. Nor has there been a comparable investigation of flower development in the moth orchid, *Phalaenopsis* Sogo Yokidian V3, one of the most elite and valuable cultivars of ornamental orchids. Here we report our strategy for the development of genomic resources for *Vanilla planifolia* to characterize this relatively large and unexplored orchid genome. In addition, we present a comparative developmental study of gynostemium development in both orchids, with a specific focus on stigma and rostellum development that represent key floral modifications important for initial pollen signaling, pollination, and post-pollination development of the ovary, ovule and fruit."
(a) University of California, Davis (b) Matsui Nursery (c) Texas A&M University (d) USDA-ARS Pacific Basin Agricultural Research Center (e) Tropical Plant Genetic Resources and Disease Research Unit

SESSION P57 - RHIZOSPHERE

P57001 New insights into environmental rhizosphere biology
Schenk, Peer M-presenter p.schenk@uq.edu.au(a) Thomas-Hall, Skye R (a) Kidd, Brendan (a,b) Sintrajaya, Regina (a) Schmidt, Susanne (a) Kazan, Kemal (b) Manners, John (b) McGrath, Ken (a) Scalia, Gerard (a) Omega, Maria (a) Rhiannon, Mondav (a) Husma, Hussein (a) Wong, Chin Lin (a) Choo, Jin Hsien (a) Vlaardingerbroek, Ido (a) Kobe, Bostjan (a)
"Plants in their natural environment are associated with soil microbial communities potentially comprising millions of species. Using custom-built environmental functional gene arrays and next generation 454 sequencing of expressed mRNA, we have used a meta-transcriptomics approach to simultaneously study the *in situ* interactions of plant roots and microbes in the rhizosphere. The obtained microbial activity profiles and their deduced biochemical pathways are strongly influenced by plant defense responses (including root exudates) and several abiotic factors (e.g. fertilizer applications leading to N_2O emissions). Two examples of plant defense responses in the rhizosphere will be presented in more detail: First, a lectin protein family from Arabidopsis directly influences microbial composition and confers resistance against pathogenic bacteria and root-knot nematodes. These highly abundant proteins form a protective barrier in the cell wall and act similarly like antibodies, by binding as dimers to carbohydrates on bacterial cells and nematode gut lining, causing effective immobilization. Second, the Mediator complex providing the link between transcription factors and RNA Polymerase II has been found to play a key regulatory role for disease resistance in the root. Individual Mediator subunits confer resistance to the soil-borne fungus *Fusarium oxysporum* and models of the Fusarium-Arabidopsis interactions will be discussed."
(a) The University of Queensland (b) CSIRO Plant Industry

P57002 A unique biotechnological tool for improving corn yield in organic farming

Yesmin, Laila-presenter Laila.Yesmin@brettyoung.ca(a) Banerjee, Manas (a)

"Organic farming is one of the valuable sectors in agriculture and use of biologicals is a common practice in organic farming to increase nutrient uptake or to control diseases. Different types of nitrogen fixing microbes such as *Rhizobium*, *Azotobacter*, *Azospirillum* and Blue Green Algae (BGA) have long been used in organic farming to supply nitrogen demand of many agricultural crops. In recent years the demand for organic produce has grown at an enormous speed in many countries. To meet the demand of the sector and to increase productivity, search for new biological is getting widespread attention. Nevertheless, new research to increase the productivity in the organic sector remains neglected and not many new biological has been introduced. Even if a number of microorganisms reside in the rhizosphere are known to have beneficial effect on plant health and growth but isolation and formulation for targeted crop takes time & resources. In the present investigation, a unique sulfur (S)-oxidizing plant growth promoting rhizobacteria (PGPR), identified as *Achromobacter piechaudii* RAY12 was used as corn growth promoter in the organic farming system. Corn (*Zea mays*) treated with bacteria RAY12 showed to improve corn performance & yield with high quality cob & kernels. These field trials were conducted in the organic farms in corn belts of USA in two consecutive years. Hence, this new biotechnological approach could be a perfect strategic tool for the organic corn growers to boost corn yield in an environment friendly way."

(a) Research & Development, BrettYoung Seeds

P57003 Analysis of NodDs and IFS genes

Cai, Qingqing (a) Zhang, Xiaoyan (a) Zheng, Xiaoxuan (b) Nan, Peng-presenter nanpeng@fudan.edu.cn(b)

" Rhizobia are known as symbiotic nitrogen-fixing bacteria that can form root nodules on legume plants. The molecular dialogue between rhizobia and legumes requires thousands of chemical signals, within which the essential components are nodulation (nod) factors from Rhizobia and flavonoid / isoflavonoid compounds from legume plants. The isoflavonoid compounds are limited primarily to leguminous plants,as inducers of the nodulation(Nod) genes in symbiotic Rhizobium bacteria to form nitrogen-fixing root nodules. Isoflavonoids are biosynthesized in a branching pathway of flavonoid metabolism, which are constructed by the isoflavone synthase (IFS). NodDs were scattered in almost all Rhizobia. We found function-unknown NodD-like proteins in Pseudomonadaceae, Actinomycete, and Cyanophyta, including more ancient nodD-like proteins in Frankia alni and Nostocwhich can also form nitrogen-fixing nodules with plants. This result may emphasize the predominant function of nodD genes in nodulation and nitrogen-fixing. We constructed the phylogenetic tree based on nodD and nodD-like protein sequences. The topology of the nodD tree is similar to the tree built by 16s rDNA, which implies that nodD evolved with the evolution of species. Beta vulgaris, a member in Chenopodiaceae family, has been deteced tumour-like deformations produced by Bradyrhizobium betae. Later two ifs cDNA in Beta vulgaris were cloned, and has been shown to produce isoflavones in pathogen-infected tissue. A number of symbiosis Rhizobia were detected in some genus of Ulmaceae, such as Parasponia, Trema, Celtis and Gironniera. It may implies if ifs genes exist in Ulmaceae. Or else it veiled a distinctive nitrogen-fixing symbiotic bioprocess."

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SESSION P58 - RHYTHMS

P58001 "PSEUDO-RESPONSE REGULATOR 9, 7 and 5 are Repressors of CCA1 and LHY Transcription in Arabidopsis Circadian Clock"
Nakamichi, Norihito-presenter nnakamichi@psc.riken.jp(a) Takatoshi, Kiba (a) Hitoshi, Sakakibara (a)
http://labs.psc.riken.jp/brt/English/index.html
"Circadian clocks regulate daily fluctuations of many physiological events in organism. In plant, an interlocking transcriptional/translational of 24h-feedback loop of clock-associated genes is thought to be clock core. PSEUDO-RESPONSE REGULATOR9 (PRR9), PRR7, PRR5, TIMING OF CAB EXPRESSION 1 (TOC1, also called PRR1), closest paralog of MYB transcription factors CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), play pivotal role in the interlocking feedback loop. Genetic study have suggested that PRR9, PRR7, and PRR5 somehow repress morning genes CCA1 and LHY, whereas activate evening gene TOC1. However, the molecular function of PRR proteins is mostly unknown. Here we demonstrate that nuclear entry of PRR5 can immediately repress CCA1 and LHY expression. Reporter assay indicated that PRR proteins repress CCA1 promoter activity. In addition, chromatin-immunoprecipitation analysis indicated the interaction between PRR proteins and upstream region of CCA1 and LHY genes during daytime in vivo. On the other hand, PRR proteins neither associated to TOC1 promoter region in vivo, nor attenuate TOC1 promoter activity. These results establish PRR9, PRR7, and PRR5 as repressors for morning genes CCA1 and LHY during daytime, which is essential to proper clock function. "
(a) RIKEN Plant Science Center

P58002 "Two new clock proteins, LWD1 and LWD2, regulate Arabidopsis photoperiodic flowering"
Wu, Shu-Hsing-presenter shuwu@gate.sinica.edu.tw(a,b) Wu, Jing-Fen (a) Wang, Ying (a,b)
"The 'light' signal from the environment sets the circadian clock to regulate multiple physiological processes for optimal rhythmic growth and development. One such process is the control of flowering time by photoperiod perception in plants. In Arabidopsis, the flowering time is determined by the correct interconnection of light input and signal output by the circadian clock. The identification of additional clock proteins will help to better dissect the complex nature of the circadian clock in Arabidopsis. Here we show LWD1/LWD2 as new clock proteins involved in photoperiod control. The *lwd1lwd2* double mutant has an early flowering phenotype, which could be complemented by either *LWD1* or *LWD2*. LWD1 and LWD2 share greater than 90% sequence similarity and function redundantly, yet their expression is independent of each other. The early flowering phenotype is contributed by the significant phase shift of *CO* and, therefore, an increased expression of *FT* before dusk. Clock genes tested have a short period length in the *lwd1lwd2* double mutant. Our data imply that LWD1/LWD2 proteins function in close proximity to or within the circadian clock for photoperiodic flowering control. We will describe the in depth characterization of LWD1/LWD2 in the aspects of their expression kinetics, subcellular localization and their impact on clock genes."
(a) Institute of Plant and Microbial Biology, Academia Sinica (b) Graduate Institute of Life Science, National Defense Medical Center and Academia Sinica

P58003 Natural Allelic Variation In Circadian Clock Function In *Brassica rapa*.
Lou, Ping (a) Xie, Qiguang (a) Xu, Xiaodong (a) Brock, Marcus T. (b) Edwards, Christine E. (b,b) Weinig, Cynthia (b) McClung, C. Robertson-presenter mcclung@dartmouth.edu(a)
http://www.dartmouth.edu/~rmcclung/index.html
"The endogenous circadian clock confers an internal periodicity that synchronizes an organism with the environmental period imposed by Earth's rotation; when internal and external periods diverge, performance measures such as net photosynthesis decline dramatically. Crop species encounter widely differing environmental conditions, including variable daylength and temperature. Thus, variation in circadian clock function has implications for agricultural productivity, especially among crops grown across wide latitudinal ranges. To date, studies of the plant circadian clock have emphasized *Arabidopsis thaliana*. We have extended this study to the crop plant, *Brassica rapa*. There is considerable variation in clock function among cultivated *Brassica rapa* accessions. To identify genes responsible for this natural variation we have analyzed a set of Recombinant Inbred Lines to identify Quantitative Trait Loci (QTL) for period, amplitude and temperature compensation of the circadian rhythm in leaf movement and for flowering time, as well as for a number of morphometric parameters, including, size of floral organs, and hypocotyl length. We are generating Heterogeneous Inbred Families to identify the genes responsible for these QTL. We also are testing candidate loci in transgenic Arabidopsis and *B. rapa*. We have developed a transgenic *B. rapa* hypocotyl system that expresses a light- and temperature-entrained circadian clock, as measured with clock-regulated reporter gene fusions. Our results suggest that there is considerable potential for the modification of circadian clock function as well as floral traits in *B. rapa* crops grown under agroecologically relevant daylength and temperature settings. Supported by National Science Foundation grant IOB-0517111. "
(a) Dartmouth College (b) University of Wyoming

P58004 Circadian clock controlled gene expression in developing soybean seeds
Hudson, Karen A-presenter kkaczoro@purdue.edu(a)
"The circadian clock controls a number of developmental and metabolic processes in plants. Soybean (*Glycine max*) seeds are not only an excellent model system for the study of seed development, but also an important commercial crop. To further understand how the circadian regulation of gene expression may be important to the development and composition of soybeans, we used the Affymetrix Soybean Genome array to identify genes with circadian expression patterns in soybean seeds at the midpoint of the seed-filling stage. We identified 300 circadian-clock regulated genes with predicted functions in many aspects of seed development, including protein synthesis and fatty acid metabolism. This set of clock controlled genes was used to identify conserved promoter motifs potentially involved in circadian regulation of gene expression. A difference in the timing of peak gene expression between seeds and leaves from the same plant was observed for a number of genes, primarily from classes of genes predicted to be involved in photosynthesis, demonstrating differential gene regulation in source and sink tissues, and organ-specific phasing of gene expression. "
(a) USDA-ARS Crop Production and Pest Control Research Unit

P58005 "Diurnal and circadian transcript profiling defines functionally conserved key clock regulated genes among arabidopsis, rice, and poplar."
Filichkin, Sergei A. -presenter filichks@onid.orst.edu(a) Priest, Henry (a) Michael, Todd P. (b) Breton, Ghislain (c) Kay, Steve (c) Chory, Joanne (d) Mockler, Todd C. (a)
"To interrogate diurnal gene expression under light/temperature cycles in rice, Arabidopsis, and poplar we used whole-genome oligonucleotide microarrays. Transcript abundance was phased to all hours over the day, with most genes peaking before dawn or dusk. Collectively, photocycles and thermocycles controlled a large proportion (~40 %) of both the rice and the poplar transcriptomes. We have identified clusters of co-expressed genes for each phase of the day in all three species. A total of 41-46% of predicted Arabidopsis-poplar-rice orthologs cycled under diurnal conditions.

Approximately 21-36% of the putative Arabidopsis-poplar-rice orthologs were phased to within 3 hrs of each other, suggesting that daily phasing of gene expression is strongly conserved in higher plants. After cycling genes were identified, the promoters of genes on the individual gene lists for each 1 hour phase of the day were analyzed using ELEMENT to identify significantly over-represented 3-8mer DNA words. The Z-score profiles for several well characterized (ME, GBOX, EE, GATA) and novel (TBX, SBX) diurnal/circadian associated cis-regulatory promoter elements were conserved among Arabidopsis, poplar, and rice. Identification of orthologous motifs in promoters of co-cycling gene clusters suggested that the diurnal/circadian regulatory network is conserved among all three species and likely in other higher plants. "
(a) Oregon State University, Corvallis, OR 97331 (b) Rutgers University, Waksman Institute, Piscataway, NJ 08855 (c) University of California, San Diego, CA 92093 (d) The Salk Institute and HHMI, La Jolla, CA 92037

P58006 Comparative analysis of rhythmic proteome in Oryza sativa.
Hwang, Heeyoun-presenter tilldawn@khu.ac.kr(a) Yong-Kook, Kwon (a) Jong-Seong, Jeon (a) Seong Hee , Bhoo (a) Tae-Ryong, Hahn (a)
"In plant daily rhythm, photoperiod is the most important environmental cue. The diurnal rhythm has the 24-hr period with day/night cycling and the circadian rhythm is regulated by a free-running internal circadian clock. In plants, these diurnal and circadian rhythms are important to be synchronized with many aspects of physiological metabolism such as growth, development, signaling and the movement of stoma, leaf and other organs. To identify the proteins involved in diurnal and/or circadian rhythm in rice, a monocot model plant, proteomic approach was carried out. Three-week-old rice seedlings were harvested with 6 hr intervals that were grown under 12 hr light/dark photoperiod condition. Among approximately 3,000 total reproducible protein spots, 354 diurnal-regulated and 53 circadian-regulated protein spots were detected. Using MALDI-TOF MS analysis, 81 spots(57 proteins) were identified. The identified proteins from detected spots were related to photosynthesis, mitochondrial electron transport, nitrogen metabolism, signal transduction, protein degradation, transcripts regulation and antimicrobial system. The comparative analysis was performed with RT-PCR and microarray data proposed by the PLANT DIURNAL PROJECT (http://diurnal.cgrb.oregonstate.edu/). The peak-time difference between transcription and translation level was confirmed. Chloroplast phospho glycerate kinase was shown consistant at the same peak-time. Phosphoribulokinase, malate dehydroganase and some enzymes related in biosynthesis showed same rhythms but 6hr interval. Oxygen evolving enhancer 1 is shown that they have 12hr interval. Chaperonin 60 beta is showed 18hr-interval. As a result, rhythmic proteins have their own time internals for transcriptional and translational expression."
(a) Graduate School of Biotechnology and Plant Metabolism Research Center, Kyung Hee University, Yongin 446-701, Korea

SESSION P59 – ROOT BIOLOGY

P59001 Mechanically induced Ca^{2+}transients may play a role in curvature-associated LR initiation.
Richter, Gregory L-presenter grichter@psu.edu(a) Monshausen, Gabriele B (b) Giroy, Simon (b,a)
"Development of the root system represents a morphogenetic program where the positioning of new lateral organs occurs through the periodic recruitment of pericycle cells to become founder cells of a new lateral root (LR) primordium. While the hormone auxin appears intimately involved in specifying LR formation, it remains unclear why some pericycle cells are specified to initiate a LR while others are not. Here we show that mechanical forces can act as one of the triggers for founder cell formation and so entrain the pattern of LR production to the environment. We observed that transient physical bending of the root was capable of eliciting LR formation to the convex side of the curve. Such mechanical stimulation triggered a Ca^{2+} transient within the pericycle, which was associated with the recruitment of cells to a LR founder cell fate. The initial establishment of the mechanically induced LR primordium was independent of an auxin supply from the shoot and was not disrupted by mutants in a suite of auxin transporters and receptor/response elements. Mechanical forces have long been proposed to act as plant morphogenetic factors, however the cellular elements that translate mechanical force to a developmental signaling cascade have remained obscure. Our observations indicate that in the case of mechanical induction of LR formation, the program of organogenesis may be triggered by mechanically elicited Ca^{2+} changes that can even suppress the requirement for many auxin-related elements normally involved in founder cell recruitment. Thus, the plant mechano-sensitive Ca2+ signaling system provides a potentially widespread mechanism whereby external and endogenous mechanical forces could be translated into morphogenetic programs during plant growth and development."
(a) Pennsylvania State University, Department of Biology (b) University of Wisconsin, Department of Botany

P59002 Root secretions mediate kin recognition in plants
Biedrzycki, Meredith L-presenter mlbiedrz@udel.edu(a,b) Jilany, Tafari A (c) Dudley, Susan A (c) Bais, Harsh P (a,b)
"The ability of an organism to monitor and react to the surrounding environment and identify potential competitors is essential for survival. For example, animals can detect the presence of others, such as kin or possible predators, and distinguish between the two, through use of visual, auditory, or olfactory cues. Plants, however, lack these conventional senses and have evolved unique chemical mechanisms such as chemical sequestration, emission of volatile organic compounds (VOCs), and allelopathy, as survival strategies to sense and communicate with other organisms. Recently, Dudley and File (2007) have demonstrated *Cakile edentuala* has the ability to discriminate between kin and nonkin root interactions, as a result, alter root allocation and may impact interspecies and intraspecies competition. Although this data demonstrates that plants have the ability to react to interactions, the question remains, how do plants sense and judge the presence of others? Taking this prior knowledge of *Cakile edentuala* behavior further, we questioned whether the root in general is important to drive kin recognition patterns. We also hypothesized whether root secretions contribute in relaying kin related chemical messages. Here we show that root secretions play an essential role in kin recognition patterns, utilizing the model plant system *Arabidopsis thaliana* (Col-0), we show that the root secretions from two kin plants negate lateral root formation compared to kin plants exposed to non-kin secretions. Our results demonstrate that plants can discriminate kin in competitive interactions and confirm that the root secretions may provide the cue for kin recognition. "
(a) Department of Plant and Soil Sciences, University of Delaware (b) Delaware Biotechnology Institute (c) Department of Biology, McMaster University

P59003 Maize Root Characteristics that Enhance Flooding Tolerance
Kindiger, Bryan-presenter Bryan.kindiger@ars.usda.gov(a) Yoshiro, Mano (b)
"Plant root systems have several cellular and molecular adaptations that are important in reducing stress caused by flooding. Of these, two physical properties of root systems provide an initial barrier toward the avoidance of stress. These are the presence of aerenchyma cells and rapid adventitIous root growth following flooding. As a first line of defense, these attributes are critical in reducing or avoiding the deleterious effects of flooding. Recent investigations have identified QTL effecting aerenchyma cell formation and adventitious root formation in teosinte, a close relative of maize, and its hybrids with maize. A brief description of the research and results of the investigation are provided."

(a) USDA ARS Grazinglands Research Laboratory (b) Japanese Natl. Inst. of Livestock and Grassland Science, Dept. of Forage Crop Breeding, 768 Senbonmatsu, Nishinasumo, Tochigi, 329-2793, Ja

P59004 Root iron acquisition in *Helianthus annus* through strategy I reducing system

Mercado, Stephanie Q-presenter smercad2@stedwards.edu(a) Grusak, Michael A (b) Quinn, Quinn J (a)

"Iron provides an essential oxidation/reduction potential in plant metabolic pathways, and is thus an essential nutrient. However, it can also be toxic. Thus, its absorption into plant tissues is highly regulated and its internal concentration is largely controlled at the level of root uptake. Iron is abundant in almost all soils, but often in insoluble forms, and plant roots affect iron solubility by one of two strategies. In dicots and the non-grass monocots, iron acquisition is facilitated by a set of mechanisms known as strategy I. These mechanisms include the FRO (ferric reductase oxidase) protein and the IRT (iron regulated transporter) gene. As a strategy I plant, *Helianthus annus* is expected to be able to take up iron effectively (and in fact to exhibit enhanced capabilities) when internal iron levels are below required amounts, but to reduce the activities of iron acquisition mechanisms when internal iron concentrations are sufficient. The focus of this study was to evaluate the change in iron acquisition, as measured through the activity of the iron reductase in *H. annus* roots, in response to different concentrations of iron in a hydroponic medium. When plants were grown in lower Fe(III) EDDHA conditions (0.5 µ;M) and assayed with Fe(III) EDTA, higher Fe reductase activity was measured. When plants were grown in higher Fe(III) EDDHA conditions (5 µ;M), lower iron reductase activity was measured. These data are consistent with earlier findings and provide direction for further research on iron reductase expression in plants drawn from different *H. annus* native populations."

(a) St. Edward's University (b) USDA-ARS Children's Nutrition Research Center

P59005 Identifying the location of GAL4 enhancer trap line insertions in *Arabidopsis thaliana*

Diaz, Jessica-presenter jessica.diaz.602@csun.edu(a) Zavala, MariaElena (a)

"*Arabidopsis thaliana* is a very important model organism for plant biology. *Arabidopsis* is a small flowering plant that has had its entire genome sequenced therefore a vast amount of genetic information about it is available. In our research, we are focusing on the root growth and development. We are identifying the location of GAL4 enhancer trap line insertions that result in fluorescence in root specific tissues of *Arabidopsis thaliana*. We are using a variety of transgenic *Arabidopsis* tissue specific markers. To identify the location of the GAL4 insertions, we are using TAIL-PCR (thermal asymmetric interlaced polymerase chain reaction, Lui 1995). TAIL-PCR is a method used to determine DNA fragments flanking known sequences of a genome. We have adapted the TAIL-PCR method for various lines of transgenic *Arabidopsis* with varying success. Once the flanking regions of the inserts are located using bioinformatic tools, we have a library of sequences that may provide useful information regarding relationships between the locations of the trap lines and the tissue specificity of roots. "

(a) California State University, Northridge Biology Department

P59006 Role of Oxalate Oxidase in Apoplastic ROS Generation and Root Growth Regulation under Water Deficit Conditions

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"Previous work on maize primary root adaptation to water deficit showed that cell elongation is maintained in the apical region of the growth zone but progressively inhibited further from the apex. In association with these growth responses, cell wall extensibility is enhanced in the apical region but decreased in the basal region. Cell wall proteomic analyses were conducted to identify proteins important for wall extensibility and elongation (Zhu *et al.* 2007, Plant Physiol. 145: 1533-48). The results revealed predominantly region-specific changes in protein profiles between well-watered (WW) and water-stressed (WS) roots. Several proteins related to reactive oxygen species (ROS) generation showed an increased abundance in the apical region of WS roots, prominent among them being putative oxalate oxidases. An increase in apoplastic ROS in the apical region of WS roots was confirmed by in-situ imaging. Apoplastic ROS may have wall loosening or tightening effects which could be region specific. In this study a transgenic maize line constitutively expressing wheat oxalate oxidase (Ramputh *et al.* 2002, Plant Sci. 162: 431-440) was used to test if enhanced oxalate oxidase activity and apoplastic ROS affects root elongation. The results showed differential effects on growth and growth-related processes in WW and WS roots. Staining experiments in the transgenic line revealed increased oxalate oxidase activity in a region-specific manner with different profiles in WW and WS roots. *Oxalate oxidase* transgene expression was higher in the apical compared to the basal region of both WW and WS roots, partly explaining the protein activity profiles. Experiments to determine the mechanisms of root growth regulation by oxalate oxidase/apoplastic ROS are in progress."

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P59007 Flavonoids and auxin-related microRNAs play critical roles in nodule and lateral root development

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"Flavonoid compounds play critical roles in lateral root development and legume-rhizobium symbiosis. However, it was not known which group of flavonoids are critical and what role they play. By metabolic engineering, we were able to alter the flavonoid profiles in transgenic roots and supplement these roots with different flavonoid compounds to physiologically and biochemically complement the altered metabolic profile and study the role of individual flavonoid compounds. We discovered that flavonoids are essential for legume-rhizobia symbiosis in both determinate and indeterminate nodulating plants. In soybean, which is a determinate nodulating plant, isoflavones serve as internal nod gene inducers to maintain continuous production of bacterial Nod Factors. Isoflavones inhibit polar auxin transport in soybean root but this inhibition is not critical to nodule primordia development. In contrast, in *Medicago truncatula*, which is an indeterminate nodulating plant, flavonoids serve as both internal nod gene inducer and auxin transport regulator. Both functions are essential for successful nodulation. In *M. truncatula*, flavones are internal nod gene inducers and flavonols are most likely the auxin transport regulators. More importantly, we were able to use several engineered strains of rhizobia that does not require flavonoids to nodulate the roots to confirm the critical mechanism of the flavonoid compounds. For the first time, we demonstrated clearly that different flavonoid compounds function differently during nodule development. Recently, we identified and characterized a group of auxin-relate miroRNAs. Their action during nodulation provided additional support that flavonoids and auxin are important early signals for nodule and lateral root primordia development."

(a) Danforth Plant Science Center (b) South Dakota State University (c) Ludong University (d) University of Missouri-Columbia

P59008 Mutation of the *Arabidopsis NRT1.5* nitrate transporter causes defective root-to-shoot nitrate transport.

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"NRT1.5 is one of the 53 Arabidopsis NitRate Transporter *NRT1* (Peptide Transporter PTR) genes, of which two members, *NRT1.1 (CHL1)* and *NRT1.2*, have been shown to be involved in nitrate uptake. Compared to the wild type, the root-to-shoot ^{15}N- nitrate translocation of *nrt1.5* mutants

were reduced. To confirm the function of NRT1.5, the nitrate contents in the xylem sap were measured. Consistent with the defect of the root-to-shoot nitrate translocation, in *nrt1.5* mutants, the nitrate contents of the xylem sap were lower than that of wild type. in situ hybridization showed that *NRT1.5* is expressed in root pericycle cells close to the xylem. In addition, localization of *NRT1.5* expression was analyzed by transgenic plants carrying GUS gene under the control of the *NRT1.5* promoter. Consistent with the in situ hybridization result, GUS activity was detected in the parenchyma cells (more likely the pericycle cells) close to the xylem vessels. Subcellular localization showed that NRT1.5 is located in the plasma membrane. Functional analysis of cRNA-injected Xenopus oocytes showed that NRT1.5 is a low-affinity, pH-dependent bidirectional nitrate transporter. Taken together, these data suggested that NRT1.5 is located in the plasma membrane of root pericycle cells close to the xylem and responsible for nitrate efflux out of pericycle cell for root xylem loading of nitrate. The root-to-shoot nitrate transport is not completely eliminated in *nrt1.5* mutants suggesting that there are multiple nitrate xylem loading mechanisms and nitrate efflux mediated by a proton coupled nitrate transporter is one of them. "
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P59009 The SHR/SCR pathway activates genes involved in asymmetric cell division in the Arabidopsis root
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"The combinatorial activity of two transcription factors, SHORT-ROOT (SHR) and SCARECROW (SCR), is required for the regulatory network that leads to the asymmetric cell division of the cortex/endodermal initials (CEI) in the Arabidopsis root. In order to elaborate a comprehensive model of SHR-mediated signaling network in tissue patterning, we employed a conditional activation system of SHR and SCR. We performed microarray analyses over a time course (0, 1, 3, 6, and 12 hours) that was designed to capture expression changes before, during and after asymmetric division in the shr and scr mutants. In addition, using cell-sorting technology, we were able to specifically examine the transcriptional effects of SHR and SCR specifically in the ground tissue. These analyses allowed us to identify transcription factor activity, signal transduction, and cell cycle progression as important biological processes regulated by these two transcription factors. Moreover, we found that cell cycle genes, activated by the SHR/SCR pathway, were highly expressed in CEI cells. These results suggest an important role for cell cycle machinery in asymmetric cell division in the ground tissue and provide a mechanistic link between the SHR/SCR pathway and cell cycle progression. "
(a) Duke University, USA (b) UC-Davis, USA (c) Cardiff University, UK

P59010 "In vitro transformation of *Stenocereus gummosus*, a Cactaceae with determinate root growth"
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"During determinate root growth the root apical meristem becomes exhausted and eventually lost. As a consequence, all cells in the root tip differentiate. *Stenocereus gummosus*, a Sonoran Desert Cactaceae, was used in this study because its primary root maintains growth for only 2-3 days after the start of seed germination (Dubrovsky, 1997, Planta, 203: 85). With the objectives to elucidate the molecular pathways that regulate determinate root growth and to understand how the root apical meristem is maintained in plants, we created subtracted libraries of *S. gummosus* cDNA that were expressed during the initial and terminal phases of primary root development. To analyze the role of candidate genes during determinate root growth, a homologous system for gene overexpression or inhibition in determinate roots is required. Because of the particularly long life cycle of desert Cactaceae - the life span of *S. gummosus* is estimated to last for more than one hundred years - it is not feasible in this species to obtain genetically transformed seeds and analyze primary roots in transgenic plants. Therefore, we are in the process of development of an artificial system which comprises obtaining transformed callus tissues and subsequently inducing transgenic roots. Previously we have optimized in vitro conditions for callus induction and root regeneration (Shishkova et al., 2007, Plant Cell Reports, 26: 547), and in the present work, results of the *Agrobacterium*-mediated in vitro transformation of *S. gummosus* will be presented. We thank S. Napsucialy-Mendivil for excellent technical help and DGAPA-PAPIIT, UNAM (project IN212509) and CONACyT (projects 79736 and 102285) for financial support."
(a) Instituto de Biotecnologia - Universidad Nacional Autonoma de Mexico

P59011 Characterization of lateral root emergence in *Arabidopsis thaliana*
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"Lateral roots are initiated in the pericycle, deep within the parent root, and must work their way through the overlying layers of cells to reach the soil. Because openings that allow lateral roots to exit could also serve as entrance points for pathogens, maintaining tight control over cell separation is likely to be advantageous. We previously hypothesized that selective degradation of the pectin-rich middle lamellae surrounding lateral root primordia occurs in part because of expression of *AtPLA2*, an auxin-regulated pectate lyase (Laskowski et al. 2006 Plant Cell Physiol 47(6):788-792). Here, we show with SEMs that lateral roots emerge cleanly, breaking between rather than within cells. The average length of the opening is approximately 200 µ;m +/- 40 µ;m, roughly the length of two epidermal cells. Dynamic confocal imaging of live roots demonstrates that auxin response, as measured by the DR5::GFP reporter, increases in cells directly overlying lateral root primordia, suggesting a mechanism that could coordinate expression of genes such as *AtPLA2* that may be involved in lateral root emergence. "
(a) Oberlin College Biology Department

P59012 A new look into old histological techniques for studying root biology using modern microscopy
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"Microscopy is an indispensable tool for studying biological sample morphology and anatomy, as well as gene expression and protein localization. Different microscopy sample preparation methods always have unique strengths and weaknesses, but often complement each other in providing better picture and information of a specific subject in question. This project attempted to compare traditional histological techniques, such as whole-mount method, paraffin section, and cryosection, and to outline their respective pros and cons based on case studies of *Ceratopteris richardii* and *Arabidopsis thaliana* roots. For example, cryosection preparation is generally faster and has better antigen preservation because initial chemical fixation and dehydration processes can be eliminated. Since antigens remain in a hydrated environment in cryosection, this method also provides better antigen accessibility in immunolabeling. However, the quality of structural appearance usually is not as good as that of paraffin or resin section. The quality of cryosection is also largely dependent on the Cryostat/microtome used and preparer's experience. The choice of sectioning methods should be coupled with the objectives of investigation. The results of this project should provide a general guideline for choosing sectioning methods in studying root biology in specific, and other biological investigations in general."
(a) Appalachian State University

P59013 Hormonal changes during the alteration of pattern and developmental competence in the root apical meristem of *Catasetum fimbriatum*.

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"The root apical meristem (RAM) of *Catasetum fimbriatum* is able to convert into bud when isolated from the plants and cultivated in a hormone-free medium. Previously, it was noticed that competence acquisition for this process was related to plant ageing, and was also coupled with the establishment of the determinate root growth and with a structural re-organization of the RAM. Based on this, the aim of this study was to investigate the endogenous levels of four cytokinins (Z, ZR, iP and iPR), two auxins (IAA and IAA-Asp), two gibberellins (GA_1 and GA_7), abscisic acid (ABA) and ethylene during the competence acquisition for conversion of RAMs of *C. fimbriatum* into buds. The results showed a strict correlation among root ageing, competence acquisition, alteration in the RAM pattern and decrease in endogenous levels of auxins and cytokinins - important hormones for the cell division progression. On the other hand, during this same period, it was observed an increasing in ethylene, ABA and gibberellins contents - hormones involved in the signaling of stress conditions or cellular differentiation. These alterations entailed loss of root tip characteristics, which resulted from developmental modifications in the quiescent center (QC) and stem cell control. A higher level of cell differentiation in old root apices promoted the isolation of a new group of meristematic cells in the former QC region, which seemed to respond to different developmental signals. Therefore, all the morphological and hormonal alterations observed during the *C. fimbriatum* root ageing appear to play an important role in the loss of root tip features, and in the RAM competence acquisition to follow a new developmental fate that, in this case, involves the establishment of an ectopic shoot meristem."

(a) Universidade de Sao Paulo

P59014 "Growth and gravitropic curvature of the maize primary root after experiencing the stress by KCl, NaCl and RbCl"

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"Salt stress profoundly affects the growth and development of plant roots. The main symptom of salt stress on root system is the growth inhibition, and resulted in the loss of production. Therefore, plant scientists have made great efforts to overcome the damage by salt stress. However, details of the effects of salt stress on root system still remain unknown. In this study, we investigated the growth and grvitropism of the primary root of maize (*Zea mays*), after the root experienced (i.e., roots were not in the salt solution any more during the measurement of gravitropism) the stress by KCl, NaCl and RbCl. As expected and well-known, the root growth was inhibited by the salts at high concentrations. However, the gravitropic curvature of the primary root was better with KCl or NaCl than that of untreated control. In contrast, the pretreatment with RbCl severely disturbed the gravitropism as well as the growth. The possible explanations for the observed differences will be discussed."

(a) Dept. of Molecular Biology, Dankook Univ.

P59015 Automated Computer Vision Measurements of Relative Elemental Growth Rates of Auxin Transport Mutant

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"As a sessile organism, a plant's ability to explore and optimally position itself within a changing environment depends on its capacity to grow throughout its lifecycle. In roots, elongation growth is restricted to a well-defined zone of the root tip. Regulated cellular expansion in this elongation zone drives root penetration of the soil and determines changes in growth rate and direction in response to environmental stimuli. We have developed a novel computer vision tool to monitor relative elemental growth rates (REGRs) of vertically growing *Arabidopsis* roots with high spatiotemporal resolution. Roots are mounted on a Nikon microcope and imaged using a 10x objective and AVT Pike camera every 30s for 1-2 h at a resolution of 1.77 µ;m per pixel. To estimate the REGRs, the rate at which cells are displaced from the root tip is needed. To obtain this information, the user selects points along the root midline which are to be tracked. Utilizing optimization theory, the tracking algorithm locks onto the image texture and follows its progression during root growth. Post processing, a flexible logistic function is fit to the velocity distribution and is differentiated to obtain the REGRs. The location of the growth zones and the rates at which cells are expanding in them, is regulated in part by the spatial distribution of growth hormones, such as auxin. To determine the role auxin plays in controlling the location and magnitude of the REGRs we assayed the profiles of three *Arabidopsis* mutants aux1-21, mdr4-1, and arg5-1, all of which are known to disrupt the auxin fluxes within the root."

(a) UW Madison

P59016 Directed root growth in response to gradients of nutrient and water availability

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"Nutrient and water acquisition are essential to plant survival. We have shown that, under conditions of phosphorous and nitrogen deprivation, *Arabidopsis thaliana* primary roots exhibit tropic growth towards areas of higher nutrient availability. We suggest that auxin redistribution is central to this tropic response. Nutrient-mediated tropism was observed utilizing a clinostat-based system to minimize the effects of gravity on root growth direction. This system was also used to investigate the root tropic response to an induced water potential gradient. We are investigating the role of root cap cells in the perception of gradients in nutrient and water availability. "

(a) Swarthmore College

P59017 Root anatomy and gene expression characterization in poplar

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"Global warming research in recent years has focused on various possibilities of carbon sequestration along with the ongoing efforts on reducing carbon dioxide emission. Because of the ability of plants to sequester terrestrial carbon, we are studying the molecular and genetic mechanisms in poplar, which controls carbon allocation. The primary aim of the project is to increase below ground carbon sequestration by altering some of the molecular mechanisms involved in root proliferation. Plant regeneration from hybrid aspen (clone 717; *Populus tremula x P. alba*) stem and apex cuttings were investigated to develop a rapid and efficient method to assess overall plant growth and development, with a focus on visualizing lateral root development in real-time. Root anatomical studies on hybrid aspen revealed a cellular arrangement consisting of xylem pole pericycle, a layer of endodermis followed by three layers of cortical cells and an external layer of epidermal cells. We have also identified highly expressing genes during root growth stages in poplar based on bioinformatics analysis on micro-array data available for poplar and Arabidopsis root. The results obtained and the methods developed during this study will be used as a baseline for future work to investigate the effect of silencing *Aux/IAA* and *ARF* genes during plant growth and development in poplar."

(a) ornl

P59018 Modifying Cytokinin Activity Through overexpression of Cytokinin Oxidase 2

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"Cytokinins are plant hormones, which have been shown to be crucial in many physiological processes in plants including promoting cell division, cell growth, cell differentiation, and local and long distance signaling. Numerous studies have been dedicated to understanding the regulatory role of cytokinins by altering their levels. The results of the experiments have led to an increased awareness of the pleotropic effects of cytokinins in plant development. In transgenic tobacco, cytokinin deficient plants exhibited smaller shoots and larger roots while in cytokinin over producers the plants appeared to have a larger shoot system and smaller roots. Cytokinin levels can be modified through the overexpression of cytokinin oxidases, which irreversibly inactivates cytokinins by cleaving the N6 Isoprenoid side chain. The cleavage leads to depletion of cytokinins by converting the most abundant active cytokinin trans-zeatin to adenine. Cytokinin oxidase 2 (CKX2) gene was modified to allow insertion into a collection of GAL4/UAS-YFP and S35 YFP plasmids. The resultant plasmids were used to transform E. coli DH5αundefined. The binary vectors were used to transform Agrobacterium strain (GV3101) and used to transform C24. Putative transformants will be selected and used to cross into the available GAL4-GFP enhancer trap lines. Results of these crosses will be reported. We expect to see altered phenotypes in response to the altered cytokinin levels in a cell and tissue specific manner. Supported by NIH NIGMS MORE to MEZ, and Provost Fund. "
(a) Ca State University Northridge

SESSION P60 – SECONDARY METABOLISM & NATURAL PRODUCTS

P60001 Acetohydroxyacid Synthases in the Biosynthesis of Lycopsamine Type Pyrrolizidine Alkaloids
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"Pyrrolizidine alkaloids (PAs) serve as a model system for studying the evolutionary origin of secondary pathways. PAs are ester alkaloids of a necine base moiety and of necic acids. For the first specific enzyme in the necine base biosynthesis (homospermidine synthase) we were able to show a polyphyletic origin of an enzyme involved in primary metabolism (deoxyhypusine synthase). Lycopsamine type PAs possess a structural unique necic acid. The occurrence of these PAs is restricted to three not closely related families (Asteraceae, Boraginaceae and Apocynaceae) making them an ideal model for evolutionary studies. Former experiments with ^{13}C-labelled glucose suggest that an intermediate in valine biosynthesis is used as a precursor of this unique necic acid. Mechanistically, this reaction should be catalyzed by an acetohydroxyacid synthase (AHAS) with extended or altered substrate specificity. Based on amino acid alignments of plant AHAS catalytic and regulatory subunits, three homologous sequences from *Eupatorium cannabinum* catalytic subunits and two sequences of regulatory subunits from the same plant were identified using degenerate primers. Heterologous co-expression of both subunits in *E. coli* host cells resulted in active enzyme. Substrate specificity and genetic analysis allowed a deeper understanding in the evolutionary origin of the PA metabolic pathway. "
(a) University Kiel, Botanical Institute

P60002 Localization and regulation of potential diamine oxidases of pyrrolizidine alkaloid biosynthesis
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"Pyrrolizidine alkaloids (PAs) are a typical group of plant secondary compounds. They are constitutively produced by various plant species as a defence against herbivores. Structurally PAs are ester alkaloids composed of a necine base and a necic acid. Transformation from spermidine to homospermidine, catalysed by homospermidine synthase (HSS), is first step in necine base biosynthesis. We assume that a diamine oxidase (DAO) catalyzes next step: The transformation of homospermidine to the cyclic necine base. Three different cDNAs of DAOs were identified from Senecio vernalis. One or more of these sequences might encode such a specialized DAO. In order to gain if one of these sequences is involved in PA biosynthesis *in situ* hybridisation as a tool of localization expression site was adopted. As a result expression one of the tested sequences was detected at specific root cells around central cylinder. This expression site conforms to results of localizing HSS, the enzyme catalyzing transformation of homospermidine which is expected to be substrate of a diamine oxidase. RT-PCRs and a Northern Blot approved results. In order to proof functional attendance of this sequence in PA biosynthesis transgenic *Senecio vernalis*- and *Nicotiana tabacum*-cultures were generated. By knocking out the DAOs separately in each line we try to intermit PA biosynthesis and reduce PA contain considerably. Furthermore studies in overexpression of two *Senecio* DAOs in *Senecio* and *Nicotiana* were performed. "
(a) Botanical Institute of Kiel University

P60003 Identification and characterization of a conserved tandem gene duplication implicated in capsaicinoid biosynthesis
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"Capsaicinoids are synthesized exclusively in the genus *Capsicum* and are responsible for the burning sensation experienced when consuming hot pepper fruits. *AT3*, which based on homology belongs to the BAHD family of acyltransferases, has been implicated in capsaicinoid biosynthesis. The recessive forms of this gene lead to the absence of capsaicinoids and coordinated transcriptional down-regulation of phenylpropanoid and fatty acid structural genes. Sequence information from representative taxa in the Solanaceae revealed that the coding region of *AT3* is highly conserved throughout the family and also uncovered a tandem duplication of this gene, designated *AT3-1* and *AT3-2* respectively. This duplication predates the diversification of the Solanaceae. The *AT3-2* locus, now a pseudogene, retains punctuated levels of amino acid conservation relative to *AT3-1*. Both Bayesian and Maximum Parsimony methods demonstrated that the paralogous gene lineages of *AT3-1* and *AT3-2* form well supported phylogenetic clades. We find support for the species complex comprising *C. annuum*, *C. chinense* and *C. frutescens*, widely reported in the literature. We also find that this species complex includes *Lycianthes*. Additionally, in *C. rhomboideum*, a recombination event between *AT3-1* and *AT3-2* modifies the putative active site of *AT3-1*, and a frame-shift mutation in the second exon may account for the absence of capsaicin biosynthesis reported in this basal species in the genus. Our data suggest that duplication of the original *AT3* representative, in combination with divergence, and pseudogene degeneration may account for the pattern of sequence divergence and punctuated amino acid conservation observed in the data."
(a) Cornell University (b) University of Wisconsin, Madison

P60004 Transcriptional Activation of a MYB gene Controls the Tissue-Specific Anthocyanin Accumulation in a Purple Cauliflower Mutant
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"Flavonoids such as anthocyanins possess significant health benefits to humans and play important physiological roles in plants. An interesting *Purple* gene mutation in cauliflower confers an abnormal pattern of anthocyanin accumulation, giving intense purple color in very young leaves, curds, and seeds. Through high-resolution genetic mapping, we have isolated the *Purple* gene and found that it encodes a R2R3-MYB transcription factor that shows tissue-specific expression, consistent with anthocyanin accumulation pattern in the mutant. The *Purple* gene is tightly linked with other two MYB transcription factors of similar function. Comparison of the DNA sequences between the WT and mutant alleles revealed that the mutation is caused by a unique sequence in the promoter region of the *Purple* allele. Analysis of *Arabidopsis* transformants with different fragments of cauliflower promoter-GUS constructs demonstrated that the unique part contains promoter enhancer sequence, causing enhanced expression of the *Purple* gene.

The resulting overexpresson of the *Purple* gene may activate the expression of a bHLH transcription factor, which together upregulate a subset of anthocyanin structural genes to produce the striking mutant phenotype. Yeast two-hybrid assays showed that the *Purple* gene encoded MYB protein exhibits stronger interaction with bHLH than other MYBs. Moreover, expression of the *Purple* allele in transgenic *Arabidopsis* also results in the tissue-specific accumulation of anthocyanins. These results strongly suggest that the change of genomic context in the promoter of the *Purple* allele is the genetic basis for the purple cauliflower mutant and the increased expression in *Purple* transcript levels accounts for the ectopic accumulation of anthocyanins in the mutant plant."
(a) Department of Plant Breeding and Genetics, Cornell University (b) Robert W. Holley Center for Agriculture and Health, USDA-ARS

P60005 Molecular and Biochemical Characterization of Polyphenol Oxidase in Walnut
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"The enzyme polyphenol oxidase (PPO) is nearly ubiquitous in Kingdom Plantae, and catalyzes the oxidation of monophenols and *o*-diphenols to quinones. While the role of PPO in postharvest browning of cut fruit is well established, its native functional role in plants remains unclear. Despite the rich diversity of phenolics in walnut leaves and hulls, PPO in walnut (*Juglans regia*) has been little studied since the early 1900s, when a potential antimicrobial/defense role for walnut PPO was posited. Based upon enzyme activity assays, we found that PPO is expressed primarily in the leaves, hulls, and flowers of walnut. We cloned a PPO-encoding gene from a walnut pistillate flower cDNA library and designated the gene *jrPPO1*. Genomic Southern analysis demonstrated that *jrPPO1* is the sole PPO gene in walnut. In order to facilitate biochemical and functional analyses of PPO, we assembled two binary vectors for use in *Agrobacterium*-mediated transformation of walnut: one vector designed for overexpression of *jrPPO1* and one vector designed to initiate post-transcriptional gene silencing of *jrPPO1*. While none of the recovered transgenic walnut plants displayed substantially higher PPO activity than the wild type, eight transgenic lines displayed PPO specific activities <1% of wild type. Using protein extracts from one PPO-silenced line and wild type walnut, we examined the substrate specificity of JrPPO1 using 14 potential phenolic substrates found in walnut leaves. Caffeic acid and catechin were found to be the preferred native phenolic substrates based upon V_{max}/K_m ratio. Future studies will focus on the comparison of wild-type and PPO-silenced lines to examine potential roles of PPO in pathogen resistance, insect herbivory, and pellicle color in walnut."
(a) California State University San Marcos, Dept. of Biological Sciences (b) University of California Davis, Dept. of Plant Sciences

P60006 A genetic approach to cyanogenesis in the model legume *Lotus japonicus*
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"Cyanogenic glucosides (CNGs) are defense compounds found in a large number of plant species. They protect the plant against herbivores as tissue damage will result in their breakdown and the release of toxic hydrogen cyanide gas. The synthesis and breakdown of CNGs has been investigated extensively biochemically, but genetic approaches, which may identify additional metabolic steps or regulators, have been under utilized. To establish the first comprehensive genetic characterization of CNG metabolism, we conducted a screen for mutants in cyanogenesis in the model legume *Lotus japonicus*. Lotus contains two CNGs named linamarin and lotaustralin, derived from valine and isoleucine respectively. An EMS mutagenized population of 47,000 M2 plants, representing 3600 M1 families, was screened using a simple colorimetric detection assay for cyanide release following tissue disruption. We identified >40 mutants with no or significantly reduced cyanogenesis. Metabolic profiling by LC-MS showed that there were three broad classes of mutants obtained, these are: i) no or reduced CNG production, ii) distortion of the relative levels of valine versus isoleucine derived CNGs and iii) deficiency in the breakdown of CNGs. The breakdown mutants contain CNGs at levels equivalent to wild-type plants but are unable to release cyanide gas upon tissue disruption. Genetic and biochemical analysis indicate that these mutants fall into several complementation groups and provide novel insight into our understanding of cyanogenic glucoside breakdown."
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P60007 "From genes to the wine bottle: genomic, transcriptomic and functional analysis of the grape R2R3-MYB family reveals expansions and diversification of members related to flavonoid synthesis. "
Matus, J.Tomas-presenter tomas.matus@gmail.com(a) Vega, Andrea (a) Aquea, Felipe (a) Ferrier, Thilia (b) Barrieu , Francois (b) Arce-Johnson, Patricio (a)
"Flavonoids are the most abundant secondary metabolites found in grape berry tissues. These molecules define quality parameters when analyzing grapes and wines as they influence colour intensity, astringency, bitterness and because of their beneficial properties on human health. Their synthesis arises from the phenylpropanoid pathway. Its key enzymes are tightly regulated by the combinatorial interaction of MYB, bHLH and WDR transcription factors, as found for many plant model species. The MYB superfamily controls a wide diversity of physiological functions and is one of the most abundant groups of regulatory factors described in plants. In Arabidopsis, a reduced group of these members regulate flavonoid synthesis. We recently identified and characterized over 110 R2R3 MYB members in the grape genome (Matus et al., 2008). Unravelling some of their functions by means of heterologous or homologous experimental approaches, plus the use of bioinformatic tools such as the PLEX database for obtaining Affymetrix-based expression profiles, revealed that while some genes seem to posses conserved functions and expression domains across species (Arabidopsis, Populus and rice), those subfamilies concerning flavonoid synthesis have expanded and may have additional properties, characteristic to the features of grapevine development. As an example, a putative grape MYB24-orthologue was found to be related to anther and flower development, the same as for Arabidopsis, but may posses an additional role throughout berry growth. The analysis of these genes in grapevine will provide the basis for field and biotechnological approaches in order to metabolic engineer these molecules in the fruit and also to improve wine potential. "
(a) Facultad de Ciencias Biologicas, Pontificia Universidad Catolica de Chile (b) Institut des Sciences de la Vigne et du Vin Centre INRA-Bordeaux-France.

P60008 Enhanced anthocyanin production in tobacco plant by overexpression of maize and Arabidopsis regulators
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"Plants produce a wide array of natural products, many of which are likely to be useful bioactive structures. These complex natural products usually occur at very low abundance and with restricted tissue distribution, thereby hindering their evaluation. To accumulate the composition of anthocyanins, regulatory genes of anthocyanin biosynthetic pathway encoding R2R3 MYB PAP1-D gene form arabidopsis and basic helix loop helix (bHLH) B-peru gene from maize were amplified by RT-PCR and then cloned individually into plant expression vector pB7WG2D. We have generated transgenic tobacco plants expressing each construct via Agrobacterium tumefaciens strain LBA4404 and verified by genomic PCR and Northern blot analysis. All transgenic plants looked similar with the wild-type plant, but accumulation of red-purple anthocyanin was only observed flower tissue.

Anthocyanin overproduction hybrid tobacco plants were developed by crossing with transgenic tobacco plants harboring each construct. Hybrid tobacco plants showed the purple pigmentation in whole plant, including the roots, stems, leaves, as well as sepals, anthers, petal and stigma, but other morphological phenotypes were not observed. Hybrid tobacco plants were strongly up-regulated the gene expression involved in the flavonoid biosynthetic pathway. "
(a) National Academy of Agricultural Science, RDA

P60009 Metabolic plasticity in isoquinoline alkaloid biosynthesis in *California poppy* cells induced by the ectopic expression of *Cj*SMT
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"Higher plants produce a divergent array of secondary metabolites. These chemicals are synthesized from simple precursors through multistep reactions. It is interesting to know how plant cells developed such complicated metabolism. Here, we report the metabolic plasticity in isoquinoline alkaloid biosynthesis in transgenic *California poppy* cells with ectopic expression of *Coptis japonica* scoulerine-9-*O*-methyltransferase (CjSMT). SMT is the enzyme which catalyzes the *O*-methylation of scoulerine to produce tetrahydrocolumbamine (THC) in berberine biosynthesis. Since *California poppy* cells have no SMT but produce benzophenanthridine alkaloids from scoulerine by the two P450s (cheilanthiforine synthase and stylopine synthase), we examined the effect of ectopic expression of CjSMT in California poppy cells (Sato et al PNAS 2001). Whereas the preliminary characterization confirmed that transgenic cells produced THC, many newly found peaks were not identified. Here, we report the identification of these newly found chemicals in transgenic *California poppy* cells. When we analyzed these compounds by LC-MS, LC-NMR etc, we found that those compounds belong to protopine and benzophenanthridine type alkaloids such as allocryptopine and 10-hydroxychelerythrine and protoberberine type such as THC minor. Interestingly, newly identified compounds had *O*-methylated residue derived from SMT reaction instead of methylendioxy ring in normally found compounds in non-transgenic poppy cells. These results suggest that transgenic poppy cells had some potential to use the new substrate to produce more diversified metabolites. Introduction of new metabolic blanch and metabolic plasticity is discussed. "
(a) kyoto university

P60010 A new route for monoterpene biosynthesis in tomato glandular trichomes: the use of neryl diphosphate rather than geranyl diphosphate as substrate
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"Trichomes are epidermal protuberances present on aerial tissues of many plants and are capable of producing an array of specialized metabolites having a wide range of known and proposed functions including coping with biotic and abiotic stresses and as attractants for pollinators and seed-dispersing animals. Many of these specialized metabolites also have significant commercial value for use as pharmaceuticals or cosmetic fragrances. We are using tomato trichomes to study the natural products these distinctive epidermal cells produce. From sequencing of a tomato (*Solanum lycopersicum* cv. M82) trichome cDNA library, we recently identified putative *cis*-prenyltransferase and terpene synthase genes that map to chromosome 8. Chromosomal substitution lines containing DNA from *S. pennellii* LA0716 at this region of chromosome 8 produced a monoterpene profile characteristic of LA0716 indicating these genes could encode proteins that catalyze the production of monoterpenes in tomato trichomes. The *cis*-prenyltransferase gene was shown to encode Neryl Diphosphate Synthase 1, an enzyme that produces neryl diphosphate (NPP) from isopentenyl diphosphate and dimethylallyl diphosphate. We also show that NPP is the substrate for the terpene synthase (β-Phellandrene Synthase 1) which produces β-phellandrene as the major product as well as several other monoterpenes. Recombinant PHS1 from M82 produced *in vitro* a monoterpene profile identical to that found in Type VI glands of M82 plants. The data indicate that in tomato glands, NPP is the precursor used for the synthesis of monoterpenes by PHS1, and not geranyl diphosphate (GPP), which was previously suggested to be the universal substrate of monoterpene synthases."
(a) Michigan State University (b) University of Michigan

P60011 Metabolomics Reveals that the Devastating *Phymatotrichopsis omnivora* (root rot) Pathogen Circumvents Traditional *Medicago truncatula* Defense Responses and Suggests Strategies for Metabolic Engineering of Resistance
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"*Phymatotrichopsis omnivora* is a devastating pathogen that causes substantial economic losses in more than 2000 dicotyledonous plant species including alfalfa. Currently, no cost effective chemical control methods nor sources of genetic resistance have been identified for *P. omnivora*. Here, a metabolomics approach was used to study the complex biochemical interactions between the model legume *Medicago truncatula* and *P. omnivora* to enhance opportunities for developing resistance to this devastating pathogen. Analyses of the interactions between 0 and 9 days post-inoculation revealed massive metabolic changes. GC/MS revealed decreased sucrose and increased mannitol, arabitol, proline and trehalose in plant roots following fungal infection. Contrary to many *Medicago*-fungal interactions, secondary metabolite profiling by UPLC-MS revealed no significant increase in medicarpin; which is the typical and predominant isoflavonoid induced during *Medicago*-fungal interactions. Interestingly, increased flavone levels were observed and particularly increased 7,4-dihydroxyflavone. We conclude that *P. omnivora* circumvents traditional *Medicago* defense responses by suppressing isoflavonoid/medicarpin biosynthesis, while simultaneously inducing flavonoid biosynthesis. Quantitative RT-PCR suggests that the suppression of the isoflavonoid pathway was at the transcript level and less likely fungal catabolic detoxification of isoflavonoids. In vitro growth inhibition assays revealed that medicarpin and 7,4-dihydroxy flavone possess significant anti-microbial activity against *P. omnivora* and suggest that increased constitutive levels of these compounds represents a strategy for future metabolic engineering of alfalfa resistant to *P. omnivora*. "
(a) The Samuel Roberts Noble Foundation, Ardmore, OK (b) Monsanto Company, St. Louis, MO

P60012 The Biosynthesis of Triterpenoid Glutinol and Friedelin in *Kalanchoe daigremontiana*
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"Triterpenoids ($C_{30}H_{50}O$), a major group of plant secondary metabolites, are synthesized by enzymatic cyclization of a common precursor (2,3-oxidosqualene). The variations in 1,2-methyl and 1,2-hydride shifts as well as deprotonation result in the remarkable structural diversity of triterpenoids. The goal of this project was to identify the amino acid residues that determine product specificity in those triterpenoid synthases catalyzing the maximum number of 1,2-shifts, leading to the formation of glutinol and friedelin. Two novel triterpenoid synthase genes were isolated from *Kalanchoe daigremontiana* and heterologous expression in yeast, followed by GC-FID and GC-MS chemical analysis, showed that they code for a glutinol synthase and a friedelin synthase. Sequence comparisons between both enzymes, together with three-dimensional structure modeling, were used to predict amino acids involved in determining product specificity. To test these predictions, chimeragenesis and site-directed mutagenesis experiments were carried out. The product profiles of the altered enzymes were significantly changed, most notably in the percentages of glutinol and friedelin. These results provide some important new insights into the mechanisms of triterpenoid cyclization, and also have implications for the

biological functions of plant triterpenoids. Glutinol and friedelin accumulate to high concentrations in the cuticular wax of *K. daigremontiana*, where they likely play an important role in protecting the plant against biotic and/or abiotic stress. As a result, understanding the genetics and biochemistry of triterpenoids will also provide us further insight into their physiological and ecological roles."
(a) University of British Columbia, Department of Botany (b) University of British Columbia, Department of Chemistry

P60013 Transcriptome coexpression analysis and comprehensive metabolite profiling led to decoding gene-metabolite correlations in *Arabidopsis* flavonoid metabolism
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" To complete the metabolic map for an entire class of compounds, it is essential to identify gene-metabolite correlations of a metabolic pathway. We used liquid chromatography-mass spectrometry (LC-MS) to identify the flavonoids produced by *Arabidopsis thaliana* wild-type and flavonoid biosynthetic mutant lines. The structures of novel and known flavonoids were deduced by LC-MS profiling of these mutants. Candidate genes presumably involved in the flavonoid pathway were delimited by transcriptome coexpression network analysis using public databases, leading to the detailed analysis of two flavonoid pathway genes, *UGT78D3* and *RHM1*. The levels of flavonol 3-*O*-pentosides were reduced in *ugt78d3* knockdown mutants. Recombinant UGT78D3 protein could convert quercetin to quercetin 3-*O*-arabinoside. The strict substrate specificity of UGT78D3 for flavonol aglycones and UDP-arabinose strongly suggest that UGT78D3 is the first flavonol arabinosyltransferase characterized. The structures of unknown *Arabidopsis* flavonols were confirmed by direct comparison of flavonol 3-*O*-arabinoside-7-*O*-rhamnosides enzymatically synthesized by UGT78D3. In *rhm1* knockout mutants, flavonol 3-*O*-rhamnoside levels were lower but levels of flavonol 3-*O*-glucosides were higher. These results suggest that the rate of flavonol glycosylation is affected by the supply of UDP-rhamnose produced by RHM1. The precise identification of flavonoids in conjunction with transcriptomics thus led to the identification of a novel gene function and a more complete understanding of a plant metabolic network. We will also discuss a gene encoding a novel anthocyanin glycosyltransferase. reference: Yonekura-Sakakibara and Tohge *et al.*, Plant Cell, 2008, 20: 2160-2176. "
(a) RIKEN Plant Science Center (b) Graduate School of Pharmaceutical Science, Chiba University

P60014 Elongation of Carotenoid Biosynthetic Pathway beyond Beta-carotene Rice
Ha, Sun-Hwa-presenter shha@rda.go.kr(a) Kim, Seong-Il (a) Liang, Ying Shi (a) Kim, Jae Kwang (a) Lim, Sun Hyung (a) Kim, Young-Mi (a) Lee, Yeon-Hee (a)
"Some of carotenoids are very functional for human health as a source of provitamin A and components to decrease age-related-macular degeneration, some cancers and heart disease. The demand for simultaneous expression of multiple genes has constantly arisen to manipulate complex carotenoid pathway in plants. Since Golden rice has showed carotenoids could be accumulated in rice endosperm that is one of plant tissues lacking carotenoid, we considered rice endosperm as the best visible system for carotenoid metabolic engineering. Two bicistronic vectors employing the rice codon-optimized 2A sequence of foot-and-mouth disease virus (FMDV) and the internal ribosome entry site (IRES) sequence of crucifer-infecting tobamovirus (CrTMV) were compared by insertion between two biosynthetic genes encoding Capsicum phytoene synthase (PSY) and Pantoea carotene desaturase (CrtI) under a single promoter, respectively. Co-expression of two genes successfully resulted to induce beta-carotene accumulation in both bicistronic systems of 2A and IRES through rice transgene approaches. Currently our research is evolving from beta-carotene to other functional carotenoids like lycopene, zeaxanthin and astaxanthin by further genetic manipulation of carotenoid biosynthesis. By this study we hope to develop high nutritional and commercially valuable rice crop or something more."
(a) National Academy of Agricultural Science

P60015 Analyzing the cuticular wax of trichome cells of *Arabidopsis thaliana*
Wu, May HY-presenter uriko200@hotmail.com(a) Jetter, Reinhard (b) Buschhaus, Christopher W.J. (c)
"The plant cuticle is a hydrophobic layer coating the epidermis of the primary plant body. The cuticle plays an important role in plant life as it protects the plant from pathogenic microbes, insects, UV-light, and water loss. Structurally, the cuticle forms a continuous seal over the outer walls of the epidermal pavement, guard, and trichome cells. Plant cuticles are composed of the fatty acid polyester cutin as well as complex mixtures of very-long-chain (VLC) aliphatic lipids, which form the component known as cuticular wax. It is a complex mixture of straight-chain C20 to C60 aliphatics and may include secondary metabolites such as triterpenoids, phenylpropanoids, and flavonoids. While the biosynthesis and composition of cuticular wax on epidermal pavement cells have been studied in detail, corresponding knowledge on the trichome and guard cell waxes is lacking to date. The goal of the present work was to investigate the composition of waxes on the surface of trichomes on Arabidopsis leaves and stems. Wax composition is typically analyzed after extracting the mixtures from intact organ surfaces with organic solvents. However, this method does not allow a distinction between wax mixtures from different epidermis cell types. Therefore, we performed a comparative study using *Arabidopsis thaliana* wild type, the trichome-less *gl1* mutant and a quadruple mutant characterized by large trichome densities. Waxes were extracted and analyzed by GC-FID and GC-MS. Differences in wax compositions could be correlated with differences in ratios between trichome and pavement cell numbers. The results of the wax analyses of the three lines will be discussed."
(a) University of British Columbia, Department of Biochemistry and Molecular Biology (b) University of British Columbia, Department of Botany and Chemistry (c) University of British Columbia, Department of Botany

P60016 Plant ecdysterone increases protein synthesis and skeletal muscle mass through PI3K-dependent signaling
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"Plant ecdysterone increases protein synthesis and skeletal muscle mass through PI3K-dependent signaling Esposito D, Gorelick-Feldman J., Kizelsztein P., Komarnytsky S., Raskin I. Plant ecdysteroids improve growth, physical performance, and glucose metabolism in mammals. One of the most common plant ecdysteroids, 20-hydroxyecdysone (20HE), is found in many plants including spinach. The present study was designed to investigate the effect of 20HE on protein synthesis in the L6 rat and C2C12 murine skeletal muscle cells, and to evaluate the relative efficacy of oral chronic 20HE treatment in rats. 20HE treatment increased protein synthesis by 20% and induced PI3K-dependent Akt phosphorylation in cell culture. 20HE had no effect on induction and rate of protein degradation. Daily oral administration of 20HE (60 mg/kg for 24 days) increased the size of gastroctnemius muscle in Wistar rats fed a high-protein diet and produced a mild increase of lean body mass compared to non-treated animals as demonstrated by dual-energy x-ray absorptiometry. Orally administrated 20HE showed no androgenic activity in a 10-day Hershberger assay performed in castrated Wistar rats. These studies confirm the anabolic effects of 20HE and begin to elucidate its cellular targets. "
(a) Rutgers University

P60017 Evolution and biosynthesis of medicinally important terpenoids curlone and the turmerones in turmeric and ginger
Koo, Hyun Jo-presenter hjk@email.arizona.edu(a) Gang, David R (a)
"Turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*) are known for their anti-inflammatory and anti-cancer activities, which are attributed to the presence of gingerols, curcumin and related diarylheptanoids. Curlone and the turmerones are sesquiterpenoids from turmeric that have been shown to possess antioxidant and antimutagenic properties. α-Zingiberene and β-sesquiphellandrene, which also possess important biological activities, have been found in both turmeric and ginger. In a database of over 50,000 ESTs from these species, we identified 20 putative monoterpene synthases and 10 putative sesquiterpene synthases, as well as P450 monooxygenases that are good candidates for the oxidation step leading to curlone and the turmerones. Corresponding cDNAs were isolated from the respective species and recombinant proteins were expressed in E. coli or yeast. In vitro enzyme assays identified the following terpene synthases: camphene synthase, α-phellandrene synthase, β-phellandrene synthase, 1,8-cineole synthase, *p*-mentha-1,4(8)-diene (terpinolene) synthase, α-zingiberene/sesquiphellandrene synthase, β-selinene synthase, β-elemene synthase, γ-amorphene synthase, and α-humulene synthase. Comparative metabolite/gene expression profiling suggested that α-zingiberene/sesquiphellandrene synthase initiates the curlone/turmerone synthesis pathway in turmeric. Results from comparative modeling of the different terpene synthases and implications regarding the evolution of terpenoid metabolism in these species will also be discussed."
(a) University Of Arizona

P60018 Convergent biosynthetic evolution in type III polyketide synthases
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http://www.salk.edu/faculty/noel.html
"Type III polyketide synthases are structurally simple, yet biochemically complex enzymes involved in the biosynthesis of a dazzling array of metabolites important in medicine and agriculture. Two classic type III polyketide synthases are the chalcone synthases (CHS) and stilbene synthases (STS). CHS is ubiquitous in plants and is essential for the biosynthesis of tannins, anthocyanins, and other flavonoids. STS is taxonomically restricted and responsible for the biosynthesis of stilbenes such as the well-known red wine compound, resveratrol. The key difference between CHS and STS is their cyclization pattern; STS catalysis involves an aldol cyclization (C2→ C7); whereas CHS choreographs a claisen cyclization (C6→C1). Previous work in our lab discovered an aldol switch responsible for shifting the cyclization specificity in CHS and STS. However, sequence comparisons of STSs from various species suggested that the aldol switch was not conserved. X-ray crystallographic analysis of STSs from grape and peanut indicate that while there is not a consensus sequence, a consensus structure is present. Mutagenesis and biochemical assays indicate that the amino acid residues responsible for shifting cyclization specificity are species-specific. These results support an evolutionary model whereby STSs independently diverged from CHS only to later converge upon the same structural solutions for choreographing their aldol cyclizations. This work highlights the evolutionary relationship between enzyme architecture and the emergence of novel biochemical activities. Understanding how nature fine-tunes enzyme activity lays the foundation for expanding the biosynthetic potential of enzymes for the production of environmentally friendly pesticides, flavors, and fragrances."
(a) Salk Institute for Biological Studies

P60019 Investigation of the biosynthesis and phytotoxicity of the non-protein amino acid *m*-tyrosine
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"*m*-Tyrosine, an isomer of the common protein amino acid tyrosine (*p*-tyrosine), is produced in a large quantities by some cultivars of *Festuca rubra* (Chewings fescue), a commonly-planted turfgrass species. *m*-Tyrosine biosynthesis occurs very infrequently in nature and, to date, no enzymes catalyzing the formation of this non-protein amino acid have been reported. Labeling experiments show that *m*-tyrosine is synthesized in *F. rubra* by hydroxylation of phenylalanine, and metabolic inhibitor studies suggest that the enzyme responsible for this reaction is a cytochrome P450. Candidate genes that may encode such a cytochrome P450 are being investigated. Whereas *F. rubra* is quite resistant to m-tyrosine, growth of most other plants is inhibited by micromolar concentrations. *Arabidopsis* root growth on agar plates is reduced 50% by 3 micromolar *m*-tyrosine. This toxic effect makes *m*-tyrosine an attractive tool for studying the regulation of amino acid metabolism in plants. An *Arabidopsis* mutant screen identified an EMS-induced mutation causing strong *m*-tyrosine resistance. Genetic mapping of this mutation revealed the *adt2-1* allele, a single-nucleotide change in *ADT2*, which encodes an arogenate dehydratase catalyzing the last step in phenylalanine biosynthesis. Enzymatic analyses using recombinant ADT2 proteins showed that the mutated form is no longer feedback-regulated by phenylalanine. Transformation of wild-type *Arabidopsis* with the *adt2-1* allele recapitulated the mutant phenotype. Interestingly, in addition to over-accumulating free phenylalanine, the *adt2-1* mutant also has higher levels of tryptophan. This finding suggests a previously unidentified regulation of aromatic amino acid biosynthesis. "
(a) Boyce Thompson Institute for Plant Research (b) Department of Plant Biology, Cornell University

P60020 "A TTG1 ortholog from Medicago truncatula is necessary for anthocyanin and proanthocyanidin biosynthesis, but not for trichome development"
Pang, Yongzhen-presenter ypang@noble.org(a) Dixon, Richard A. (a)
"The Transparent Testa Glabra 1 (TTG1) from Arabidopsis encodes a WD40 repeat protein which regulates biosynthesis of anthocyanins, proanthocyanidins and mucilage in the seed, and the development of trichomes and root hairs. We have cloned and characterized a TTG1 ortholog from Medicago truncatula (MtWD40-1) via a transposon tagging approach. Deficiency of MtWD40-1 expression blocks anthocyanin accumulation in leaves, formation of other flavonoids in a range of tissues, and proanthocyanidins, mucilage levels in the seed, but does not impair trichome development. MtWD40-1 is expressed constitutively, with highest expression in the seed coat where its transcript profile temporally parallels those of proanthocyanidin biosynthetic genes. Over 150 Medicago Affymetrix probe sets were down-regulated more than 2-fold in seed of an M. truncatula line harboring a transposon insertion in the MtWD40-1 gene, including the flavonoid pathway genes dihydroflavonol 4-reductase and anthocyanidin synthase, as well as the proanthocyanidin-specific genes anthocyanidin reductase and epicatechin-specific UDP-glucosyltransferase. MtWD40-1 complemented the anthocyanin, PA and trichome phenotypes of the Arabidopsis ttg1 mutant, and the anthocyanin phenotype in M. truncatula hairy root under mutant background. "
(a) The Samuel Roberts Noble Foundation

P60021 Analysis of the flavonoid biosynthetic pathway in grapevines using a hairy root transformation system
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http://intranet.csiro.au/intranet/stafflookup2.asp?name=&first=&location=GH
"The flavonoid biosynthetic pathway leads to the production of important secondary metabolites such as anthocyanins, proanthocyanidins and flavonols in plants. These compounds are synthesised in grapes and are important for providing colour and mouth feel in wine. Many of the genes encoding the biosynthetic enzymes and several transcription factors regulating this pathway have been identified in grapevine. Further characterisation of these genes requires forward and reverse genetic studies. However, as grapevine is a woody perennial crop, it is difficult and

time-consuming to generate transgenic plants. In this study, a hairy root transformation system was used for further characterisation of the genes of proanthocyanidin biosynthetic pathway. Hairy root culture is a valuable tool for secondary metabolism pathway studies because of the stable and extensive production of secondary metabolites, their rapid growth, and ease of generation. This presentation will discuss the effects of constitutively expressing and/or silencing biosynthetic and regulatory genes of the grape proanthocyanidin biosynthetic pathway on the metabolite profile and gene expression pattern of this pathway in transgenic hairy roots. For example, hairy roots constitutively expressing anthocyanidin reductase and leucoanthocyanidin reductase genes, which are responsible for the production of the extension units required for proanthocyanidin biosynthesis, have been used to analyse the contribution of the different extension units to the total proanthocyanidin profile and metabolite flow in the flavonoid biosynthetic pathway. "
(a) CSIRO - Plant Industry (b) Department of Primary Industries (c) Heidelberg Institute of Plant Science

P60022 Comparative analysis of the triplicate proanthocyanidin regulators in *Lotus japonicus*
Yoshida, Kazuko-presenter yoshida1106@gmail.com(a) Wakui, Eri (a) Kume, Nao (a) Nakaya, Yumi (a) Yamagami, Ayumi (a,b) Nakano, Takeshi (b,c) Sakuta, Masaaki (a)
"Proanthocyanidins (PAs), which are flavonoid compounds widely distributed in the plant kingdom, protect against environmental stress. The accumulation of PAs is regulated by a ternary transcriptional complex comprising the R2R3MYB transcription factor, a basic helix-loop-helix (bHLH) transcription factor, and a WD40 repeat (WDR) protein. Recently, multigene families of the R2R3MYB-type PA regulators were isolated and characterized. Although their roles as transcription factors that up-regulate PA biosynthetic genes have been elucidated, the significance of their redundancies and functions in planta is unknown. In this study, we characterized recently duplicated putative PA regulators of *Lotus japonicus*, LjTT2a, b, and c, to elucidate their functions in planta and determine differences in transcriptional activation properties. Transgenic studies demonstrated that LjTT2a could induce ectopic PA accumulation in *Arabidopsis*. Further analysis of the *LjTT2* multigene family using a transient expression system revealed differences in transcriptional activities in cooperation with WDR and bHLH proteins isolated from *L. japonicus*. In-depth characterization of chimera constructs of three LjTT2s, as well as site-directed mutagenesis in R2MYB domains were performed."
(a) Ochanomizu University (b) RIKEN Advanced Science Institute (c) PREST, Japan Science and Technology Agency

P60023 Biosynthesis of Multiple Flavonoid Compounds in Maize through the Action of a Flavonoid 3'-Hydroxylase
Sharma, Mandeep-presenter mxs781@psu.edu(a) Moises, Cortes-Cruz (b) McMullen, Michael (c) Snook, Maurice (d) Chopra, Surinder (a)
"The phenylpropanoid pathway in plants leads to the synthesis of several flavonoid end products that include anthocyanin and phlobaphene pigments in maize. Flavonoid 3'-hydroxylase (F3'H) plays a key role in generating flavonoid pigment diversity by 3'-hydroxylation of B-ring. In maize, mutations in the pr1 locus lead to the accumulation of pelargonidin (red) as oppose to cyanidin (purple) pigments in the aleurone cells. We have isolated a putative maize f3'h (Zmf3'h1) gene. Maize populations segregating for pr1 and Pr1 were developed and a genetic ratio of 3:1 was observed for Pr1:pr1. Genetic mapping of the Zmf3'h1 gene confirms the previously known map position of the pr1 locus on chromosome 5L. Further, genetic complementation experiments using CaMv 35S::F3'H1 gene construct established that the putative protein product is capable of performing 3'-hydroxylation reaction both in vitro and in vivo. Transcripts of Zmf3'h1 were detected in floral as well as vegetative tissues of Pr1 plants. On the other hand, pr1 plants did not show any detectable level of Zmf3'h1 mRNA indicating that the pr1 allele used here had a defect that affects transcription of the gene. The defect was identified as an insertion of dinucleotide repeats in upstream promoter region. Further, we show here that Zmf3'h1 is under the independent transcriptional control of both MYB and MYC types of transcription factors. The expression of Zmf3'h1 was also required for the biosynthesis of luteoforol, one of the flavan-4-ols that is precursor of the phlobaphenes. In addition, we show that pr1 plays a role in biosynthesis of maysin and 3-deoxyanthocyanidins. Maysin and 3-deoxyanthocyanidins have insecticidal and antimicrobial properties, respectively. "
(a) Pennsylvania State University (b) Applied Biotechnology Center, CIMMYT (c) USDA-ARS, University of Missouri (d) USDA-ARS, RBRRC, University of Georgia

P60024 "Functional and Structural Characterization of a Flavonoid Glucoside 1,6-Glucosyltransferase from *Catharanthus Roseus*"
Masada, Sayaka-presenter p002552@phar.nagoya-cu.ac.jp(a) Terasaka, Kazuyoshi (a) Okazaki, Seiji (b) Mizushima, Tunehiro (a) Mizukami, Hajime (a)
"Sugar-sugar glycosyltransferases play an important role in structural diversity of small molecule glycosides in higher plants. We isolated a cDNA clone encoding a sugar-sugar glucosyltransferase (CaUGT3) catalyzing 1,6-glucosylation of flavonol- and flavone glucosides for the first time from Catharanthus roseus. CaUGT3 exhibited a unique glucosyl chain elongation activity forming not only gentiobioside but also gentiotrioside and gentiotetroside in a sequential manner. Expression analysis suggested that CaUGT3 may be involved in biosynthesis of defense-related flavonoid glycosides in the leaf tissues of *C. roseus*. In addition, we investigated the functional properties of CaUGT3 using homology modeling and site-directed mutagenesis and identified amino acids positioned in the acceptor binding pocket as crucial for providing enough space to accommodate flavonoid glucosides instead of flavonoid aglycones. These results provide basic information for understanding and engineering the catalytic functions of sugar-sugar glycosyltransferases involved in biosynthesis of plant glycosides."
(a) Graduate School of Pharmaceutical Sciences, Nagoya City University (b) Venture Business Laboratory, Nagoya University

P60025 Transcriptome analysis of Hardy Rubber Tree (*Eucommia ulmoides* Oliv.)
Uefuji, Hirotaka-presenter uefuj001@bio.eng.osaka-u.ac.jp(a) Sakurai, Nozomu (b) Fukusaki, Eiichiro (c) Kobayashi, Akio (c) Suzuki, Hideyuki (b) Shibata, Daisuke (b) Nakazawa, Yoshihisa (a,c) Ogata, Yoshiyuki (b) Fujii, Fumiko (b) Harada, Yoko (a) Inoue, Sumihiro (a) Chen, Ren (a) Mariko, Fujimoto (c) Kazumasa, Hirata (c) Bamba, Takeshi (c)
"*E. ulmoides* is native to China and the only species in the family Eucommiaceae. This plant attracts attention for accumulating a massive amount of *trans*-1,4-polyisoprene (TPI) throughout the plant. TPI is a structural isomer of *cis*-1,4-polyisoprene (CPI) and a low-temperature thermoplastic material used in commercial applications; CPI is well-known as the main component of natural rubber. To elucidate the molecular mechanisms underlying the TPI production, and to manipulate it, functional genomic information is required. In this study, two cDNA libraries were constructed from young current-year stems of *E. ulmoides*; one was from outer tissues including epidermis, cortex, phloem and TPI-producing laticifer cells, and the other was inner tissues including xylem and pith. A total of 27,752 cleaned EST sequences were obtained from random sequencing of 38,167 cDNA clones. The ESTs were assembled into 10,520 unigenes composed of 4,302 contigs and 6,218 singletons. Several putative isoprenoid biosynthetic enzymes including putative TPI synthases were identified from the EST collection. Interestingly, 6 kinds of proteins similar to small rubber particle protein (SRPP) were also found. SRPP has been considered to be a part of the CPI biosynthetic machinery in latex of Para rubber tree (*Hevea brasiliensis*). We are currently carrying out microarray analysis of *Eucommia* gene expression to identify enzymes, transcriptional regulators, etc. involved in TPI production and laticifer formation. This will be presented in detail. This work was supported by the New Energy and Industrial Technology Development Organization (NEDO)."
(a) Hitachi Zosen Corporation (b) Kazusa DNA Research Institute (c) Osaka University

P60026 Molecular characterizatiom of a flavone synthase gene in sorghum
Du, YEGANG-presenter du920848@hkusua.hku.hk(a) Lo, CLIVE (a)
"More than 9000 different flavonoids have been described in plants and flavones are one of the major classes of flavonoids. They exhibit diverse functions in plant physiology, biochemistry and ecology. In addition, they are known to have significant pharmacological properties including anti-oxidation, anti-anxiety and antiestrogenic effects as well as prevention of coronary heart diseases. In this study, three flavones, luteolin, apigenin and tricin, were identified in infected seedlings of different sorghum cultivars which are susceptible or resistant to Colletotrichum sublineolum, the causal agent of sorghum anthracnose. The levels of the different flavones were quantified by liquid chromatography-tandem mass spectrometry in multiple-reactions-monitoring mode. In bioassay experiments, luteolin showed higher inhibitory effects on spore germination of C. sublineolum than apigenin. A pathogen-inducible gene, SbFNSII, which was predicted to encode a P450 enzyme, was demonstrated to be involved in flavone synthesis in transgenic plants. Glycosides of luteolin and apigenin were identified in SbFNSII-overexpressing Arabidopsis lines. To our knowledge, SbFNSII represents the first example of a flavone synthase gene in monocots."
(a) School of Biological Scienecs, The University of Hong Kong, Hong Kong

P60027 Identification of genes from *S. bicolor* involved in the biosynthesis of the allelochemical sorgoleone
Pan, Zhiqiang-presenter zhiqiang.pan@ars.usda.gov(a) Baerson, Scott R (a) Rimando, Agnes M (a) Dayan, Franck E (a) Duke, Stephen O (a)
"Root systems of members of *Sorghum* spp. such as the crop *Sorghum bicolor* exude an allelochemical known as sorgoleone, which is likely responsible for much of the allelopathy observed within this genus. Previous studies suggest that the biosynthetic pathway of this compound initiates with the synthesis of an unusual C16:3 fatty acid possessing a terminal double bond, which serves as the starter unit for one or more alkyresorcinol synthases. The condensation reactions catalyzed by the alkylresorcinol synthase enzyme using malonyl-CoA extender units result in the formation of a 5-pentadecatrienyl resorcinol intermediate. This resorcinolic intermediate is then methylated by an O-methyltransferase, and subsequently dihydroxylated to yield the reduced form of sorgoleone (a hydroquinone). To clone genes involved in the biosynthesis of this compound, an EST database constructed from isolated *S. bicolor* root hair cells was mined, and candidate sequences representing all of the required enzyme classes were identified. The progress to-date on the functional analysis of these genes will be presented. "
(a) USDA, ARS, Natural Products Utilization Research Unit

P60028 Large Scale Survey of Tocol Content in Barley Germplasm
Wise, Mitchell L-presenter mlwise@wisc.edu(a)
"Tocols, compounds showing vitamin E activity, are biosynthesized exclusively by photosynthetic organisms. Although the precise benefits of vitamin E are not known, diets deficient in vitamin E are associated with cardiovascular disease. The tocols consist of eight naturally occurring compounds: α,β,γ,δ-tocopherol and α,β,γ,δ-tocotrienol. These consist of a phenolic, polar chromanol head group, the four isomers differing in the methylation pattern on the phenolic moiety. The tocopherols are conjugated to a saturated phytyl side chain whereas the tocotrienols are conjugated to an unsaturated geranylgeranyl side chain. Among the cereal grains barley has moderately high levels of tocochromanols and usually contains all eight congeners. The few published reports on tocol concentrations in barley indicate that their levels are highly variable and this variablility is correlated primarily to genotype. This study examines the tocol content in 864 germplasm lines of barley from year one of the Barley Coordinated Agriculture Project (Barley CAP). The total tocol content ranged from 21.9 to 103 mg/kg with a mean of 59.9 mg/kg. As expected α-tocotrienol represented the dominate tocol congener, ranging from 4.8 to 61.6 mg/kg with a mean of 32.5 mg/kg. These preliminary results demonstrate that tocol production in barley is highly variable. The data does not allow analysis of environmental effects, however, there is clearly a large genetic effect. Noteworthy is the range of γ-tocotrienol found in the Virginia Tech germlines. These ranged from 4.5 to 13.1 mg/kg with a mean of 8.3 mg/kg, almost twice the mean of the next highest location. The basis for this higher γ-tocotrienol level is not clear although it can be noted that these particular lines are winter barley varieties "
(a) USDA, ARS, MWA, Cereal Crops Research Unit

P60029 Profiling of lignans in transgenic *Arabidopsis thaliana* expressing piperitol/sesamin synthase CYP81Q1 from sesame
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"[Introduction] Sesamin, which is one of lignans synthesized in plants, accumulates in sesame (*Sesamum indicum*) seeds. Because sesamin has physiological effects such as an antioxidative effect, it is now in demand as a health food. Our goal is effective production of sesamin by transgenic plants whose lignan biosynthetic pathways have been modified to accumulate sesamin. However, the lignan biosynthetic pathways in plants are not fully understood until now. Recently, lariciresinol, which is also one of lignans, was detected in *Arabidopsis thaliana*[(1)]. This result showed existence of the lignan biosynthetic pathways in *Arabidopsis thaliana*. In this study, we analyzed lignans in transgenic *Arabidopsis thaliana* expressing piperitol/sesamin synthase (CYP81Q1) from sesame to obtain new information for effective production of sesamin in transgenic plants. [Result] We analyzed lignans in Arabidopsis thaliana for wild type and CYP81Q1 transgenic lines using capillary LC-ESI-MS/MS and UPLC-FLR. As a result, the contents of pinoresinol which is a precursor of sesamin were lower in the CYP81Q1 transgenic lines compared to that in the wild type. Moreover sesamin was detected in the CYP81Q1 transgenic lines, whereas it was not detected in the wild type. These results showed that pinoresinol was metabolized to sesamin in the CYP81Q1 transgenic lines via introduced sesamin synthetic pathway. (1) Nakatsubo, T. *et al.*, *J. Biol. Chem.* 2008, **283**:15550-7 "
(a) Grad. Sch. Eng., Osaka Univ. (b) Suntory HD (c) Suntory Inst. Bioorg. Res.

P60030 Structural novelties of a recently discovered isopentenyl monophosphate kinase
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"Isoprenoids are a group of compounds that exist in all organisms, and are important in plants for membrane structure (sterols), photoprotection (carotenoids), chemical defense (terpenes), and growth regulation (giberrellins). Isopentenyl diphosphate (IPP) is one of the central building blocks used to make all of these compounds, and this molecule is therefore critical for survival. In 2006, a group discovered an enzyme present in the thermophilic archaeon *Methanocaldococcus jannaschii* that was able to phosphorylate isopentenyl monophosphate (IP), thereby producing IPP. This protein, named isopentenyl phosphate kinase (IPK), is interesting because it is a newly discovered branch of the mevalonate pathway. In this work, the crystal structure of IPK from *M. jannaschii* has been solved in its apo form, substrate-bound form, and product-bound form. This protein is the newest structural member to the amino acid kinase (AAK) superfamily, a family of proteins that phosphorylates small molecules with carboxylate and phosphate functional groups. These structures not only highlight dynamic and conformational changes that most likely occur throughout the kinase reaction, but also demonstrate that this enzyme can be engineered to perform another more valuable reaction. The collection of structural and kinetic data for IPK also suggests an unusual role for one of the residues in this enzyme when compared with functional residues in other members of the

AAK family."
(a) The Salk Institute (b) University of California: San Diego

P60031 Molecular cloning and characterization of iridoid 1-*O*-glucosyltransferase from *Gardenia jasminoides*
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"*Gardenia jasminoides* produces geniposide, a well known iridoid glucoside. In the iridoid biosynthetic pathway, cyclopenta-[c]-pyran skeleton derived from geranyl diphosphate is glucosylated. However, this glucosylation step and iridoid 1-*O*-glucosyltransferase are still unclear. We attempted to characterize glucosylation activity toward iridoids and isolate a cDNA encoding a iridoid glucosyltransferase from *G. jasminoides*. *G. jasminoides* cell suspension cultures converted exogenously supplied genipin to geniposide (genipin 1-*O*-glucoside), and also geniposide to gardenoside (8-hydroxygenipin 1-*O*-glucoside). The glucosylation activity was dependent on culture stage of cells. The glucosylation activity was not affected by addition of methyl jasmonate to the cell cultures. In *Catharanthus roseus* cell cultures, which also produce many iridoids and terpenoid indole alkaloids derived from iridoids, genipin was converted to geniposide, but the activity was much lower than that of *G. jasminoides*. Thirteen cDNAs encoding plant secondary product glycosyltransferases (PSPGs) were cloned from cultured *G. jasminoides* cells, using a RACE-PCR method based on the highly conserved C-terminal region of PSPGs. We prepared the recombinant enzymes expressed in *E. coli* and analyzed their genipin glucosylation activities. GjUGT2 catalyzed the formation of geniposide from genipin, and also exhibited an 1-*O*-glucosylation activity toward 7-deoxyloganetin and produced 7-deoxyloganin, whereas hardly or did not accept other phenolic compounds such as *p*-nitrophenol, quercetin, esucletin, and *trans*-zeatin as substrates. To our knowledge this is the first report describing cDNA cloning of the iridoid-specific glucosyltransferase, and provide useful information on the iridoid biosynthetic pathway."
(a) Graduate School of Pharmaceutical Science, Nagoya City University (b) College of Pharmacy, Kinjo Gakuin University

P60032 Chromosomal locations and expression analyses of glucosidase and glucosyltransferase genes involved in benzoxazinone metabolism in wheat
Sue, Masayuki-presenter sue@nodai.ac.jp(a) Nomura, Taiji (b)
"Benzoxazinones (Bxs) are major secondary metabolites in young wheat, rye, and maize, and are considered to be included in the plant resistance against herbivores and pathogens. The five sequential reactions generate DIBOA, one of the major Bxs, from indole-3-glycerol phosphate. UDP-Glc:Bx glucosyltransferase (GT) is responsible for the formation of non-toxic Bx-glucosides in intact plants, and β-glucosidase (Glu) hydrolyzes the glucosides to release the toxic aglycones when the cells are disrupted by infection and wounding. Bxs are accumulated in plants at a high level only in the early stage of growth. Because common wheat is a hexaploid (2n=6x=42, genome formula: AABBDD), there should be at least three homoeologous genes for each step of the reactions. To investigate the regulatory mechanisms of such transient occurrence of Bxs in wheat, individual analyses of the homoeologous genes are required. In this study, we cloned four genes encoding each of GT and Glu from common wheat (*TaGTa-TaGTd* and *TaGlu1a-TaGlu1d*). Their chromosomal locations were determined by the genomic PCR of aneuploid lines of common wheat using specific primers for each homoeolog. *TaGlu1*s and *TaGT*s were shown to be located on homoeologous group-2 (2A, 2B, and 2D) and group-7 (7A, 7B, and 7D) chromosomes, respectively. Quantitative RT-PCR analyses clearly showed that the *TaGlu1* and *TaGT* homoeologs on the B-genome chromosomes are transcribed at the highest level among the four homoeologs. Transcription levels of *TaGlu* and *TaGT* orthologs in three diploid progenitors (2n=2x=14) of hexaploid wheat were similar in ratio to those observed in hexaploid wheat, where the transcription levels in B-genome progenitor were higher than those in A- and D-genome progenitors."
(a) Tokyo University of Agriculture (b) Donald Danforth Plant Science Center

P60033 Transcriptome analysis of the regulatory network of *Or*
Men, Xiao-presenter menxiaoaria@gmail.com(a) Sun, Tian-Hu (a) Dong, Kang (a) Li, Li (b) Lu, Shan (a,b)
"The *orange* (*or*) gene identified from cauliflower (*Brassica oleracea* var. *botrytis*) encodes a 305-amino acid protein which contains a DnaJ cysteine-rich domain at the C-terminus. It represents a gene family highly conserved among flowering plants. An insertion of a *copia*-type LTR-retrotransposon into this gene leads to massive accumulation of β-carotene in the curd, turning it from white to orange, and also a delayed plant development. To investigate the effects of this mutant gene (*Or*, dominant) on carotenogenesis as well as on plant growth and development, the *Or* gene was transformed into wild type *Arabidopsis thaliana* plants and the global expression profiles of the *Or* transformants and vector-only control were compared. Transcriptome analysis revealed that 70, 102, and 136 genes were up-regulated for more than 2-fold in rosette leaves, cauline leaves, and floral buds, respectively. Among these genes, 40 were up-regulated in both cauline leaves and floral buds, where *Or* is highly expressed. These up-regulated genes include ERF/AP2 transcript factors, zinc finger proteins, auxin-responsive and calcium-binding elements, enzymes, and structural proteins. Remarkably, sequence analyses revealed that different putative *cis*-elements exist in the upstream flanking regions and each of which might govern a subset of these 40 genes. Detailed investigation of the regulatory network to decipher the molecular mechanisms underlying *Or*-regulated carotenoid metabolism and plant development is in progress."
(a) School of Life Sciences, Nanjing University (b) Robert W. Holley Center, Cornell University

P60034 "Functional analysis of an *Eucommia trans*-1,4-polyisoprene synthase in tobacco plants"
Harada, Yoko-presenter nakadou@bio.eng.osaka-u.ac.jp(a) Uefuji, Hirotaka (a) Nakazawa, Yoshihisa (a,b) Bamba, Takeshi (b) Chen, Ren (a) Inoue, Sumihiro (a) Li, Xuehong (a) Gyokusen, Koichiro (c) Fukusaki, Eiichiro (b) Kobayashi, Akio (b) Hirata, Kazumasa (b)
"*Eucommia ulmoides* Oliver known as hardy rubber tree is native to central and southern China. *E. ulmoides* accumulates *trans*-1,4-polyisoprene (TPI) in leaves, barks, roots, and pericarps. It is expected that TPI is used as a biopolymer resource because of its unique properties that is thermoplastic. TPI is thought to be synthesized by sequential condensation of isopentenyl diphosphate (IPP) with allylic prenyl diphosphates, which would be catalyzed by a family member of *trans*–prenyltransferase (TPT). However, the genes encoading long-chain elongating TPT have not yet been reported. In this study, we identified nine *trans*-isoprenyl diphosphate synthases (TIDSs) by analyzing degenerate RT-PCR products and ESTs from *E. ulmoides*. We investigated the function of TIDS1, the most potential candidate, in tobacco plants. We introduced TIDS1 driven by the CaMV *35S* promoter into tobacco. High molecular weigh polymer (about 10^4-10^5*M*) was detected in a toluene extract from TIDS1-overexpressing lines by size exclusion chromatography, whereas it was not detected in wild type. This fraction was subjected to 1H NMR analysis, and signals characteristic to TPI were observed. These results suggest that TIDS1 functions as a TPI synthase. This work was supported by the New Energy and Industrial Technology Development Organization (NEDO)."
(a) Hitachi Zosen Corporation (b) Osaka University (c) Kyushu University

P60035 Regulation of Anthocyanin Biosynthesis in Red Cabbages
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"The color of red cabbage is due to the accumulation of large quantity of anthocyanins. To investigate the general regulatory control of anthocyanin

production in red cabbages, the expression of anthocyanin biosynthetic genes and regulators from eight commercial cultivars was examined. While the four green varieties had negligible amounts of anthocyanins under normal growth condition, the four red varieties contained varied amounts of anthocyanins to levels up to 1.60 mg g-1 fresh weight. HPLC analysis of the four red varieties revealed that they produced similar composition of various forms of cyanidin glucosides, but at different concentration. Molecular analysis indicated that all the red cabbages shared common pattern of regulatory control for the anthocyanin biosynthesis. While the early genes (from *PAL* to *F3H*) of anthocyanin biosynthetic pathway showed similar expression levels between green and red cultivars, the late structural genes, *F3'H*, *DFR*, *LDOX*, *UGT* and *GST*, were constitutively up-regulated during all the developmental stages of red varieties. The expression of these structural genes also dramatically increased in green and red cabbages under nutrient stresses. The increased expression of the late structural genes coincided with a coordinated increase in transcript levels of a bHLH gene *BoTT8* and a MYB transcription factor *BoPAP1*. These results indicate that the activation of two regulatory factors by unknown mechanisms up-regulates all the late structural genes for the onset of anthocyanin biosynthesis in the red cabbages. Moreover, the amount of total anthocyanins in red cabbages was found to be positively correlated with total antioxidant power, implicating the potential health benefit of red cabbages to human health."
(a) Department of Plant Breeding and Genetics, Cornell University (b) Robert W. Holley Center for Agriculture and Health,USDA-ARS, Cornell University

P60036 Changes of capsaicinoid concentrations in chili peppers in response to plant growth in carbon dioxide enriched atmospheres.
Sicher, Richard C-presenter Richard.Sicher@ars.usda.gov(a)
http://www.ars.usda.gov/Main/site_main.htm?modecode=12-75-51-00
"Capsaicinoids are lipophilic, secondary metabolites in chili peppers that produce a burning sensation on contact with animal tissues. In addition to their widespread use as flavoring ingredients in food preparation, capsaicinoids are used for pain relief, in self defense sprays, to treat neuralgia and they may have antitumor activity. The two most common and biologically active capsaicinoids are capsaicin (8-methyl-N-vanillyl-6-nonenamide) and dihydrocapsaicin. The current study hypothesized that carbon dioxide enrichment would modify the capsaicinoid content of chili pepper fruit. Pepper plants [Capsicum annum L. cv. Bugang] were grown to maturity in matching growth chambers providing ambient and twice ambient carbon dioxide treatments. Between 90 and 100 days after planting whole plants were separated into leaves, stems, fruits and roots prior to determining leaf area and dry matter distribution. Red and green colored fruit were analyzed for capsaicinoid content after seed removal, freeze drying and milling to a fine powder. Capsaicin and dihydrocapsaicin were determined using an HPLC procedure employing an RP-C18 column and a PDA detector. Total dry matter was 33% greater ($P \geq 0.05$) but total fruit dry weight did not differ ($P \geq 0.05$) in the elevated compared to the ambient carbon dioxide treatment. Consequently, harvest index was decreased by carbon dioxide enrichment. Capscaicinoid levels in pepper fruits were decreased by carbon dioxide enrichment ($P \leq 0.01$) and also differed by fruit color ($P \leq 0.01$) and by harvest ($P \leq 0.01$). We concluded that increasing concentrations of carbon dioxide in the atmosphere may adversely affect the pungency of chili peppers in the future. "
(a) usda,ars,psi,csgcl

P60037 Characterization of glycyrrhizin transport in cultured *Glycyrrhiza glabra* cells
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" Glycyrrhizin is the major triterpenoid saponin produced by *Glycyrrhiza glabra*, and stored in the roots and stolons. There are many reports describing cDNA cloning and characterization of glycyrrhizin biosynthestic enzymes, but almost nothing is known about the membrane transport mechanism of glycyrrhizin into vacuoles. We examined a transport mechanism of glycyrrhizin by using cultured *G. glabra* cells, because they cannot synthesize glycyrrhizin *de novo*. We showed that the *G. glabra* cells were able to uptake glycyrrhizin from the medium. Inhibitor experiments suggested that glycyrrhizin uptake was ATP dependent. Anion blockers, pH gradient destroyers, and glycyrrhetinic acid inhibited glycyrrhizin uptake. To clarify the molecular mechanism of glycyrrhizin transport, membrane vesicles of the *G. glabra* cells were fractionated using a discontinuous sucrose gradient. The tonoplast-enriched vesicles exhibited ATP-dependent glycyrrhizin uptake activity. Further characterization of glycyrrhizin transport using various inhibitors revealed that this transport activity was inhibited by sodium orthovanadate, a typical inhibitor of ATP-binding cassette (ABC) transporters, but was hardly affected by various inhibitors, such as DCCD, CCCP, or bafilomycin A_1, suggesting involvement of an ABC transporter in the accumulation of glycyrrhizin in the vacuole. Competition experiments using various triterpenoids of both aglycone and glycoside varieties suggested that this ABC-type transporter recognizes the oleanane ring of aglycone as its substrates. This is, to our knowledge, the first biochemical characterization of the membrane transport of triterpenoid saponin into the vacuoles. "
(a) Graduate School of Pharmaceutical Sciences, Nagoya City University (b) Iwate Medical University

P60038 Growth Inhibitory Activities of Aquatic Vascular Plants against *Microcystis aeruginosa* and Fourier Transform Infrared Analysis
Choi, Hong-Keun (a) Kwon, Suong-Ho-presenter totoron2@hotmail.com(a,c) Sang-Kyu, Park (a,b)
"We tried to figure out the growth inhibitory activities of aquatic vascular plants against *Microcystis aeruginosa* (UTEX 2385) using spectra-chemical data and statistical analysis. Thirty three plants (25 species) were extracted by 80% methanol using for growth experiment for M. aeruginosa. *M. aeruginosa* was cultured with 25^0C, 150 rpm, 2,000 lux, Light/Dark = 16 / 8 chamber in L16 liquid medium that checked optical density between 620~810nm on 2days and calculated concentration of chlorophyll-a. And we obtained the profiles using Fourier Transform Infrared (FT-IR) spectroscopy. FT-IR spectra collected over the 4,000 cm-1 ~ 650 cm-1 and processed using the spectrum GX (PerkinElmer) in ATR mode. Cultured data were calculated into inhibition activity based on blank and normalization, and spectra baseline were corrected as the smallest absorbance was equal to 0. The normalized spectra were calculated by using the Savitzky-Golay algorithm. These data were compared by discriminant analysis (DA) and correlation coefficient (MATLAB). The regression function was gained from CANDISC-DA result based on some FT-IR data. By the correlation coefficient analysis, four groups shows the highest relationship; 3,798 cm^{-1} ~ 3,757 cm^{-1}, 3,747 cm^{-1} ~ 3,313 cm^{-1}, 2,224 cm-1 ~ 1,500 cm^{-1} and 1,062 cm^{-1} ~ 1,000 cm^{-1}. In addition, few representative peaks (such as 3,662 cm^{-1}, 3,779 cm^{-1}, 1,620 cm^{-1} and 1,253 cm^{-1}) has positive relation with inhibition activity ($R^2 > 0.2$). We confirmed a verified relationship between growth inhibition activities of several aquatic vascular plants against *M. aeruginosa* (UTEX2385). Also we suggest that FT-IR can be used for a fast screening technique in terms of growth inhibitory compound screening of aquatic vascular plants bioassay."
(a) Ajou University (b) Professor (c) Graduate Student

P60039 Peroxynitrite scavenging activity of betalains isolated from beetroot and its reaction products
Maeda, Makiko-presenter m-maeda@abs.agr.hokudai.ac.jp(a) Sakihama, Yasuko (a) Hashidoko, Yasuyuki (a)
"Betalains, one of the classes of four major pigments in plants, are water soluble, nitrogen-containing pigments, which have two types, red-violet betacyanins and yellow betaxanthins. Distribution of betalains is limited in Caryophyllales except for Caryophyllaceae and Molluginaceae, and betalains and anthocyanins are mutually exclusive. Compared with other plant pigments, physiological functions of betalains in plant cell are not

studied well, remaining to be elucidated. Recently, betalains as antioxidant agents against reactive oxygen species (ROS) have been reported and their functionalities have been received increased attention in food science. Reactive nitrogen species (RNS) potentially attack biomolecules, leading to oxidative damage as well as ROS. Betalains are oxidized by ROS, but reactivity of betalains toward RNS has not yet been reported. Here we examine ability of betalains to quench RNS in vitro to clarify roles of these pigments in plant cell. Two major betalains isolated from root of red beet (*Beta vulgaris* L.) had absorption maxima at 538 nm and 474 nm, identical with betacyanin and betaxanthin respectively. Absorption spectra of them after the addition of nitric oxide (NO) showed no significant change, but when $ONOO^-$ was added, their original absorption peaks rapidly decreased and new absorption peak was observed at 350 nm. The products of betalains reacted with $ONOO^-$ were analyzed by HPLC at 350 nm, and polar peaks originated from $ONOO^-$-scavenged betalain were newly detected. The peak intensity was positively dependent on concentration of added $ONOO^-$. From these results, betalains had $ONOO^-$ quenching ability. We are now attempting to elucidate their structures to reveal the reaction mechanism of betalains to quench $ONOO^-$. "
(a) Faculty of agriculture, Hokkaido University

P60041 Cloning and characterization of genes involved in α-tomatine pathway
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"Tomato (Solanum lycopersicum) plants synthesize the steroidal saponin α-tomatine, constituted by a polycyclic aglycone, which is a steroid in steroidal saponins or a triterpene in triterpenoid saponins, attached to a polysaccharide chain. This compound acts in planta as a phytoanticipin against fungi, bacteria and insects. Moreover, it shows interesting pharmacological properties including antibacterial, antifungal, antiviral, antitumoral and anticholesterolemic activities. The biosynthetic pathway of α-tomatine is still weakly understood despite the agrochemical and pharmaceutical importance of this secondary metabolite. Since the pathway of steroids is the starting point of tomatine biosynthesis, molecular cloning of cycloartenol synthase (CAS1), (S)-adenosyl methyltransferase (SMT1), cycloeucalenol cycloisomerase (CYC1) genes was pursued in S. lycopersicum and S. habrochaites by homology-based PCR method or EST clones assembly. In addition to the 2274-bp full-length clone (CAS1), an alternative in-frame splice form of the CAS transcript (CAS1-β), which lacks 106 nucleotides, was found. The expression patterns of both forms are being studied in different tissues. In silico analysis allowed the identification of a beta-D-xylosidase (Xyl) gene putatively involved in the catabolic pathway of α-tomatine. Expression patterns of Xyl were achieved in different organs, and Gateway technology RNAi approaches are being pursued for function analysis of this gene. "
(a) CNR-IGV, Institute of Plant Genetics, Portici, Italy

P60042 Optimization of the transient transformation of *Catharanthus roseus* cells by particle bombardment and its application to the subcellular localization of hydroxymethylbutenyl 4-diphosphate synthase and geraniol 10-hydroxylase
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"The monoterpene indole alkaloids (MIA) synthesized in *Catharanthus roseus* are highly valuable secondary metabolites due to their pharmacological properties. *In planta*, the MIA biosynthetic pathway exhibits a complex compartmentation at the cellular level whereas subcellular data is sparse. Therefore, we developed a high efficiency GFP imaging approach to systematically localize MIA biosynthetic enzymes within *C. roseus* cells by biolistic. The biolistic transient transformation protocol has been optimized with a 12-fold increase compared to previous protocols allowing us to clearly identify the fusion GFP expression patterns in numerous cells. Using this improved protocol we characterized subcellular localizations of hydroxymethylbutenyl 4-diphosphate synthase (HDS), a methyl erythritol phosphate (MEP) pathway enzyme and geraniol 10-hydroxylase (G10H), a monoterpene-secoiridoid pathway enzyme both involved in early steps of MIA biosynthesis. Besides showing the accumulation of HDS within plastids of *C. roseus* cells, we also provides for the first time evidences of the presence of HDS in stromules that are long stroma-filled thylakoid-free extensions in close vicinity with other organelles such as endoplasmic reticulum (ER) or mitochondria in agreement with their proposed function in enhancing interorganelle metabolite exchanges. We also demonstrated for the first time that G10H is an ER anchored protein, consistent with the presence of a transmembrane helix at its amino-extremity. These new results are of importance for the better understanding of the metabolic exchanges between plastids and other organelles during the biosynthesis of the terpenoid moiety of MIA in *C. roseus*, and possibly during the biosynthesis of other isoprenoid-derived metabolites in plant kingdom."
(a) Universite Francois Rabelais de Tours, EA 2106 "Biomolecules et Biotechnologies Vegetales"; IFR 135 "Imagerie fonctionnelle"

P60043 Construction of transgenic plant lines of *Achyranthes japonica* Nakai over-expressing candidate genes for 7-dehydrocholesterol reductase
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"For functional studies of candidate genes for 7-dehydrocholesterol reductase (DHCR) in phytoecdysteroid metabolism, an agrobacterium-mediated transformation system was established in *A. japonica* and a transgenic plant line over-expressing the gene was constructed. Prior to transformation, tissue culture condition was optimized. When the effect of explant source was tested for regeneration of multi-shoot, cotyledonal node (10-days old seedling) showed the regeneration efficiency of 53.0% in the combination of 0.3 uM NAA and 4.4 uM BA in MS medium, and stem node (4-5 leaf pair plant) had an efficiency of 50.0% in the combination of 0.06 uM NAA and 4.4 uM BA. For rooting 0.6 uM NAA was used without cytokinin. To obtain over-expression lines of *DHCR*, the gene was constructed in binary vector pB7WG2D harboring *bar* and *gfp* genes, and introduced into plant cells using agrobacterium LBA4404. The multi-shoots regenerated after transformation were selected on a medium containing 5 mg/L of PPT, and then the expression of *gfp* was detected by green fluorescence for primary screening of transformants. The introduced DNA of *DHCR* in putative transgenic plantlets was confirmed by PCR of genomic DNAs."
(a) College of Appiled Life Science, Jeju National University (b) Subtropical Hoticulture Research Insitute, Jeju National University (c) Gene & Material Bank for Citrus Breeding, Jeju National University

P60044 Microarray analysis of phenylpropanoid metabolism in the model legume *Lotus japonicus*
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"The model legume Lotus japonicus produces a large variety of natural products via the phenylpropanoid pathway, including flavonoids and anthocyanins common to many plants, but also isoflavonoids found almost exclusively in legumes. These phytochemicals are of particular interest as their inclusion in the human diet is considered to have health promoting effects. Whilst many of the biosynthetic genes are known there is limited knowledge concerning the regulation of these genes. Further adding to the regulatory complexity, many of the biosynthetic genes exist as large families. To enable better understanding of the regulation of these biosynthetic pathways a customised microarray chip has been produced containing approximately 1500 genes representing 75 transcription factor (sub-)families and 365 putative genes related to the biosynthesis and

transport of phenylpropanoid-related compounds. Such a microarray allows detailed analysis of individual biosynthetic gene family members in different tissue or under different physiological conditions and may help identify the regulatory genes controlling individual aspect of phenylpropanoid metabolism. "
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P60045 Distribution and biosynthesis of 20-hydroxyecdysone in plant of *Achyranthes japonica* Nakai
Boo, Kyung Hwan (a) Ko , Seung Hee (a) Jeon, Gyeong Lyong (a) Lee, Doseung (a) Jin, Seong Beom (b) Cho, Somi K. (a,b) Kim, Jae Hoon (b,c) Hong, Quanchun (d) Lee, Hyo Yeon (b,c) Riu, Key Zung-presenter kzriu@cheju.ac.kr(b,c)
"Phytoecdysteroids (PEs) are increasingly interesting for their potential role in plant defense against insects. To understand the mechanism regulating their levels in plants, the fluctuation, distribution and biosynthesis of 20-hydroxyecdysone (20E) were examined in *A. japonica*. The total amount of 20E per individual plant was maintained at a constant level while the concentration of 20E in a plant decreased rapidly during vegetative growth. In addition, there was no significant difference between developmental stages in the incorporation rate of [2-^{14}C]-mevalonic acid (MVA) into 20E. These results showed that the biosynthesis of 20E was not developmental-stage-specific, and was maintained constantly in spite of dynamic changes in its concentration. In the experiments on 20E distribution within a plant, the reproductive organs showed a much higher concentration of 20E than vegetative tissues, which led to the postulation that these organs might be the major sites of 20E biosynthesis. However, the data from an incorporation study with [2-^{14}C]-MVA showed no significant difference in the biotransformation rate between these organs. This indicated that 20E could be synthesized in any tissue, and that its biosynthesis was not organ-specific. There was an obvious discrepancy between the level of 20E and its biosynthetic activity in each organ. This implied that the level of 20E in an plant organ might be primarily regulated by source- sink partitioning rather than by *de novo* biosynthesis. "
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P60046 Investigation Into The Molecular Basis For Differential Anthocyanin Accumulation in Raspberry and Grapevine Berries
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"Red raspberries (*Rubus idaeus*) are considered an antioxidant, health-promoting food. Anthocyanin flavonoid compounds have previously been shown to contribute to 25% of the total antioxidant capacity in raspberry fruit extracts. Flavonoid-3'5'-hydroxylase (F3'5'H) is a member of the cytochrome P450 superfamily that catalyzes hydroxylation reactions to yield dihydromyricetin-type (DHM) flavonoids. Raspberries and other Rosaceae family members such as apple (*Malus* spp.) and rose (*Rosa* spp.) preferentially accumulate dihydroquercetin-type (DHQ) anthocyanins while lacking the DHM types. Repeated PCR-based efforts to heterologously clone the gene encoding F3'5'H from raspberry were unsuccessful. A TBLASTN search of translated apple whole genome sequence data was conducted with the grapevine (*Vitis vinifera* L.) F3'5'H amino acid sequence. Subsequent alignments showed that the best apple hits had highest amino acid similarities to grapevine F3'H rather than F3'5'H. Consequently, we hypothesize that the raspberry genome lacks an F3'5'H ortholog but whole genome sequencing will be required to verify this. We are undertaking gain-of-function complementation tests by transforming raspberry with the grapevine F3'5'H driven by the 35S or the native grapevine F3'5'H promoter. We are currently conducting experiments in parallel to develop a protocol for anthocyanin induction in raspberry callus via treatments with single and/or combinatorial environmental and chemical factors. To validate complementation of F3'5'H deficiency, we will verify changes in anthocyanin composition by LC-MS and evaluate antioxidant capacity by ORAC assay in transgenic callus lines."
(a) University Of British Columbia (b) Instituto Agrario di San Michele all'Adige

P60047 An Exploration of Diterpene Biosynthesis in the Euphorbiaceae.
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http://https://keaslinglab.lbl.gov/wiki/index.php/Jim_Kirby
"Terpenes constitute one of the most structurally diverse groups of natural products found in nature. In plants they range from essential and relatively universal primary metabolites, such as sterols, carotenoids, and hormones, to more unique secondary metabolites that serve roles in plant defense and communication. A large number of terpenes have been isolated from plants of the Euphorbiaceae family and the most interesting of these from a therapeutic standpoint contain diterpene (C20) backbones. Specific Euphorbiaceae diterpenes of medical interest include the latent-HIV activator prostratin (and related phorbol esters), the analgesic resiniferatoxin, and a family of anticancer drugs related to ingenol. In spite of the large number of diterpenes isolated from these plants and the similarity of their core structures, there is little known about their biosynthetic pathways. Here, we have chosen four Euphorbiaceae species to investigate terpene biosynthesis and report on the distribution of diterpene synthases in these plants. Other than the universal kaurene synthase, the only diterpene synthase isolated to date from the Euphorbiaceae has been casbene synthase, responsible for biosynthesis of the macrocyclic diterpene casbene in the castor bean (Ricinus communis). We have since discovered genes encoding casbene synthases in our selected Euphorbiaceae species and have demonstrated casbene production in engineered microbial hosts. The prevalence of casbene synthase in the Euphorbiaceae and implications for diterpene biosynthesis in this plant family is discussed. "
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P60048 The role of cis-prenyltransferase in the plant rubber biosynthetic pathway
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"Rubber is an important plant derived commodity required for the manufacture of numerous products essential for modern life. Surprisingly, little is known about the proteins/enzymes involved in the biosynthesis of this important compound. However, recent proteomic- and genomic-based analyses have led to the identification of a cis-prenyltransferase (CPT) as candidate plant rubber biosynthetic enzyme. Furthermore, in vitro cross-linking studies with a rubber substrate analog probe revealed that CPT was localized to the rubber particle, the known site of rubber synthesis and sequestration. As further support for its role in rubber biosynthesis, the CPT gene from the rubber producing species *Taraxacum kok-saghyz* (TKS), showed patterns of temporal and tissue specific expression that strongly correlated with patterns of rubber accumulation in this plant species. To confirm the in vivo role of CPT in rubber biosynthesis, CPT gene overexpression and RNAi studies are being conducted in transgenic TKS. CPT over and underexpresing lines have been generated and are currently being analyzed for changes in rubber polymer molecular weight and rubber yield. The results should provide insight of the CPT role in plant rubber biosynthesis."
(a) University Of Nevada (b) University of Minnesota (c) USDA, Western Region Research Center

P60049 Functional analysis of the gene family that encodes for the 1-deoxy-D-xylulose-5-phosphate synthase gene involved in the synthesis of plastidic isoprenoids in maize

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"The methyl-D- erythritol 4-phosphate pathway (MEP) is responsible for the biosynthesis of diverse number of natural compounds such as major antioxidants (carotenoids and tocopherols), anti-oncogenic drugs and several of the major plant hormones. This pathway has become a clear target to develop new herbicides and antimicrobial drugs and to improve compounds of nutrient, medical and agricultural value Understanding of the mechanisms that regulated this pathway in plants is important. The first enzyme of the MEP pathway is the 1-deoxy-D-xylulose-5-phosphate synthase (DXS). In plants DXS is one of the limiting steps of the pathway. In most plants DXS is encoded by a family of genes. In maize, independent DXS genes has been reported displaying differential expression. We present the characterization of the genes encoding putative DXS proteins. Three independent DXS-like genes have been identified that belong to specific clades. To support the functionality of these genes, their subcellular localization was determined. We have analyzed the capacity of each of these genes to complement enzymatic function in *E. coli*. These genes exhibit a unique expression pattern, supporting particular functions for each gene in the synthesis of particular isoprenoids. At the transcriptional level the maize *dxs* genes display a specific tissue expression pattern. At the protein level the maize DXS is feedback regulated at the post-translational level in response to a blockage or decrease of the pathway flow. We have corroborated that in response is similar to the Arabidopsis DXS, suggesting that this mechanism of regulation is conserved in evolution. "

(a) University of Mexico (b) el Centre for Research in Agricultural Genomics (CRAG), Barcelona

SESSION P61 – SEED BIOLOGY

P61001 Flower and grain specific promoters from wheat for modifying grain characteristics

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"Grain-specific promoters are essential tools for the improvement of grain quality using transgenic approaches, or for functional genomics studies of grain development. We identified and cloned several promoters from wheat, which are active either in different grain tissues or in early grain and flowers shortly before fertilization. Transcriptional GUS fusion constructs were transformed either into rice and barley using *Agrobacterium tumefaciens*-mediated transformation, or in wheat using microprojectile bombardment. *TdPR60* promoter was activated specifically in endosperm transfer cells and adjacent starchy endosperm in wheat and barley starting from 9 days after pollination. It offers the potential to improve grain quality by modifying the quality and quantity of nutrient transfer to the grain. The *TdPR61* promoter activity was localised in the region surrounding the embryo as early as 6 DAP and in the embryo at later stages of grain development. It can be used to engineer sterility through the early grain abortion. *TdPRPI* promoters were active in female gametophyte before fertilisation; the maximum activity was detected in aleurone and adjacent to aleurone cell layers at 5 to 7 DAP and decreased at 15 DAP. These promoters will be useful for improvement of the disease resistance at early stages of grain development and in minimising grain abortion under stress conditions. Another promoter, *TdPRGL7* was activated in ovule before fertilization. The GUS expression was detected in embryo and endosperm and the promoter was active until 40 DAP. The promoters have been cloned in the pMDC32 vector in the position of 2x35S promoter and obtained vector derivatives can be used for the transformation of rice, barley and wheat to target gene expression to different grain tissues. "

(a) Australian Centre for Plant Functional Genomics Pty Ltd, University of Adealide

P61002 Flux in the coding and small RNA transcriptomes during soybean seed and seedling development

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"Soybean cotyledons and seed coats undergo major developmental transitions during seed development. Changes in the transcriptome from a few days after flowering through maturation and in the cotyledons during early seedling growth have been revealed using microarrays. In addition, we have explored the flux of small RNA populations for several stages and tissues of soybean seed and seedling development by using Illumina Sequence-by-Synthesis deep sequencing yielding over 50 million reads. The small RNAs are being characterized by enumerating the species present and alignment of each to the curated miRNA Sanger database and to the non-redundant nucleotide and protein databases of NCBI. Many match known miRNAs from other organisms in the database, while many others appear to be novel siRNAs or miRNAs. We have also developed statistical techniques to identify differentially expressed small RNAs. Similar to the coding transcriptome, the small RNAome reveals many organ, tissue specific and developmental shifts in the population of small RNAs. Finally, we are correlating the changes in small RNA populations to those of the mRNAs as elucidated by microarray analyses and additionally by digital gene expression high throughput sequencing during normal seed development and in selected mutant isolines. The information is being used to identify possible targets of the siRNA and miRNAs. Naturally occurring soybean siRNAs that control the activity of chalcone synthase in a tissue specific manner only during seed coat development are examples of endogenous small RNAs that produce a physiological effect and visible trait (inhibition of pigmentation) in soybean. Supported by CRI and SDBC programs of Univ. of Illinois, USDA, USB, and ISA. "

(a) University Of Illinois

P61003 Characterization of new molecular players in ABA action during seed germination.

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"Seed biology is a highly topical subject in Plant Molecular Biology research. Many of the molecular and physiological events within the seed, specially those related to seed germination, remain to be elucidated. Essential regulatory molecules, such as abscisic acid (ABA) and jasmonic acid (JA), have been related to seed germination although most of the molecular bases of the ABA and JA action during germination are currently unknown. Furthermore, it is known that alteration in a concrete signal transduction pathway may affect plant sensitivity to another hormonal signaling pathway. In this way, the JA-insensitive mutants *coi1-16* and *jar1-1* additionally show ABA-hypersensitive phenotypes being a good strategy to isolate mutants affected in ABA responses. Based on this observation, we have developed a screening strategy to find novel ABA mutants during germination using the JA insensitive background *coi1-16*. Screening of 105.000 M2 seedlings from 17 M1 EMS-mutagenized *coi1-16* families, yielded 72 M2 new putative mutants able to suppress the *coi1-16* ABA-hypersensitive phenotype (*sac* mutants: *suppressor of ABA hypersensitivity of coi1*). The genetic characterization of these mutants uncovered new alleles of the previously identified *abi3* and *abi4* mutants, providing a proof of concept of the feasibility of our screening strategy. In addition, we have isolated one hypermorphic mutation *(sac13)* on higher arm of Chromosome 1, affecting a protein phosphatase type-2C that negatively regulates ABA signaling pathway during seed germination. Another mutant *(sac1)* is located on higher arm of Chromosome 5 where no gene implicated in ABA responses is located. The phenotypical and molecular analysis of the corresponding mutants

are under study. "
(a) Centro Hispano-Luso de Investigaciones Agrarias. Universidad de Salamanca. Spain

P61004 "Protein disulfide isomerase-2 (PDI2) interacts with, and localizes to, diverse components of the nucleus and secretory pathway, including the embryo arrest transcription factor (MEE8) involved in seed biogenesis"
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"Protein disulfide isomerase (PDI; EC 5.3.4.1) belongs to the super-family of thiodisulfide oxidoreductases. PDI catalyzes the formation, reduction and rearrangement of disulfide bonds in target proteins of eukaryotes. Most PDIs have six domains: a signal peptide, two thioredoxin catalytic sites (CXXC), two fold domains, and a KDEL endoplasmic reticulum (ER) retention signal. The classical PDI resides in the ER and mediates the folding of nascent polypeptides of the secretory pathway. PDIs also serve as redox-response regulators and chaperones outside the ER. Recently, we showed that PDI5 (At1g21750) chaperones and inhibits cysteine proteases during trafficking to vacuoles prior to programmed cell death of the endothelium in developing seeds (Ondzighi et al 2008). Here we describe PDI2 (At5g60640) and its involvement in proper seed development by interacting with maternal effect-embryo arrest transcription factor (MEE8). RT-PCR and PDI2-specific immunoblot and -fluorescence microscopy were used to show that PDI2 is expressed in seed integuments, leaves, immature stamens and gynoecia, and roots. A native PDI2-promoter-N-terminus-GFP fusion was highly expressed in developing seeds, root tips, and entire 5-d-old seedlings. Immunoelectron microscopy with GFP- and PDI2-specific antisera demonstrated that PDI2 is found in the ER, nucleus, Golgi, vacuole, plasma membrane and apoplasm of seed and root cells. Using the yeast-two hybrid assay and coimmunoprecipitation, PDI2 interacted primarily with MEE8, and secondarily with response to dessication-RD2 factor, syntaxin, Rho factor, and binding protein-BiP1. The pdi2 T-DNA insertion mutant, which lacks the PDI2 protein, exhibited a leaky phenotype of darker and shriveled seeds, indicating an impact on proper seed biogenesis.ı"
(a) University of Hawaii (b) University of Colorado

P61005 Laser capture microdissection of Arabidopsis seed compartments: RNA profiling provides new insight into seed development
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"Development of the Arabidopsis seed is programmed by a complex network of regulatory genes and pathways partitioned in a cell type-specific manner. Access to genes responsible for various biological processes is essential to further our understanding of seed biology. To obtain insight into the expression patterns of genes that underlie seed development, we used a combination of laser-capture microdissection and DNA microarray technology to profile RNA populations from 36 cell and tissue types from all stages of developing Arabidopsis seeds. RNA profiling of the embryo proper, suspensor, micropylar endosperm, peripheral endosperm, chalazal endosperm, chalazal seed coat and distal seed coat has allowed a more rigorous understanding of the functional relationships between the cell types during development and exposed previously undiscovered cellular processes separated in a spatiotemporal manner. Because coordinated development of the embryo, endosperm and seed coat is required for the overall growth of the seed, specific cell and tissue type RNA profiles of each region were examined. The data validates previously known processes occurring within the seed including cell patterning of the embryo proper. However, laser capture microdissection also provides new insight into compartments like the endosperm, whose function is still relatively unknown. Dynamic changes in gene expression within the three endosperm domains is specified through alterations in processes like seed filling and hormone metabolism which are partitioned both in space between the three domains as well as in time through seed morphogenesis and maturation. Novel roles for the endosperm and its relationship to other compartments within the Arabidopsis seed will be discussed. "
(a) UC Davis (b) UC Los Angeles

P61006 SEED LONGEVITY - *Nelumbo nucifera*
Shen-Miller, Jane-presenter shenmiller@biology.ucla.edu(a)
"Seeds of sacred lotus, *Nelumbo nucifera*, have--a record viability of 1300 yr--whereas the half-life longevity of seeds of most other crops is 5-15 yr. Features, some ***unique***, that probably contribute to lotus seed-longevity: **1)** Pericarp (fruit coat) ***impermeable to water***. **2)** Embryo axis: **a]** ***Protected*** enclosed by hefty cotyledons. **b]** ***Green*** at maturity. **c]** ***Emergence of shoot*** at germination. **3)** *Heat-stable proteins* share homologies mostly with those of ***anaerobes, facultative aerobes, hyperthermophiles***: **a]** Pericarp (***dead tissue***): G-protein, chaperone, stress, defense (*ADP-ribosylation-factor, cyclophilin, Hsp17, Hsp18, lysozyme-pre*). **b]** Cotyledon protein levels ***comparable*** to those of embryo axis: as antioxidant, for development, metabolism, repair/signaling (*Cu/Zn-SOD, GA-oxidase2, trioseP-isomerase, ABC-Fe-transporter*); ~24% of total remain after 549-y of aging. **c]** Embryo axis, ***amount unchanged*** after 549-y, ~40% heat-stable for defense, development, metabolism, protection, repair, stress: ***a.*** Some remain ***polymeric on SDS gel*** (similar to prions of mad-cow disease): *ABC-P-transporter, 1-Cys-peroxiredoxin, DNA-directed-RNA-polymerase, EF-Tu, GroEL, Hsp70, Hsp90, Nucleolin, Rubisco-ssu, VitB6-synthase*. ***b. Dual functional***: endo-exo, metabolism-editing, transcription-editing, food-defense, ***blue/red-light-reception***, or as '1-gene-2-protein' chaperonins (*cellulase, fusion-enolase, maturaseK-intronII, vicillin-antibacterial,* ***phototropin-phytochrome****, GroEL-GroES*). ***c.*** Intron-containing proteins, ***inteins***: possibly in cell maintenance, repair (*enolase1, maturaseK-intronII, reverse-gyrase*). Together, these hefty adaptive features of *Nelumbo* seeds, like those of early-evolving prokaryotic 'generalists' (cyanobacteria), may enable their long-term survival in diverse environments. "
(a) UCLA, Inst Geophys Planet Phys-Ctr Study Evol Orig Life, Dept Ecol Evol Biol

P61007 Expression of a hybrid wheat high-molecular-weight glutenin subunit gene in sorghum
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"The wheat seed storage proteins, referred to as the high-molecular-weight glutenin subunits (HMW-GS), are known to have significant impact on flour quality. To test the effect of HMW-GS on the end-use quality of sorghum flour, we introduced a plant expression cassette that harbors a HMW-GS gene that encodes for an 842 amino acid protein fusion consisting of the N-terminal 124 amino acids derived from HMW-GS Dy10 and the C-terminal 718 amino acids of HMW-GS Dy5. This hybrid gene is flanked by the native Dy10 promoter and 5prime UTR and is terminated by the Dy5 3prime UTR (Nat. Biotechnol 14:875). A total of 20 independent transgenic sorghum events have been generated via Agrobacterium-mediated transformation. Southern blot analysis revealed the transgenic events harbored between one to five inserts. Northern blot analysis indicated extremely high expression of the foreign gene during seed development. Moreover, 2D gel analysis conducted on mature seed showed three unique protein spots relative to wild-type seed. These spots were confirmed to HMW-GS by tandem mass spectrometry. Selected transgenic sorghum events are currently being bulked-up to allow for down-stream functionality testing of the derived flour. In separate complementary set of sorghum transformations we are introducing genetic cassettes designed to specifically down-regulate the accumulation of the sorghum alpha and gamma kafirins, both individually and simultaneously. The long-term goal is to stack the HMW-GS trait with the modulated kafirins events, as a means to address both end-use functionality and digestibility. "
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P61008 A genetic locus and gene expression patterns associated with the priming effect on the upper temperature limit for lettuce seed germination

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"Seeds of lettuce (*Lactuca sativa* L.) are subject to thermodormancy (failure to germinate) at warm temperatures (above 25°C). Seed priming (controlled hydration followed by drying) alleviates thermodormancy by increasing the upper temperature limit (UTL) of germination. We conducted a quantitative trait locus (QTL) analysis of seed germination responses to priming using a recombinant inbred line (RIL) population derived from a cross between *L. sativa* cv. Salinas x *L. serriola* accession UC96US23. A major QTL from UC96US23 associated with high temperature germination capacity (*Htg6.1*) was previously identified in this population. Priming significantly increased the UTL of germination of the RIL population, and a single major QTL was responsible for 47% of the phenotypic variation in UTL due to priming. This QTL collocated with a previously identified one (*Htg6.1*) associated with high temperature germination and with *LsNCED4*, a gene encoding a key enzyme (9-*cis*-epoxycarotenoid dioxygenase) in the abscisic acid (ABA) biosynthetic pathway. Expression of *LsNCED4* after imbibition for 24 h at high temperature was greater in non-primed lettuce seeds compared to primed seeds. In contrast, expression of genes encoding regulated enzymes in the gibberellin and ethylene biosynthetic pathways (*LsGA3ox1* and *LsACS1*, respectively) was enhanced by priming and suppressed by imbibition at elevated temperatures. High imbibition temperatures may independently suppress expression of *LsGA3ox1* and *LsACS1*, preventing germination even in the absence of increased ABA synthesis. Both developmental and feedback relationships regulating hormonal biosynthetic pathways appear to be involved in seed priming effects on germination temperature sensitivity."

(a) University of California

P61009 Vacuolar processing enzyme plays an essential role in the formation of the glutelin crystalline structure in rice seed

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"The vacuolar processing enzyme (VPE) in rice seeds participates in the cleavage of proglutelin into two glutelin subunits in protein storage vacuoles (PSV). However, the physiological role of VPE in rice seeds has been unknown. In order to clarify the physiological function of VPE, the endosperm in the VPE deficient mutant, *glup3*, was observed by both immunoelectron and the immunofluorescent microscopic analyses. DNA sequencing of the VPE gene in three *glup3* lines revealed amino acid replacements or the formation of stop codon in the coding region of the respective gene. The VPE activity in the developing seeds from *glup3* lines was reduced remarkably compared to wild type. In *glup3* endosperm accumulated the proglutelin in PSV. These results suggest that the accumulation of proglutelin in PSV affects the crystallization of glutelin. The PSV in wild type shows ununiform and α-globulin distributes heterogeneously. On the other hand, the PSV in *glup3* endosperm showed spherical, and α-globulin and glutelin precursor were distributed homogeneously within PSV. And *glup3* PSVs lacked the crystalline lattice structure, which is a typical PSV of wild type. These findings suggest that the VPE-dependent processing of glutelin precursor in rice is essential for the PSV structure and the crystallization of glutelin molecules in PSV. The growth retardation in the *glup3* seedlings suggests that the storage of mature glutelin as crystalline structure in PSV is required for the rapid use of glutelin as a source of amino acids during early stages of seedling growth. "

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P61010 Effect of temperature and humidity on seed stability in the *A. thaliana atem6-1* mutant

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"Mature dry seeds can be stable for very long periods of time, and intracellular glasses could potentially play a role in seed stability. Intracellular glasses in seeds are likely made of carbohydrates and proteins, particularly sucrose and LEA proteins. If LEA proteins contribute to glass stability, wild type seed could contain glasses with an elevated glass transition temperature compared to LEA mutants. The major goal of this project was to use accelerated aging assays to determine if environmental conditions differentially affect the germination of the wild-type *Arabidopsis* seeds and those of the *atem6-1* mutant. If ATEM6 protein contributes to glass stability, wild-type seeds might be anticipated to germinate more readily after incubation at a high temperature and/or humidity because of the elevated glass transition temperature. If so, this could provide information about how ATEM6 affects seed development and stability. Seeds were held at a stable temperature and incubated in chambers with varying relative humidities. A higher germination rate in the wild type seeds at high humidities would suggest that the mutant's intracellular glasses may be more affected by the humidity than the wild type glasses. The experiment was also performed at a constant humidity while varying the temperature, looking again for altered germination. Results indicated that high humidity did have an effect on the germination rate of the *atem6-1* mutant seeds compared to wild-type. However, the mutant and wild-type did not show a difference in germination rates at high temperatures, suggesting that humidity has a larger effect on the germination rate in the *atem6-1* mutant."

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P61011 Temporal analysis of protein phosphorylation changes in the soybean seed proteome after pod detachment

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"Partitioning of photosynthate among carbohydrates, oils, and proteins within plant seed is a highly regulated process. To systematically characterize the role of protein phosphorylation in response to photosynthate supply, global quantitative phosphoproteomics was performed on developing seed from detached soybean pods. Soybean pods, 28 days after flowering, were detached from the stem in biological triplicate and seeds were harvested at 0, 6, 24, and 48 hours after pod excision. Whole seed proteins for each harvested time point were isolated under denaturing conditions, and fractionated by high-resolution two-dimensional gel electrophoresis. Phosphoproteins were detected in gel using Pro-Q Diamond stain followed by laser imaging. Gels were then stained with colloidal Coomassie to visualize total protein. Spot detection and quantitation was performed using ImageMaster software. Differential expression (p-value <0.01) was established for 108 phosphoproteins and 326 non-phosphoproteins based on statistical significance of protein expression changes between the control (0 hours) and at least two experimental (6, 24, 48 hours) samples. To date, 75 phosphoproteins and 129 non-phosphoproteins have been identified by liquid chromatography-tandem mass spectrometry. Among the differential proteins identified was the well-characterized phosphoprotein, mitochondrial pyruvate dehydrogenase alpha subunit, which increased eight-fold by 48 hours. Novel phosphoproteins were also identified including cytosolic triose-phosphate isomerase, which showed a six-fold increase six hours after pod excision and decreased to twice the zero time point level by 24 hours. These data suggest regulation of carbon assimilation and partitioning in seed may be more complex than previously thought."

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P61012 Role of ISA3 in starch granule synthesis in the endosperm of rice

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http://www.nias.affrc.go.jp/index_e.html

"Debranching enzymes play a dual role in the synthesis and degradation of starch in plants. We characterized in detail an *isa3* mutant of Nipponbare

rice, screened from transposon insertion mutant lines, and confirmed that ISA3 plays a significant role in the degradation of transitory starch in leaves during the night. Zymogram analysis with recombinant ISA3 expressed in *E. coli* showed hydrolytic activity on β-limit dextrin, amylopectin and starch, but no hydrolytic activity was detectable against amylose. However, zymogram analyses with crude extracts of mature leaf blades or developing seeds of rice failed to detect ISA3 activity. We investigated whether ISA3 plays a role in starch synthesis in the developing endosperm by a combination of microscopic and transgenic analyses. Overexpression of *ISA3* in the *sugary* mutant, which is deficient in ISA1 activity, did not convert phytoglycogen to starch granules in the amyloplasts. The chain length profiles of amylopectin showed that the proportion of DP 7 and DP 10-19 in *isa3* was lower than that in the wild type. The gelatinization properties of starch analyzed by DSC showed that the peak temperature of *isa3* starch was lower than that of the wild type. Based on these results, we conclude that ISA3 plays a role in starch synthesis in the endosperm. Stroma-targeted GFP illustrated *in vivo* that starch granules in *isa3* endosperm were more heterogeneous in size and shape. Our recent studies revealed that amyloplasts divide simultaneously at multiple sites and by budding. We speculate on the possibility that ISA3 may play a role in localized hydrolysis of starch in the vicinity of constriction sites and budding neck, thus facilitating amyloplast division processes."
(a) National Institute of Agrobiological Sciences

P61013 "Functional Analysis of AtTudor, a microtubule-associated RNA binding protein in Arabidopsis"
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"The 4SN-Tudor domain protein is an almost ubiquitous eukaryotic protein with four Staphylococcal nuclease domains at the N-terminus and a Tudor domain towards the C-terminus. It was first reported as a transcriptional co-activator, p100, which stimulates the activity of several transcriptional factors. Recently, 4SN-Tudor was found to be a component of the RNA interference silencing complex, RISC, in mammals, Drosophila and Caenorhabditis elegans and as a component of a cytoplasmic-localizd nucleolytic activity of hyperedited RNAs. In plant, it has been found that 4SN-Tudor domain proteins are cytoskeleton- associated RNA binding protein and involved in storage protein RNA transport and localization in rice. In this study, we investigated Arabidopsis 4SN-Tudor protein, AtTudor. Our results indicated the expression of AtTudor2 in seeds was evidently higher than in other tissues. Mutation in the AtTudor2 gene affects seed germination. Furthermore, we found that the expression of a key enzyme for GA biosynthesis is affected evidently in attudor2 mutant. Together, our results suggest that AtTudor may be involved in GA biosynthesis and seed germination regulation in Arabidopsis."
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P61014 Over-expression of *AtCYS6* retards seed germination and seedling growth in *Arabidopsis*
Hwang, Jung Eun-presenter ibbi0121@naver.com(a) Lee, Sang Yeol (a) Lim, Chae Oh (a)
"Cystatins are a group of proteins inhibiting cysteine-proteinases that have been identified in wide range of organisms including plants. In plants, cystatins (phytocystatins; PhyCys) have been suggested to play a role as storage proteins and to interfere in programmed cell death, defence mechanisms, regulation of endogenous proteases and catabolism, germination and seed maturation. In *Arabidopsis* genome seven phytocystatins have been reported, and we isolated *AtCYS6* and analysed its expression patterns and functions. *AtCYS6* was highly expressed in flower and seedlings, and was also accumulated in seeds. This was confirmed in transgenic plants bearing an P_{AtCYS6}*::GUS* construct, where we found that expression from the *AtCYS6* promoter was strongly induced during seed germination and post-germination periods. The GUS staining patterns in transgenic *Arabidopsis* seedling reflected the expression patterns of the AtCYS6 protein. In addition, constitutive over-expression of the *AtCYS6* gene retarded seed germination and seedling growth, whereas these were enhanced by suppression of T-DNA insertional knock-out mutant (*cys6-2*). The retarded seed germination induced by over-expression of the *AtCYS6* gene caused a general retardation in overall plant growth and development that was reversed in the *cys6-2* mutant. Additionally, stored cysteine peptidase activities were inhibited by *AtCYS6* in transgenic *Arabidopsis*. From these data, we propose that *AtCYS6* participates in the control of germination and seedling growth by regulating stored peptidases activities."
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P61015 Acquisition of Desiccation Tolerance During Seed Development in *Phalaenopsis*
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"Long-term seed storage is a strategy for preserving the biodiversity of the Orchidaceae, currently imperiled by habitat destruction. Like most agricultural seeds, orchid seeds are orthodox in storage characteristics. They can be germinated asymbiotically only in tissue culture. Although naked mature seeds can be surface-sterilized with some loss of viability, the preferred method is surface sterilization of intact pods and culture of the enclosed seeds. This method could also be used to prepare seeds for storage if the timing of acquisition of desiccation tolerance were known. The aim of this work was to identify pod characteristics that correlate with seed desiccation tolerance during development in *Phalaenopsis*. Blooms were hand-pollinated and pod diameter was monitored until dehiscence. Pods were periodically harvested to test for seed water content, embryo development, and germinability both before and after desiccation. We found that pods at 90 DAP contained viable but non-desiccation tolerant embryos whereas the majority of seeds from 120 DAP pods were both viable and desiccation tolerant. Our results suggest that orchid seeds acquire desiccation tolerance during the last quarter of development, when pods are drying in situ. Further work is aimed at defining the exact time of acquiring desiccation tolerance."
(a) Biology Dept.., Grand Valley State University

P61016 The Small GTPase Rab5a is essential for intracellular transport of glutelin precursor from Golgi apparatus and endosomal membrane organization in developing rice endosperm
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"Rice glutelins are synthesized on the endoplasmic reticulum (ER) as a precursor, which is transported via the Golgi to the protein storage vacuole. The *glup4* rice line which over-accumulates the glutelin precursor contains a mutation in the structural gene of the small GTPase Rab5a, which participates in vesicular membrane transport. In addition to the secretion of glutelin and the presence of numerous small glutelin containing protein bodies, three independent *glup4* allelic lines showed the novel appearance of a large membranous complex containing glutelin and globulin located adjacent to the plasma membrane. Analysis of marker proteins of specific endosome compartments showed a significant disruption in endomembrane organization in *glup4* . Transgenic plants expressing GFP fusion proteins of BP-80 and β-galactosyltransferase, marker proteins of the prevacuolar compartment (PVC) and Golgi apparatus, showed native fluorescence in these novel structures. Immunofluorescence studies showed that the ER lumenal chaperones BiP and PDI, the tonoplast aquaporin α-TiP and plasma membrane aquaporin PiP as well as β-glucan were also associated with these structures. These results indicate that the formation of the novel structure in *glup4* endosperm was due to a significant disruption of the endomembrane system by the inactivation of Rab5a. Overall, Rab5a functions not only in the intracellular transport of glutelin precursor from Golgi to the PVC or protein storage vacuole in rice endosperm but also in the maintenance of the general structural organization of the endomembrane system in developing rice seeds."

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P61017 The role of cysteine-rich prolamines for the formation of prolamine protein body in rice

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"The second major seed storage proteins in rice seeds are the prolamines which are composed of cysteine-rich (10-kD, 15-kD, and 16-kD polypeptides) and cysteine-poor (13-kD molecules) species. Although these proteins lack ER-retention signals, they accumulate and assemble as inclusion granules in the lumen of endoplasmic reticulum (ER). When viewed by electron microscopy, the spherical ER-derived PBs display a series of concentric rings surrounding an electron dense core. Temporal expression studies showed that the cysteine-rich prolamine are initially deposited within the ER lumen to form the electron-dense core followed by the accumulation of cysteine-poor prolamines which form the peripheral structure of PB. Immunocytochemical analysis demonstrated that the cysteine-rich 10-kD prolamine resides in the central region of PB, whereas the cysteine-rich 15-kD prolamines are localized uniformly throughout the PB. However, the cysteine-rich 16-kD species are predominantly distributed to the peripheral region of the PBs together with the cysteine-poor 13-kD polypeptides. The differences in the distribution of cysteine-rich prolamine species may reflect different roles in prolamine deposition process within the ER lumen. In the endosperm of *esp3* mutant, which is nearly devoid of cysteine-rich prolamines, the ER-derived PBs lacked the normal spherical morphology but were, instead, hypertrophic and malformed. These results indicate that cysteine-rich prolamines play an important role in the formation of ER-derived PBs."

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P61019 Identification of microRNAs Potentially Associated with Seed Germination and Dormancy

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http://hort.oregonstate.edu/isb/

"microRNAs are involved in developmental programs of plants including seed germination and postgerminative growth of seedlings. Our laboratory has shown that *AUXIN RESPONSE FACTOR10* (*ARF10*), a target of miR160 is involved in crosstalk between auxin and ABA. Seeds expressing miR160-resistant mutant *ARF10* (*mARF10*) exhibited ABA hypersensitivity (Liu et al., 2007; *The Plant Journal*, 52, 133-146). De-regulation of *SQUAMOSA PRMOTER-BINDING PROTEIN13* (*SPL13*) from miR156 had a delay in seedling establishment, which is most likely through the interaction between miR156 and miR172 regulation cascades (Martin et al., unpublished). These results indicate that miRNA regulation of transcription factors plays a critical role in gene regulation at early stages of the plant life cycle. To identify miRNAs and their targets closely related to seed germination and dormancy, we have performed bioinformatic analysis using existing seed dormancy- and germination-associated microarray data and the list of predicted miRNA targets (Arabidopsis Small RNA Project, http://asrp.cgrb.oregonstate.edu/db/). A database of publicly available microarray data (180 data sets) has been assembled from germinating and dormant Arabidopsis seeds. Each sample array was labeled as either germinating or dormant based on the physiological state of the seeds. These seed genes were compared with the known miRNA target genes. This approach has identified multiple miRNA target genes potentially associated with seed germination and dormancy. Multiple CCAAT box-binding factors, miR169 targets are currently investigated."

(a) Oregon State University

P61020 A gene encoding an ABA biosynthetic enzyme (*LsNCED4*) maps precisely with a QTL (*Htg6.1*) for thermodormancy of lettuce seeds

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"Seeds of cultivated lettuce varieties (e.g., *Lactuca sativa* L. cv. Salinas) exhibit thermodormancy (fail to complete germination) when imbibed at warm temperatures (above ~30°C). In contrast, an accession of *L. serriola* (UC96US23), the wild progenitor of lettuce, can germinate at temperatures up to 38°C when imbibed in the light. We identified a quantitative trait locus (termed *Htg6.1*) from UC96US23 that accounted for up to 70% of the variance for high temperature germination capacity. Fine mapping of this locus using a dense genetic map derived from over 15,000 microarray-based sequence polymorphism markers identified *LsNCED4*, encoding 9-*cis*-epoxycarotenoid dioxygenase, as being localized within 1 cM of *Htg6.1*. *LsNCED4* is highly homologous to *AtNCED6* from Arabidopsis, which is a regulated gene in the ABA biosynthetic pathway. Expression of *LsNCED4* increased during imbibition at high temperatures only in seeds of Salinas and of *L. serriola* genotypes susceptible to thermodormancy. Promoter sequences of *LsNCED4* from different genotypes are being examined to identify possible causes of differential temperature sensitivity. Mutants in *LsNCED4* identified via TILLING are being characterized to determine whether this gene is solely responsible for the effect of *Htg6.1*. We previously assayed the effects of high temperature imbibition on expression of selected genes in the ABA, GA, and ethylene biosynthesis and action pathways. Global transcriptome analyses are being conducted to identify relationships among these hormonal pathways in regulating thermodormancy. This project is supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2008-02509."

(a) University of California, Davis (b) Syngenta Seeds

P61021 Expression profiling of candidate genes of lettuce seeds that have been osmoprimed to alleviate temperature and far-red light dormancy.

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"Lettuce (*Lactuca sativa* L.) seeds exhibit dormancy when imbibed under high temperature or far-red light. Osmopriming alleviates these dormancies but typically reduce the longevity of the seed. Previous studies in our laboratory showed that genes associated with photoperceptive pathways and biosynthesis and signaling of phytohormones (ABA and GA) are involved in germination of primed seed. Natural variation exists among different cultivars and recombinant inbred lines (RIL) in response to high temperatures and far-red light during germination. In this study, we selected and analyzed RIL families that constitute the phenotypic extremes of a distribution response when exposed to far-red light or high temperatures during imbibition and germination. Each family was primed with one of three different priming solutions, PEG, PEG + fluridone (ABA biosynthesis inhibitor) or PEG + GA. The primed seed was imbibed under three conditions that included control (20°C red light, 20-Rc), far-red light exposure for 24 hr followed by dark at 20°C (20-FRc-Dc), or dark at 31.5°C (31.5-Dc). Total RNA was extracted at different time points during imbibition. Gene expression of 85 candidate genes was profiled using a multiplex-PCR, capillary electrophoresis-based technology (GenomeLab GeXP, Beckman-Coulter, Inc.) Priming the seed with either fluridone or GA significantly improved germination under the conditions studied. The expression profiling identified common and unique targets of the priming treatments that indicate both ABA and GA synthesis/metabolic pathways are involved in seeds in which dormancy was alleviated. "

(a) Cal Poly Pomona University

P61022 Modulation of Expression of Thioredoxin Is Linked to Fundamental Properties and Important Applications in Wheat Grain
Li, Yong-Chun (b) Ren, Jiangping (b) Frick, Oscar L. (c) Yin, Jun (b) Buchanan, Bob B.-presenter view@nature.berkeley.edu(a) Cho, Myeong-Je (a) Zhou, Sumei (b) Kim, Yong-Bum (a) Wong, Joshua H. (a) Niu, Hongbin (b) Kim, Hyun-Kyung (a) Morigasaki, Susumu (a) Lemaux, Peggy G. (a)
"Work with cereals (barley and wheat) and a legume (*Medicago truncatula*) has established thioredoxin *h* (Trx *h*) as a central regulatory protein in seeds. Trx *h* acts by reducing disulfide (S-S) groups of diverse seed proteins (storage proteins, enzymes and enzyme inhibitors) thereby facilitating germination. Early *in vitro* protein studies were complemented by overexpressing Trx *h* in the endosperm of barley seeds, which showed accelerated germination and early or enhanced expression of associated enzymes (α-amylase and pullulanase). More recent studies have utilized transgenic approaches in wheat to alter the expression of Trx *h* genes in the endosperm: (1) a hordein promoter and its protein body targeting sequence led to overexpression of Trx *h5*, and (2) an antisense construct of Trx *h9* (Arabidopsis designation) resulted in underexpression of that gene in the cytosol. Wheat with overexpressed Trx *h5* showed changes commensurate with earlier *in vitro* work: increased solubility of disulfide proteins and lower allergenicity of the gliadin fraction. Underexpression of Trx *h9* led to effects opposite to those observed for overexpression Trx *h5* in barley, namely retardation of germination and delayed or reduced expression of associated enzymes in developing wheat seeds. Seeds from transgenic wheat lines with underexpressed Trx showed a dramatic delay in preharvest sprouting when grown in the greenhouse or in the field without a decrease in final yield. These results are further evidence that levels of Trx *h* in cereal endosperm determine fundamental properties of as well as potential applications in the seed. "
(a) University Of California, Berkeley (b) Henan Agricultural University (c) University of California, San Francisco

SESSION P62 – SMALL REGULATORY RNA'S

P62001 Structural requirements for miRNA processing in Arabidopsis thaliana
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"MicroRNAs (miRNAs) are small RNAs around 21 nt in length widely distributed among multicellular organisms. They recognize target mRNAs through base complementarity and guide them to degradation or translational arrest. In plants, miRNAs are processed from stem-loop structures located in long primary transcripts by DICER-LIKE 1. They are subsequently loaded into the effector complex RISC (RNA-Induced Silencing Complex), which contains an ARGONAUTE protein. Plant miRNA precursors are longer and also show further size heterogeneity than their animal counterparts. Probably due to this diversity, little is still known on the structural requirements for miRNA precursor processing in plants vis-a-vis animals. Here, we study the sequence requirements for miR319a biogenesis, a miRNA largely conserved in plants. We analyzed the secondary structure of miR319a precursor and carried out site-directed mutagenesis experiments to modify specific regions and residues. Overexpression of miR319a in plants causes pleiotropic developmental defects, such us uneven leaf shape and curvature. Transgenic lines that express mutant miRNA precursors with processing defects failed to accumulate mature miR319 and consequently did not show any developmental defects. We show that the terminal loop of the miR319a precursor is dispensable for the miRNA biogenesis since its modification did not affect the accumulation of mature miR319. On the other hand, when we carried out specific deletions or mutations on the precursor stem we observed that its' processing could be completely impaired. These results allowed us to identify regions of the precursor that are required for miR319 biogenesis in plants. The structural requirements for miR319 processing in Arabidopsis will be discussed."
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P62002 Bioinformatic prediction of target genes for proposed small activating RNAs in *Arabidopsis thaliana*
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http://www.tu-braunschweig.de/ifg/ag/hehl
"Recent studies in mammalian systems suggest that small RNA molecules may be involved in transcriptional activation. To identify putative target genes for proposed activating small RNAs (asRNA) in *A. thaliana*, we used the AthaMap database recently updated by positional information of small RNA target sites. These were derived from a massive parallel signature sequencing approach of the small RNA transcriptome. A total of 403,173 genomic positions of small RNAs have been mapped in the *A. thaliana* genome (Buelow *et al*., 2009, Nucleic Acids Res. 37: D983-986). The small RNAs were derived from a seedling and an inflorescence library enabling the identification of putative target sites for inflorescence-specific small RNA molecules. Correlation with inflorescence-specific microarray expression data identified several possible target genes of predicted asRNA molecules."
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P62003 Mis-regulation of a nat-siRNA Pair in Sperm Cells Results in Single Fertilizations.
Ron, Mily-presenter milyron@berkeley.edu(a) Alandete-Saez, Monica (a) McCormick, Sheila (a)
"We noticed that plants with T-DNA insertions in a gene we call T10 had reduced seed set (50-80% in homozygotes). Reciprocal crosses with WT indicated the male gametophyte was affected, although pollen of homozygous t10 plants appeared normal and could germinate, suggesting a defect in fertilization. T10 is in reverse orientation to the adjacent gene that we call M. The 3 UTR of T10 protrudes ~80 bp into the last exon of M, creating a transcript overlap. We hypothesized that T10 and M generate a pair of natural antisense small interfering RNAs (nat-siRNA) and that the transcript of T10 down-regulates M transcripts in sperm cells. Q-PCR with flowers confirmed that the T10 transcript was almost absent from the t10 mutant, while M transcripts were increased. Promoter-reporter lines confirmed that T10 is expressed specifically in sperm cells while M is expressed in both the vegetative and sperm cells. Co-expressing T10 and a GFP-M fusion in leaves resulted in down-regulation of GFP and a M-specific small RNA form was detected, supporting our hypothesis. Moreover, plants in which M was overexpressed in sperm had phenotypes similar to t10 mutants. DIC imaging of the undeveloped seeds 2-3 days after pollination showed that in the t10 mutant single fertilizations are prevalent (~40% of either embryo or endosperm) compared to their incidence in other known mutants. These results suggest that proper regulation of M in sperm cells is important for fertilization and supports the idea that sperm randomly fertilize the egg or central cell. "
(a) Plant Gene Expression Center and Dept. of Plant and Microbial Biology, USDA/ARS-UC-Berkeley,

P62004 In silico and expression-based identification of microRNAs in rice
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"MicroRNAs (miRNAs) are a class of small RNAs (smRNAs) as ribo-regulators targeting protein-coding and non-coding transcripts. MiRNA precursors possess the hallmarks of extensive foldback structure and generation of smRNAs by DICER-LIKE 1 (DCL1) or DCL4 from the stem of hairpins. We initiated the genome-wide identification of rice miRNAs by the machine learning technique of Support Vector Machines (SVMs). Non-coding sequences from rice genome were scanned using SVM features to look for miRNA candidates with characteristic hairpin structures in Expressed Sequence Tag (EST) data and clustering of sense and antisense smRNA signatures on the predicted hairpins from extant smRNA transcriptome data.

Most published rice miRNAs were found by our SVM, while novel miRNA candidates were also discovered and undergoing confirmation by Northern blot, 5'- and 3'-RACE. Interestingly, rice miRNAs spawn abundant antisense smRNAs too, as shown for Arabidopsis miRNAs (Luo et al., 2009. PLoS Genet 5(4): e1000457. doi:10.1371/journal.pgen.1000457). In addition, the hairpins for a subset of miRNAs, for example miR809 and miR1884, appear to share significantly high sequence similarities with other miRNAs, including miR441, miR446 and miR806 in an antisense manner indicated by sequence alignment. This suggests that inverted duplication of miRNA genes can be the source for *de novo* generation of new miRNA genes."
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P62005 RNAi induced silencing of each gene in the *Arabidopsis thaliana RIBOSOMAL PROTEIN* S15a gene family
Stewart, Chad D-presenter cdstewart2003@yahoo.ca(a) Bonham-Smith, Peta C (a)
"Ribosomes are large, ubiquitous two-subunit ribozymes, responsible for catalyzing peptide bond formation between amino acids in nascent polypeptides. In Arabidopsis, the cytoplasmic ribosome is comprised of a large 60S and small 40S subunit along with 81 ribosomal proteins (RPs) encoded by multigene families (254 genes). Expression of each RP isoform, from each family, of which only one is incorporated into any one ribosome, may be related to stress, developmental or environmental pressures. Four small subunit cytoplasmic (type I) and mitochondrial (type II) RPS15a isoforms are encoded by six genes, *RPS15aA-F*, with one gene, *RPS15aC*, not transcriptionally active. RNAi constructs have been made for each type I gene, *RPS15aA, D* and *F* and each type II gene, *RPS15aB* and *E*, as well as a type I family knockout construct. RNAi sequences, of 150-200 bp, were generated from the 3' UTR of each gene to minimize cross silencing, while the type I family knockout is a 400 bp fragment of the *RPS15aA* ORF exhibiting a high degree of sequence similarity that should result in cross silencing. Previous results, in our lab, from *RPL23a* knockout experiments suggest a link between auxin homeostasis and ribosome biogenesis via micro RNAs. Results from *RPS15a* RNAi silencing will be presented in light of this model."
(a) University of Saskatchewan

P62006 Microarray based approaches to analyze transcriptomes and microRNAs in response to foliar glyphosate application in Festuca arundinacea
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"There is a lack of knowledge on the complexity of glyphosate effects on transcriptome of different plant species. In this study, transcriptome and microRNA (miRNA) profiling of Festuca arundinacea, cv. Falcon in response to varying levels of glyphosate treatments using the Affymetrix GeneChip Wheat Genome and plant miRNA arrays were analyzed. One hundred seventy two genes were found to be significantly altered following exposure to varying glyphosate levels. These included genes involved in photosynthesis, chlorophyll a/b-binding protein (CAB) genes, signal transduction, and the pathogenesis related proteins. As for miRNA chip analysis, thirty-four of the 853 plant miRNA probes show differential regulation upon various glyphosate treatments. The Affymetrix and miRNA microarray results were validated by using RT-PCR and qRT-PCR analyses. Correlation between those two platforms indicated based on differentiation of gene expressions such as transcription factors and ribosomal proteins. The data suggest distinct differential regulation of genes observed in F. arundinaceae in response to glyphosate application."
(a) Sabanci University

P62007 Control of gene expression through regulation of miRNAs
Wiggins, B. Elizabeth-presenter bewiggi@monsanto.com(a) Ivashuta, Sergey (a) Banks, Isaac R (a) Roberts, James K (a) Heck, Greg (a)
"MicroRNAs (miRNAs) and short interfering RNAS (siRNAs) are master regulators of gene expression. These short, non-coding RNAs, typically 21 nt in length, act post-transcriptionally to guide cleavage of a target transcript based on sequence complementarity between the small RNA and mRNA. Expression of hundreds of genes in plants, such as key regulatory genes like transcription factors, is modulated by miRNAs. We have been developing methodologies to specifically manipulate small RNA/target interactions by interfering with productive RISC-complex formation. As a consequence, targets of miRNA activity are up-regulated. This ability to specifically apply miRNA/siRNA-based regulation has been demonstrated using transient systems in *Nicotiana benthamiana* and stably transformed *Arabidopsis*. The strategy of precisely regulating endogenous gene expression may provide a useful tool for engineering transgenic crop plants with improved performance characteristics. "
(a) Monsanto Company

P62008 Characterization of pri-miRNA structures important for efficient miRNA processing in *Arabidopsis thaliana*
Song, Liang-presenter lxs926@psu.edu(a) Fedoroff, Nina (b)
"MicroRNAs (miRNAs) are small, non-coding RNAs that are involved in various aspects of plant development and physiological responses. MiRNAs are processed sequentially from pri-miRNAs and pre-miRNAs. The structural features of pri-miRNA that are important for efficient miRNA processing have yet to be identified. To address this question, we introduced a series of mutations that alter the secondary structure of two pri-miRNAs, pri-miR171a and pri-miR167a. The mutated and wild-type pri-miRNAs were over-expressed in *Arabidopsis* and the phenotypes of the transgenic plants were analyzed. Over-expression of the wild-type pri-miRNA resulted in moderate to severe phenotypic effects, such as reduced shoot branching caused by over-expression of miR171a, or reduced silique length caused by over-expression of miR167a. When the base-pairing at the base of the miRNA-containing stem-loop was fully abolished, the transgenic plants were similar to those transformed with the vector alone. When the bulges and mismatches at the base of the miRNA-containing stem-loop were replaced by perfectly matched base pairs, the transgenic plants exhibited less severe phenotypes compared to those over-expressing wild-type pri-miRNAs. In contrast, removal of the bulges near the terminal loop in the miRNA-containing stem-loop did not significantly reduce the severity of the phenotypes observed upon over-expression of the wild-type pri-miRNA. Consistent with the phenotypic effects, lower levels of mature miRNA were observed in plants expressing pri-miRNA structural variants causing less severe phenotypes. Overall, these observations suggest that a stem with bulges and mismatches at the base of the miRNA-embedded hairpin is the most important structural feature for optimal miRNA processing."
(a) Plant Biology Program, The Pennsylvania State University (b) Huck Institute of Life Sciences and Biology Department, The Pennsylvania State University

P62009 Profiling of the small RNA transcriptome in the biomass-related tissues of Miscanthus X giganteus
Alabady, Magdy S.-presenter msalabad@uiuc.edu(a) Hudson, Matthew E (a,b) Ming, Ray (a,c) Moose, Stephen (a,b)
"The growing emphasis on the role of small RNA in regulating plant development creates an interest in discovering the role contributed by different classes of small RNA in regulating the biomass biosynthesis in Miscanthus x giganteus, which is recently considered a biomass-producer candidate for the bio-fuels industry. Small RNA data sets generated by Massively Parallel Signature Sequencing (MPSS) of small RNA (sRNA) extracted from three Miscanthus tissues: leaves, inflorescence, and rhizome tips, were computationally analyzed. Globally, the three sRNA libraries contained 12. 4 million reads representing 6.7 million signatures. Approximately, 86% of the signatures were singletons while 14% have 2 and more reads. Among the non-

singleton signatures, 45,399 (5%) were conserved among the three tissues. In average, both siRNA (23-24 nucleotides) and miRNA (21-22 nucleotides) constituted 67% and 18% of the total small RNA. Digital gene expression analysis identified 20 significantly differentially expressed miRNA families between the three tissues. Computationally, 24 miRNA, 30 ta-siRNA, and 44 Nat-siRNA novel candidates were identified. Interestingly, several of the differentially regulated and novel candidate miRNAs are targeting major cell wall related genes. In order to gain deeper insights into the role of both siRNA and miRNA in regulating the biomass biosynthesis and to confirm our findings, more small RNA libraries constructed from the main biomass tissue at different developmental stages are being profiled. In conclusion, the ultimate objective of this work is to identify the role of sRNA in the post-transcription regulation of the genes associated with the cell wall biogenesis and to provide a detailed description of the Miscanthus sRNA transcriptome."
(a) Energy Biosciences Institue, University Of Illinois (b) Department of Crop Science, University of Illinois (c) Department of Plant Biology, University of Illinois

P62010 Analysis on the phloem long-distance transport of microRNA by grafting experiment
Kasai, Atsushi-presenter kasaia@cc.hirosaki-u.ac.jp(a) Kanehira, azumi (a) Harada, Takeo (a)
"Small interfering RNAs (siRNAs) moves to spread RNA silencing as the phloem-transmissible signal. Therefore, it is tempting to speculate that microRNAs (miRNAs), given their similarity in structure to siRNAs, could also move systemically to regulate gene expression. However, there has been no direct evidence of their long-distance transport through the phloem. On the other hand, there are recent reports in which grafting studies concerning miR399 showed the implication with being phloem-mobile signal, although direct proof for their presence in the phloem is as yet missing. Here, we used Arabidopsis At-miR172A under control of the companion-cell specific Comenelina Yellow Mottle Virus (CoYMV) promoter in the vector pBI121 (CoYMV:MIR172). We transformed *N. benthamiana* with CoYMV:MIR172 and then four independent T2 transgenic lines were selected. Five days after germination, each apical bud and root were sampled and extracted total RNA. Although quantitative real-time PCR by TaqMan MicroRNA Assays (Applied Biosystems) showed the presence of endogenous mature miR172A in control *N. benthamiana*, the CoYMV:MIR172 transgenic lines contained higher miR172 level in both the apical bud and root than that of control. Then, we investigated the phloem long-distance transport of miR172A using micrografted plants between the wild-type as scion and the CoYMV:MIR172 1, showed the highest miR172 level, as rootstock. The scion showed approximately two-fold higher of mature miR172 than the control, whereas the miR172 precursor transcript was obtained from only the rootstock. Taken together, these results indicate that mature miR172 could transport long-distantly via phloem from source to sink. This work was supported by PROBRAIN in Japan."
(a) Hirosaki University

P62011 Identification and characterization of a new phosphate starvation-induced mircoRNA gene family
Chiang, Su-Fen-presenter su0421@gate.sinica.edu.tw(a) Lin, Shu-I (a,b) Lin, Wei-Yi (a,c) Chen, June-Wei (a) Shih, Arthur Chun-Chieh (d) Chiou, Tzyy-Jen (a,b)
"A new miRNA gene family consisting of two genes was identified from phosphate (Pi)-starved *Arabidopsis* plants by high-throughput sequencing of small RNA. They possessed a potential hairpin secondary structure and could produce a small RNA duplex with 2-nt overhang at the 3' end, the primary criteria of miRNA definition. Moreover, the biogenesis of these miRNAs depended on HEN1 and DCL1, the important components required for the maturation of miRNAs. Their expression was upregulated specifically by Pi deficiency but not by any of the other nutrient deficiencies tested. 5'-RACE analysis identified the targeting of miRNAs to the mRNA encoding a kelch repeat-containing F-box protein. Like the miRNAs, the steady-state level of target mRNA was also increased in response to Pi deficiency, suggesting the fine-tuning or complex regulation of target gene by miRNAs. The homologues of these miRNA genes could be identified in many other dicotyledon plant species, but could not be found in the monocotyledon plants, indicating that the conservation of miRPi may be restricted within the dicotyledons. Conservation of this family implies the importance of miRNA-mediated regulation for the survival under the Pi deficient environment. Further analyses are being carried out to dissect the biological function of these miRNAs."
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P62012 Genome-wide analysis of *Arabidopsis* small RNAs in response to phosphate deprivation
Lin, Shu-I-presenter pcoo2402@gate.sinica.edu.tw(a,b) Hsieh, Li-Ching (c) Shih , Arthur Chun-Chieh (d) Chen , June-Wei (a) Lin , Wei-Yi (a,e) Tseng , Ching-Ying (a) Chiang , Su-Fen (a) Li , Wen-Hsiung (c) Chiou , Tzyy-Jen (a,f)
"Phosphate (Pi) is an essential element whose cellular concentration and systemic distribution in plants is tightly regulated. Maintenance of Pi homeostasis is coordinated by sophisticated and delicate control of Pi sensing and signaling, uptake, allocation and recycling, which is critical for plants to survive through periods of limited Pi supply. Previously, we revealed the crucial role of microRNA399 (miR399) in regulating Pi homeostasis and demonstrated that miR399 functions as a long-distance signal to coordinate the adaptive responses between shoots and roots under Pi deprivation. In this study, high-throughput sequencing of Arabidopsis small RNAs was employed to extend our understanding of small RNA-mediated Pi starvation responses. The genes regulated by these small RNAs are expected to mediate the development and regulation of adaptive responses to external Pi fluctuation. About 3.5 million sequence reads corresponding to 0.6-1.2 million distinct sequence tags from Pi-sufficient or -deficient root or shoot samples were mapped to the *Arabidopsis* genome. Here, we report the identification of several Pi-responsive small RNAs, including known and novel miRNAs, ta-siRNAs and other unique classes of small RNAs. The potential functions of these small RNAs were investigated and discussed. Integrating the function of these small RNAs will provide an additional aspect into the overall regulatory network of adaptive responses to Pi deprivation."
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P62013 Autoregulation of *PAP1/MYB75* via miR828 and TAS4-siRNA during phosphate deficiency
Lin, Wei-Yi-presenter r94621101@ntu.edu.tw(a,d) Lin, Shu-I (b,c) Hsieh, Li-Chi (e) Chen, June-Wei (b) Li , Wen-Hsiung (e) Chiou , Tzyy-Jen (a,b)
"Development of anthocyanin is enhanced when plants encounter Pi deficiency. Accumulation of anthocyanin results from the upregulation of several transcription factors that activate the enzymes in the anthocyanin biosynthesis pathway. PAP1/MYB75 and PAP2/MYB90, the two major transcription factors activating anthocyanin biosynthesis, are upregulated by Pi deficiency. In this study, we revealed the upregulation of miR828 and *TAS4* siRNAs in Pi-starved shoots. *TAS4* siRNAs are generated by the cleavage of miR828 on *TAS4* transcript. TAS4-siR81(-) has been reported to target the mRNAs of *PAP1/MYB75, PAP2/MYB90* and *MYB113*. Analysis of a *PAP1/MYB75* activating line revealed that PAP1/MYB75 functions upstream to

activate the expression of miR828 and TAS4-siR81(-). On the other hand, the increase in the expression of *PAP1/MYB75, PAP2/MYB90* and *MYB113* as well as the anthocyanin content in *tas4* and *mir828* knockout mutants provide further evidence for the feedback regulation of those MYB transcription factors via miR828 and TAS4-siR81(-). On the basis of these observations, we propose an autoregulatory mechanism in which an adequate expression level of *PAP1/MYB75, PAP2/MYB90* and *MYB113* and a proper accumulation of anthocyanin are maintained during Pi deficiency."
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P62014 Battles between plant genome and its parasite through the action of a small RNA
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"Transposons are genomic parasites, which constitute a major component of eukaryotic genome. RNA silencing machinery is a defense system against them, and suppresses transposons to stabilize the hosts genome. It is a nature of transposons as a selfish DNA element to increase their copies, despite the presence of this defense system. This implies the existence of a pathway, which enables transposons to escape hosts defense. A similar situation is observed among the relation between viruses and their hosts. It is known that RNA silencing is a defense machinery of host against plant RNA viruses and viruses often encode silencing suppressor proteins, which suppress hosts RNA silencing and establish infections. We are focusing on a miRNA, which regulates the expression of a component of hosts silencing machinery. This miRNA is originated from recently produced inverted repeats and these inverted repeats seem to locate within a family of DNA transposons dispersed at 5 loci in rice genome. Thus, the regulation through the action of this miRNA evokes a model how transposons escape from silencing machinery of host. Similar to this situation, some RNA viruses utilize RNA silencing pathway to overcome the defense of host. We will discuss a possible mechanism of transposons escaping from hosts silencing and its contribution to genome evolution in rice. "
(a) Graduate school of Bioagricultural Sciences, Nagoya University (b) Japan Science and Technology Agency PRESTO

P62015 Identification and characterization of an Argonaute 7 Gene in *Medicago truncatula*
Zhou, Chuanen-presenter chuanen.zhou@gmail.com(a) Han, Lu (a) Tadege, Million (b) Mysore, Kiran (b) Wang, Zeng-yu (a)
"Trans-acting siRNAs (ta-siRNAs) are endogenous siRNAs that direct the cleavage of complementary mRNA targets. It has been shown that TAS3 ta-siRNA plays an important role in plant development through ARGONAUTE 7 (AGO7) which negatively regulates the expression of two auxin response factors (ARF3/ARF4). Arabidopsis *ago7* mutants cause premature expression of adult vegetative traits due to accelerated juvenile-to-adult transition. In the past several years, *Medicago truncatula* has been developed into a model legume species. *M. truncatula* has some interesting characteristics that cannot be studied using other model species like Arabidopsis and rice, such as compound leaf, complex inflorescence architecture and nitrogen fixation. By screening a large population of *Tnt1* retrotransposon tagged *M. truncatula* lines, two individual *mtago7* mutants were identified. Mutants showed ectopic leaflets in juvenile phase, lobed leaf margin in adult phase and infertility. Scanning electron microscopy and anatomical results showed that the surface of leaf margin cells of mtago7 mutants became smooth and lost the pleats. MtAGO7 contains two conserved domains (PAZ and PIWI domain) and the expression was highest in flower, stem and slightly less in juvenile and adult leaves. The expression pattern indicated that MtAGO7 possibly played an important role during the development of flower and leaves. Since MtAGO7 may be involved in the TAS3 tasiRNA formation in *M.truncatula*, the identification of target genes is ongoing. "
(a) Forage Improvement Division, The Samuel Roberts Noble Foundation (b) Plant Biology Division, The Samuel Roberts Noble Foundation

P62016 Characterization of an Arabidopsis MiR156b activation tagged mutant reveals SPL15 diverse roles
Wei, shu-presenter weishu@agr.gc.ca(a) Gruber, Margaret Y. (a) Khachtourians, George G (b) Hegedus, Dwayne D. (a) Parkin, Isobel A. P. (a) Hannoufa, Abdelali (a)
"The *Arabidopsis thaliana* miR156 plays biological roles in plant growth and development via negative regulation of plant-specific transcription factor SPL (SQUAMOSA PROMOTER BINDING PROTEIN LIKE) genes. Divergent roles of some individual *SPL* genes have been reported. In this study, an Arabidopsis T-DNA activation tagged mutant *sk156* exhibited altered morphology and increased levels of seed carotenoids violaxanthin, lutein, zeaxanthin and β-carotene compared to wild type (WT). Our studies revealed that *miR156b* activation due to the insertion of a single T-DNA activation tag was responsible for the morphological alterations and elevated seed carotenoids in *sk156*. Studies on *SPL15* loss-of-function mutants and sk156 plants expressing a mutated *SPL15* gene (*SPL15m*) under control of the cauliflower mosaic virus (CaMV) 35S promoter revealed that *miR156b* activation-induced *SPL15* suppression is involved in the observed morphological changes and elevated levels of seed carotenoids in the *sk156* mutant. CaMV-driven *SPL15m* expression in the mutant resulted in restoration of many observed phenotypes compared with WT Arabidopsis. Our results indicate that the *SPL15* gene has diverse and partially redundant functions found for some other SPL family members and plays diverse roles in miR156-regulated gene networks. "
(a) Agriculture and Agri-Food Canada (b) University of Saskatchewan

SESSION P63 – SYSTEMS BIOLOGY

P63001 Identification and Functional Analysis of Protein Phosphatase 2C in Rice and Arabidopsis
Zhong, Yuan-presenter yzhong@millersville.edu(a) Xue, T. (b) Wang, D. (b) Zhang, S. (b) Ehlting, J. (c) Ni, F. (b) Jakab, S. (a) Zheng, C. (b)
"A comprehensive computational analysis identified 80 and 78 protein phosphatase 2Cs (PP2Cs) genes in Arabidopsis (AtPP2Cs) and rice (OsPP2Cs), respectively. Phylogenic analysis divided PP2Cs in Arabidopsis and rice into 13 and 11 subfamilies, respectively, which are supported by the analyses of gene structures and protein motifs. Comparative analysis between the PP2C genes in Arabidopsis and rice identified common and lineage-specific subfamilies and potential gene birth-and-death events. Gene duplication analysis reveals that whole genome and chromosomal segment duplications mainly contributed to the expansion of both OsPP2Cs and AtPP2Cs, but tandem or local duplication occurred less frequently in Arabidopsis than rice. Some protein motifs are widespread among the PP2C proteins, whereas some other motifs are specific to only one or two subfamilies. Expression pattern analysis suggests that 1) most PP2C genes play functional roles in multiple tissues in both species, 2) the induced expression of most genes in subfamily A by diverse stimuli indicates their primary role in stress tolerance, especially ABA response, and 3) the expression pattern of subfamily D members suggests that they may constitute positive regulators in ABA-mediated signaling pathways. The analyses of putative upstream regulatory elements by two approaches further support the functions of subfamily A in ABA signaling, and provide insights into the shared and different transcriptional regulation machineries in dicots and monocots. Our results have established a solid foundation for future studies on the functional divergence in different PP2C subfamilies. "
(a) Millersville University of Pennsylvania (b) Shandong Agricultural University (c) University of Victoria

P63002 MAPK target networks in *Arabidopsis thaliana* revealed using functional protein microarrays
Popescu, Sorina C-presenter scp78@cornell.edu(a) Popescu, George V (b) Bachan, Shawn (b) Michael, Gerstein (b) Snyder, Michael (b) Dinesh-Kumar, Savithramma P (b)
"In eukaryotes signaling molecules from MAPK cascades assemble in a combinatorial fashion to specify a rich repertoire of outputs. A significant challenge is to identify the signaling components and to understand the rules that govern MAPK complex assembly. Methods for the high-throughput screening, such as protein microarrays, have been very successful in characterizing protein post-translational modifications. Protein microarrays, applied to the discovery of signaling molecules in yeast, animal and plant systems, have been instrumental in defining novel components of cellular pathways. We have initiated the development of an unbiased, large scale methodology for the identification and functional characterization of plant proteins, the *Arabidopsis* Functional Protein Microarrays (FPM). Recently, we have studied the *Arabidopsis* MAPKK/MAPK/Substrates signaling pathways using FPMs containing 2,158 unique proteins. Previous to our work, only a limited amount of information has been available on direct targets of MAPKs. We identified 570 putative MAPK targets, with a majority of transcription factors involved in development and respons to stress. A subset of the putative phosphorylation targets with known or predicted roles in response to pathogen infection, WRKY and TGA transcription factors, were subsequently confirmed *in vivo*. Our predicted MAPKK/MAPK/Substrate phosphorylation network constitutes a valuable resource to understand the function and specificity of signaling systems. **Reference: Popescu, S**., Popescu G., Bachan, S., Zhang Z., Gerstein M, Snyder M, Dinesh-Kumar S., MAPK target networks in Arabidopsis thaliana revealed using functional protein microarrays. Genes & Dev., 2009. 23(1): p. 80-92. "
(a) Boyce Thompson Institute (b) Yale University

P63003 Genome-level identification of tissue-specific gene expression networks in *Populus*
Drost, Derek R.-presenter ddrost1@ufl.edu(a,b) Benedict, Catherine I (b) Novaes, Carolina R.D.B. (b) Berg, Arthur (c) Novaes, Evandro (b) Brown, Ryan S. (b) Maia, Jessica M. (d) Dervinis, Christopher (b) Peter, Gary F. (a,b) Kirst, Matias (a,b)
"The main goals of systems biology are to identify the genetic elements that determine phenotypes, and understand how they interact in 'networks' that accurately predict phenotypic response to genetic and environmental perturbations. Currently, the attainment of these goals in plants has been limited, and little consideration has been given to the role of systems networks in regulating developmental differentiation of specialized organs and tissues in plants. We have begun to address these shortcomings through an integrative analysis of variation in genotypic, whole-genome gene expression, and transcription-factor binding sites (TFBS) data produced from a segregating pseudo-backcross pedigree of *Populus trichocarpa* and *P. deltoides*. Using genetical genomics and systems biology approaches, we demonstrate that genetic regulation of transcription is highly variable between these species in three distinct tissues (secondary xylem, expanding leaves, and mature roots), and show that variation in tissue-specific expression programs is expansive. We utilize these tissue-specific gene expression data to generate co-transcriptional networks *a posteriori*, and on the basis of gene expression QTL hotspots and TFBS, show these networks to be enriched for groups of genes that function in biologically coherent pathways and processes. Finally, we demonstrate that these networks may play key roles in tissue-specific developmental programs associated with the phenotypic diversity between *P. trichocarpa* and *P. deltoides*."
(a) Plant Molecular and Cell Biology, University of Florida (b) School of Forest Resources and Conservation, University of Florida (c) Department of Statistics, University of Florida (d) Institute for Genome Sciences & Policy, Duke University

P63004 Comparative analysis of maize mesophyll and bundle sheath gene expression in cell specific mutants *hcf136* and *bsd2*
Covshoff, Sarah-presenter sc603@cam.ac.uk(a) Hua, Hong (b) Liu, Peng (b) Owens, Thomas G (c) Brutnell, Thomas P (d)
"*Zea mays* (maize) is a C_4 plant in which photosynthetic activities are partitioned between two physiologically distinct cell types, mesophyll (M) and bundle sheath (BS). During maize leaf development, M and BS cells assemble distinct transcript and protein profiles via a poorly understood differentiation mechanism. We hypothesize that M and BS transcript accumulation is affected by novel cellular environments resulting from differential complex formation, redox potential and sugar and energy metabolite concentrations found in these cell types. To address this possibility, we present a comparative analysis of global M and BS gene expression from mutants severely and selectively disrupted in M (*hcf136*) or BS (*bsd2*) cell development. M and BS cells were isolated from *hcf136* and *bsd2* and their RNA profiles with respect to wild-type were determined by microarray analysis. Hierarchical clustering was applied to the BS_*hcf136*, BS_*bsd2*, M_*hcf136*, and M_*bsd2* transcriptomes to identify regulons affected by disruptions to M and BS cell metabolism. Gene clusters were subsequently mapped to gene ontologies and biochemical pathways were identified. Representative pathways affected by selective disruption of M and BS development are discussed in light of the cellular environment hypothesis."
(a) Department of Plant Sciences, University of Cambridge (b) Department of Statistics, Iowa State University (c) Department of Plant Biology, Cornell University (d) Boyce Thompson Institute for Plant Research

P63005 Label-Free Relative Quantitation of Developmentally Expressed Soluble and Membrane-Bound Proteins in Developing Fruit Juice Sac Cells of Citrus *sinensis*
Katz, Ehud (a) Fon, Mario G (a) Eigenheer, Richard A (b) Phinney, Brett S (b) Sadka, Avi (c) Blumwald, Eduardo-presenter eblumwald@ucdavis.edu(a)
"Pathways associated with fruit development and ripening are unique to plants and vary between species. Developmental, physiological, anatomical and biochemical differences contribute to the operation of unique biochemical pathways, genes and proteins. Our initial efforts towards the characterization of the Washington navel fruit proteome identified 1,400 juice sac cell-specific proteins and enzymes [Planta (2007):226:989-1005]. This effort facilitated the identification of a large number of biosynthetic pathways associated with development and fruit quality traits. Following these efforts, we have now developed a label-free method for the identification of proteins and the quantification of changes among different samples. Accurate LC-MS/MS data were acquired with a Finnigan LTQ-FT for the differentially expressed proteins in the three developmental stages, then label-free techniques were employed to quantify differences between proteins of the three subsets: Both label-free spectral counting and comparison of peak intensities using the Sieve program were used. Statistical analyses were conducted with Spotfire software. A citrus genome-wide ESTs database and the NCBI-nr (green plants) database were used to identify proteins, which were subsequently classified according to their putative and assigned functions to known biosynthetic pathways. A number of proteins were found to differ between the three developmental stages of orange fruits, indicating significant shifts in some pathways suggesting mechanisms for the metabolic changes affecting fruit qualityt. Among these; sugar ion homeostasis, amino acid synthesis and, hormonal balance, citrate metabolism, etc. will be presented and discussed."
(a) Dept of Plant Sciences, University Of California, Davis CA 95616 (b) Genome Center, University of California, Davis CA 95616 (c) Dept of Fruit Tree Sciences, Volcani Center, ARO ,Israel

P63006 Plant synthetic biology: design of a synthetic signal transduction system to build a plant sentinel
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"Synthetic biology is a broad approach that uses tools such as rational design of proteins, genetic networks and living systems for a specific purpose.

Plants sense and respond to stimuli using a type of input-output system. We developed a synthetic plant detection trait using computationally designed receptors and a synthetic signal transduction system. Sensing and processing a specific stimulus typically involve transmission of high energy signals among various protein components, resulting in complex networks, with plants using differentially expressed components from multigene families. A synthetic histidine kinase (HK) signal transduction system was produced in plants using three proteins. Highly specific input is generated with extra-cellular, computationally re-designed receptors, which transmit a signal intracellularly via synthetic HKs, and the signal translocated to the nucleus using a modified bacterial response regulator (PhoB-VP64) that activates transcription of a synthetic plant promoter. The synthetic signaling system is modular, allowing us to rewire plant sensing and response systems. Having established a eukaryotic synthetic signal transduction system we linked this sensing genetic circuit to a visible readout gene circuit to produce plants that detect parts per trillion of their ligands, in this case the explosive TNT (2,4,6-trinitrotoluene), and respond with a visible or remotely detectable readout. Because our system uses modular computer-designed receptors to generate input, these phytodetectors offer a simple and inexpensive means to address unmet needs to monitor human surroundings for substances such as pollutants, explosives, or terrorist agents."
(a) Colorado State University, Dept. of Biology (b) Duke University Medical Center, Dept. of Biochemistry (c) Precision BioSciences, Inc. (d) National Institute of Environmental Health Sciences

P63007 A systems approach to understanding C4 photosynthetic differentiation in maize
Li, Pinghua (a) Majeran, Wojchiech (b) Wang, Lin (a) Fernie, Alisdair (e) Myers, Chris (c) Liu, Peng (f) Turgeon, Robert (b) Sun, Qi (c) Nelson, Tim (d) van Wijk, Klaas (b) Brutnell, Thomas P-presenter tpb8@cornell.edu(a,b) Ponnala, Lalit (c) Tausta, Lori (d) Reidel, Edwin (b) Nunes-Nesi, Adriano (e) Friso, Giulia (b) Connolly, Brian M (b) Gandotra, Neeru (d) Kebrom, Tesfamichael (a)
"The maize leaf is an excellent model system to study photosynthetic development as a proximal-distal gradient from base (youngest) to tip (oldest). To exploit this system in understanding C4 photosynthetic differentiation we have conducted a detailed histological, physiological and molecular survey of a maize seedling leaf undergoing the sink-source transition. This survey was used to define four developmental zones of a 9 day old leaf: immature, transition, photosynthetic and mature. Quantitative proteome analysis of the leaf developmental zones, including isolated bundle sheath strands, was performed using large scale spectral counting by high sensitivity tandem mass spectrometry. We have generated over 100 million Illumina reads from cDNA libraries created from RNA isolated from various leaf segments and from laser capture microdissected bundle sheath and mesophyll cells. These data are being integrated with a survey of over 50 primary metabolites and detailed EM sections of the leaf that were taken from the same developmental zones. Collectively, these studies revealed major shifts in primary and secondary metabolism and protein biogenesis associated with leaf development, as well as C4 cell-specific differentiation of photosynthesis and carbon metabolism. The tools that are being developed to interrogate these datasets and the major biological findings will be discussed. "
(a) Boyce Thompson Institute, Cornell University (b) Department of Plant Biology, Cornell University (c) Computational Biology Service Unit, Cornell University (d) Department of Molecular, Cellular and Developmental Biology, Yale University (e) Max Planck Institute for Molecular Plant Physiology (f) Department of Statistics, Iowa State University

P63008 Over-expression of a novel Arabidopsis phosphatase causes accelerated flowering and increased seed yield
Sun, Feng -presenter epusun@hotmail.com(a) Lim, Boon Leong (a)
"In plants, sugar metabolism plays important roles in vegetative growth and reproductive development. Altered expression of genes that encode carbon metabolic enzymes like sucrose synthase (SUS), sucrose phosphate synthase (SPS) and invertase (INV) could affect plant floral, architectural, and reproductive traits. Here we identified a novel phosphatase that affects sugar metabolism. Over-expression (OE) lines of this phosphatase, driven by the CaMV 35S promoter, were created in Arabidopsis. The OE lines showed accelerated flowering, increased siliques numbers (30-50%) and seed yield (40%-50%), but a decrease in leaf numbers (54%-56%). Neither photoperiod (long day and short day illumination) nor extended darkness treatments influenced the accelerated flowering phenotype. LC-MS-MS measurement of sugars in the rosette leaves of 20 day-old plants revealed elevated sucrose (1.3-1.8X of wild type) and glucose (1.9-2.6X of wild type) in OE lines, respectively ($p<0.01$). mRNA expression profiles in the leaves of wild-type plants, T-DNA lines, and OE lines at 20 day-old were analyzed using the NimbleGen ATH6 30K cDNA microarray. Many genes involved in sugar metabolism and allocation process were found to be affected. Metabolomics studies by non-targeted Fourier MS are being carried out to produce a clearer picture of the effect of the transgene. "
(a) The University of Hong Kong

P63009 Thee applications of proteomics to studies on signaling and signal-responsive processes in plants
Aki, Toshihiko-presenter aki210@mvb.biglobe.ne.jp(a,b) Hamamoto, Kentaro (a) Yanagisawa, Shuichi (a,b)
"We show three proteome analyses of plant proteins using the nanoLC/MS/MS system that ensures the highest sensitivity for protein identification. The first one is nuclear and nucleic acids-associated proteome analysis of rice proteins. This analysis indicated that more than 600 proteins, including about 150 proteins that were likely involved in signal transduction processes, were putative nuclear proteins. Furthermore, in combination with the results of in silico screening that included identification of Arabidopsis homologs and investigation of sugar-responsive expression of the corresponding genes, we identified three proteins possessing the WD40- or ARM-motif as evolutionarily conserved and glucose-responsive nuclear proteins. The second one was comparative proteome analysis for characterization of the greening process of rice seedlings. Since several lines of evidence for reliability of the MS intensity-based estimation of relative protein levels were obtained, changes in respective protein levels were estimated using MS intensities derived from each protein. Taken together with the result of DNA microarray analysis, changes in the amounts of more than 800 proteins were compared with those in levels of the corresponding mRNAs. The result suggested that modulations of levels of several proteins might be regulated at the translation step or degradation process. The third one was performed as proteome analysis of the phloem sap proteins to search for proteins that function as long-distance signal molecules. We found three FT-like proteins using phloem sap prepared by the insect laser method. These three instances indicate that the proteome analysis with the high sensitivity is a simple but powerful approach for screening of proteins with specific characteristics."
(a) graduate school of agricultural and life sciences, the university of tokyo (b) jst, crest

P63010 Parallel phenotypic analyses of several thousand Arabidopsis T-DNA lines of plastid-targeted genes
Lu, Yan-presenter luy@msu.edu(a) Ajjaw, Imad (a) Shachar-Hill, Yair (a) Curtis, Wilkerson G. (a) Last, Robert L. (a) Savage, Linda J. (a) Coku, Ardian (a) Imre, Kathleen M. (a) Benning, Christoph (a) Della Penna, Dean (a) Ohlrogge, John B. (a) Osteryoung, Kathleen W. (a) Shin-Han, Shiu (a)
http://www.plastid.msu.edu
"Plastids are present in virtually all plant cells and they participate in many biosynthetic processes. The Arabidopsis chloroplast is an excellent target for functional genomics approaches. To investigate plastid functions with a systematic approach, several thousand *Arabidopsis thaliana* homozygous T-DNA lines of nuclear-encoded chloroplast-targeted genes are being analyzed. Multiple morphological, physiological and chemical assays (amino

acids, fatty acids and starch) are being used, with data stored in a relational database. Data analysis of a pilot study showed that such industrial approaches can reliably identify mutant phenotypes (Lu et al., 2008, Plant Physiol. 146:1482-1500). As of April 2009, ~3500 T-DNA lines have been analyzed and novel mutants identified. Statistically robust correlations among levels of metabolites that are not known to share direct biosynthetic origins raise the possibility that these metabolic pathways may be co-regulated. In addition, phenotypic associations between mutants of well- and poorly-understood genes allow the identification of potentially new components in a pathway and to develop functional hypotheses for under-characterized genes."
(a) Michigan State University

P63011 Analyzing the plant golgi proteome to better understand its role in plant cell wall sysnthesis
Ito, Jason-presenter jito@lbl.gov(a) Parsons, Harriet (a) Heazlewood, Joshua L (a)
"The plant cell wall is mainly composed of the polysaccharides; cellulose, hemicelluloses and to a lesser extent, pectin. While cellulose is synthesized in the plasma membrane, the non-cellulosic polysaccharides hemicelluloses and pectin are synthesized by glycosyltransferases in the Golgi apparatus and are subsequently sent to the plant cell wall through vesicular transport. Non-cellulosic polysaccharide biosynthesis in the plant Golgi apparatus is a highly complex process and requires various substrates and cofactors for construction of the polysaccharide backbones and the addition of oligosaccharide sidechains and methyl and acetyl groups. Despite the importance of the Golgi apparatus in plant cell wall biosynthesis, the complement of Golgi-localized proteins that are involved in this vital process has been poorly characterized to this date. An analysis of the *Arabidopsis thaliana* genome revealed that 351 putative glycosyltransferases could play roles in plant cell wall polysaccharide biosynthesis (Henrissat et al., 2001), which highlights the need for a comprehensive study of proteins that reside in the plant Golgi apparatus. By purifying Golgi from cell cultures of *Arabidopsis thaliana* through the use of centrifugation techniques, sucrose density gradients and surface charge-based organelle separation by free-flow electrophoresis and using mass spectrometry to identify the set of Golgi proteins, we can expect to significantly expand our knowledge of the role of plant Golgi in plant cell wall biosynthesis. Reference: Henrissat, B., Coutinho, P.M. and Davies, G.J. (2001) A census of carbohydrate-active enzymes in the genome of *Arabidopsis thaliana*. *Plant Mol Biol*. 47(1-2):55-72. "
(a) Lawrence Berkeley National Laboratories

P63012 Discovering protein-protein interactions that serve to integrate chloroplasts into the protein interaction networks of *Arabidopsis thaliana* leaf mesophyll cells
Johng, Dorhyun C (a) Harris, Gary C-presenter gharris@wellesley.edu(a) Koniger, Martina (a)
"Intracellular communication networks in eukaryotic cells involve extensive protein-protein interactions both within and between organelles. In plant cells the chloroplast plays a variety of metabolic roles and possesses a complex population of membrane bound and soluble proteins that can be localized to discrete regions of the organelle. The protein composition of the chloroplast and its metabolic activities must be tightly regulated by communication networks that integrate the chloroplast into the networks and sub-networks that connect the various organelles to each other, and to the cytosol. In an effort to identify weak protein-protein interactions involving the chloroplast, isolated *Arabidopsis thaliana* chloroplasts were incubated with biotinylated crude extracts of leaf proteins and collected by centrifugation through a layer of silicone oil. The bound proteins were collected by virtue of their tight binding to avidin beads. The biotinylated proteins were then fractionated by polyacrylamide electrophoresis and analyzed by tandem mass spectrometry. The results revealed that a complex array of proteins bind to chloroplasts under the condition employed in this assay (5mg/ml protein, 25 C, pH 7.3). We will also report on the bioinformatic construction of a virtual chloroplast interaction network generated using interlogs of proteins that have been assigned to the outer envelope membrane of the *Arabidopsis thaliana* chloroplast. "
(a) Wellesley College

P63013 NMR and algal metabolomics - identification of nutritionally-induced biomarkers
Brandsma, Jordyn B-presenter jordyn.brandsma@gmail.com(a) Bestman, Hank D (a) Ooms, Kristopher J (b) Westerhuis, Johan A (c)
"NMR spectroscopy coupled with appropriate statistical procedures constitutes a reliable and robust technique to detect differences in metabolic profiles. Using this technique we have demonstrated significant differences in the polar metabolite profiles of the unicellular freshwater alga *Chlamydomonas reinhardtii*, growing either autotrophically or heterotrophically. *Chlamydomonas* (wild type strain CC-125 mt^+) were grown autotrophically under light (500 µ;mol/m<>/sec PAR) in continuous culture systems (200 mL) with a 2-L/min airflow (1800 ppm CO_2). Heterotrophic conditions were created by adding sodium acetate to the nutrient medium (10 mM final concentration) and placing the culture system in the dark. Polar metabolites were extracted in NMR solvent (8:2 D_2O:CD_3OD) according to published protocols (Ward, J.; *et al. Phytochem.*, **2003**, *62*, 949-957), with the addition of a 3-kDa cut-off filtration step (Wishart, D.; *Trends Anal. Chem.*, **2008**, *27*, 228-237) and analyzed using NMR spectroscopy (400 MHz). Partial least squares discriminant analysis, PLSDA, (Barker, M.; *et al. J. Chemom.*, **2003**, *17*, 166-173, Westerhuis, J. A.; *et al. Metabolomics*, **2008**, *4*, 81-89) with cross model validation and permutation testing was used to identify statistically different spectral features. Twenty metabolites have been uniquely identified from the ^{1}H NMR spectra (Weljie, A. M.; *et al. Anal. Chem.*, **2006**, *78*, 4430-4442). Based on our experiments thus far 3-hydroxybutarate, cysteine and tyrosine have been identified as important biomarkers for the metabolic changes occurring when the algae are grown under the two different nutritional conditions."
(a) The King's University College, Department of Biology (b) The King's University College, Department of Chemistry (c) University of Amsterdam, Swammerdam Institute for Life Sciences

P63014 Protein and metabolite maps of soybean to improve yield and value-added traits: Investigation of metabolite changes during seed filling
Valliyodan, Babu-presenter valliyodanb@missouri.edu(a) Brechenmacher, Laurent (a) Cheng, Jianlin (b) Xu, Dong (b) Stacey, Gary (a) Nguyen, Henry T. (a)
"Profiling soybean gene products will lay the foundation for a systems biology approach to key processes such as seed development, which will lead to the genetic improvement of yield and seed composition. Also, these approaches help in the search of novel bioactive compounds leading to the production of new generation soybean products for human health and nutrition. It is well known that environmental cues influence developmental phenotypes in plants. Different biotic stresses such as fungal diseases and abiotic stresses such as drought and flooding also elicit phenotypic responses from the genome. Thus, by studying the gene products, a direct correlation between response and specific peptides, and/or metabolites can be made. This will lead to crop improvement either through breeding or transgenic efforts. One of the major goals of this project is to construct soybean proteome and metabolome maps and there by contribute towards understanding of the biochemical network involved in seed development and stress responses. As an initial step, we have conducted metabolite analysis during early and late seed filling stages and in matured seeds to catalogue major metabolite changes during seed filling and maturation in soybean. Soybean standard variety Williams 82 was grown under controlled conditions and the seeds were harvested at the right developmental stages and processed for the analysis. We have used Liquid chromatography/Mass spectrometry (LTQ-Orbitrap XL) for the chromatographic separation and identification of the metabolites. The preliminary investigation has identified changes in several metabolites across the developmental stages. Also, these approaches help to initiate to construct a

base line metabolite catalogue for soybean seeds. "
(a) National Center for Soybean Biotechnology and Division of Plant Sciences, University of Missouri (b) Computer Science Department, University of Missouri

P63015 "Isolation of Proteins Interacting with Floral Regulators FT and TFL1 of Aarbidopsis, Using Yeast Two-Hybrid Screen"
Sim, Soon Ae-presenter sasim8001@hanmail.net(a) Kang , Ju Hwan (a) Song, Young Hun (a) Shin, Su Young (a) Lim, Chae Oh (a) Lee, Sang Yee (a) Hong, Jong chan (a)
"In plants, flowering is a major developmental transition that is critical to reproductive success. In Arabidopsis FLOWERING LOCUS T (FT) and TERMINAL FLOWER 1 (TFL1), the two proteins having homolgy to mammalian phosphatidylethanolamine-binding protein, are key controllers of flowering, determining when and where flowers are made. While FT and TFL1 are similar each other in protein sequence they perform opposite functions: FT promotes flowering strongly while TFL1 represses flowering. Residues responsible for the opposite activities of FT and TFL1 were mapped previously by examining plants that overexpress chimeric proteins. However, features of binding partners that distinguish between FT and TFL1 and thus contributing to their opposite functions have not been discovered yet. Here we report the isolation of FT and TFL1 interacting transcription factors by genome wide screening of Arabidopsis transcription factors for interacting proteins. Using high-throughput yeast two hybrid screening we have identified 43 FT and 9 TFL1 interacting proteins from 1400 cloned transcription factors. FT-interacting proteins include 5 bZIP, 2 MYB, 10 G2-like , 9 TCP proteins and others while TFL1 interactors also contain 2 bZIP, 2 C2H2, 1 HB and 4 TCP proteins. From the few selected TFs we could observe FT- and TFL1-specific interactions. This study was supported by CFGC of 21C Frontier Research Program, EB-NCRC, BK21 program, and KOSEF grant (MOST: R01-2007-000-11232-0). "
(a) Gyeongsang National University

P63016 Comparative functional genomics of the heat stress response
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"The heat stress response is assumed to be a highly conserved process, yet there have been few studies investigating variation in this response among plant species. We used a comparative network approach to determine the conserved and unique aspects of the heat stress response among Soybean, Arabidopsis, and Poplar and link that information to underlying biochemistry and leaf-level gas exchange parameters. Photosynthetic heat stress response curves were conducted from 22^{o}C to 45^{o}C and leaves were sampled at the start of the curve (baseline), optimum CO_2 assimilation, 30% inhibition of optimum, and at 45% inhibition of optimum for each species. Soybean had a broader photosynthetic response curve relative to Arabidopsis and Poplar suggesting that it was more thermotolerant under these experimental conditions. A coexpression network was constructed from 48 microarrays (4 treatments x 4 replicates x 3 species) resulting in modules functionally enriched for heat stress response (HSPs and antioxidant system), membrane integrity, transcriptional regulation, and core metabolism. The Arabidopsis heat stress module was expressed after photosynthesis was inhibited, whereas Soybean expressed the heat stress module prior to inhibition. ROS concentrations were significantly correlated ($p = 2.3\ e^{-8}$) with expression of the heat stress module for both species and this was immediately followed by an increase in Peroxidase and Ascorbate Peroxidase activity, but not in Superoxide Dismutase or Glutathione Reductase activity. HSP101 protein was apparent at photosynthetic inhibition for Arabidopsis but not for soybean. These data suggest that soybean has a unique heat stress response system or regulation of that system and experimentation pursuing both hypotheses will be presented."
(a) Oak Ridge National Laboratory

SESSION P64 – TRANSLATION RESEARCH, FROM BENCH TO FIELD

P64001 Plant senescence: a paradigm of translational plant sciences
Westbrook, Jessica (a) Gan, Susheng-presenter sg288@cornell.edu(a)
"Plants exhibit both mitotic and post-mitotic senescence. Leaf senescence is a typical post-mitotic senescence while cease of cell proliferation in early fruitlet formation and shoot apical meristem arrest are examples of mitotic senescence. Leaf senescence limits crop yield and biomass accumulation and contributes to much postharvest loss of vegetables and ornamental plants. Mitotic senescence in fruitlets limits fruit size. We have studied the regulatory mechanisms underlying senescence in Arabidopsis, and have translated knowledge gained from the model system into crops for agricultural improvement. For example, we identified a master regulator that controls leaf senescence in Arabidopsis, and based on this finding, we cloned and manipulated the orthologs in various crops including soybean. Soybean plants display a significantly delayed leaf senescence phenotype and more than 10% increase in seed yield. By delaying mitotic senescence in fruitlets, cell numbers are increased in Arabidopsis, tobacco and tomato fruits, respectively, and the size of tomato fruits is significantly increased. These translational research on plant senescence is approaching commercialization. "
(a) Cornell University, Department of Horticulture

P64002 Functional Characterization of Novel Transcripts from Strawberry (*Fragaria spp*).
Chatterjee, Mithu (a) Zhang, Qian (b) Clancy, Maureen A (a) Bermudez-Lozano, Claudia (a) Dhingra, Amit (c) Ricaurte, Sasha A (a) Davis, Thomas M (b) Folta, Kevin M-presenter kfolta@ifas.ufl.edu(a)
http://www.strawberrygenomics.com
"The Roscaeae Family contains many economically important fruit, nut, ornamental and lumber species. Analysis of the constellation of transcripts from strawberry shows that many (as high as 15% by some estimations) do not share sequence identity with transcripts from other species. This set represents the strawberry unknowns, genes expressing products that defy convenient classification. Some do not even present coding sequence. A substantial 454-based deep sequencing of transcripts from many tissues, developmental states and treatments has provided ample representation of transcripts to define the *Fragaria* unknown-ome. Individual genes have been placed back into diploid strawberry and *Arabidopsis thaliana* in an overexpression contexts, leveraging the power of these two rapid transformation systems. Transcripts were suppressed using RNAi in strawberry to test for loss-of-function phenotypes. A comprehensive phenotypic analysis of transgenics has led to the identification of genes affecting a series of processes, from leaf development to flower development, to rosette size and plant architecture. The study identifies a set of transcripts that may contribute to the unique qualities of the Rosaceae, placing previously unidentified transcripts in association with specific facets of plant physiology and development. "
(a) Horticultural Sciences Department and the Graduate Program in Plant Molecular and Cellular Biology, University of Florida, Gainesville, FL (b) Plant Biology Department, University of New Hampshire, Durham, NH (c) Department of Horticulture and Landscape Architecture, Washington State University, Pullman, WA

P64003 Development of soybean oil low in palmitic acid and elevated in stearic and oleic acids
Sato, Shirley-presenter ssato1@unl.edu(c) Park, Hyunwoo (a,b) Graef, George (a) Fromm, Mike (a,c) Clemente, Tom (a,b)
"Most commodity soybean oil has a fatty acid profile of app. 10% palmitic acid, 4% stearic acid, 17% oleic acid, 55% linoleic acid, and 10% linolenic acid. Oil high in oleic acid, and elevated in stearic acid, with a concomitant reduction in palmitic acid and polyunsaturated fatty acids offers improved functionality for both food and industrial applications. To produce soybean oil with such a fatty acid profile we stacked a transgene that carries a silencing element designed to simultaneously down-regulate in a seed-specific fashion a palmitoyl thioesterase (FatB) gene and a delta 12 desaturase gene, with a stearoyl ACP thioesterase seed-specific transgenic cassette, via sexual crossing. The parent that carries the silencing transgene was derived from a soybean event designated 335-13 which produces an oil high in oleic acid (>85%) and low in palmitic acid (<4%). This event, 335-13, was crossed to soybean events that carry the stearoyl ACP thioesterase gene that displayed stearic acid levels ranging from 8% up to 17%. To date we have identified homozygous lineages from such crosses (F4 generation) that produce oil with fatty acid profiles of stearic acid at 17.8% and oleic acid at 68.0%."
(a) Department of Agronomy & Horticulture, University of Nebraska Lincoln (b) Center for Plant Science Innovation, University of Nebraska Lincoln (c) Center for Biotechnology, University of Nebraska LIncoln

P64004 Modification of Maize Carotenoid Composition through Coordinated Effects of Biosynthesis Pathway QTL During Endosperm Development
Kandianis, Catherine-presenter cbkandianis@gmail.com(a) Kim, Eun-Ha (b) DellaPenna, Dean (b) Johnson, G. R. (a) Rocheford, Torbert (c,a)
"Enhanced production of carotenoid compounds through modification of plant secondary metabolism has the potential to increase the nutritive value of cereal grains by augmenting provitamin A (ProVA) and antioxidant levels. Successful manipulation of the pathway in maize grain has been achieved by coupling the biochemical model for carotenoid biosynthesis in homologous species with an understanding of the genetic variation that regulates this pathway in maize. Carotenoid production is quantitatively inherited and is thus dependent upon the interplay of synthesis, conversion and degradation. Achieving target ProVA concentrations through genetic selection prompts the use of QTL found to affect all of these processes. Included in this suite of maize genes are lycopene epsilon cyclase (lcye), carotenoid reductase b1 (crtR-b1), and carotenoid cleavage dioxygenase 1 (ccd1). As genetic variation in the above loci is associated with differences in the carotenoid concentrations of harvested grain, it is definite that QTL effects originate during kernel development. We postulate that allelic variants for these QTL may differ in time of expression and/or magnitude of effect, and accordingly change carotenogenesis profiles. Using the known haplotypes for these QTL (Harjes, 2008; Yan, 2008; Bermudez-Kandianis, in preparation), developing kernels from a select set of maize inbred and hybrid lines were profiled from 12 to 60 days after pollination. The change in effects of individual alleles and entire haplotypes on various carotenoid pools was estimated along the timecourse. Results indicate that day by allele-specific interactions exist, and suggest that the analysis could be used to uncover synergistic allelic combinations for increased carotenoid production, and reduced degradation. "
(a) University of Illinois at Urbana-Champaign (b) Michigan State University (c) Purdue University

SESSION P65 – TROPICAL PLANT BIOLOGY

P65001 Acclimation and adaptation of leaf carotenoid composition and biosynthesis in tropical plant species
Matsubara, Shizue-presenter s.matsubara@fz-juelich.de(a) Krause, G. Heinrich (b,c) Aranda, Jorge (c) Virgo, Aurelio (c) Beisel, Kim G

(a) Jahns, Peter (b) Winter, Klaus (c)
"The composition of carotenoid pigments, involved in two essential and contrasting processes of photosynthesis (light harvesting and photoprotection), is highly conserved among higher plants. One branch of the carotenoid biosynthetic pathway (b,b-branch) gives rise to b-carotene, zeaxanthin, antheraxanthin, violaxanthin and neoxanthin, whereas another branch (b,e-branch) leads to formation of lutein. While our understanding of biosynthesis and functions of different carotenoids in photosynthesis has greatly advanced for model plants, regulation of leaf carotenoid biosynthesis and accumulation can vary in different plant species, as exemplified by some tropical plants that contain a-carotene (a-Car) and lutein epoxide (Lx), two additional carotenoids synthesized in the b,e-branch. Based on their occurrence and sun-shade responses studied in several species, roles of a-Car and Lx in improving light harvesting, as opposed to those of b-carotene and zeaxanthin in photoprotection, have been proposed. In this study we conducted a survey of photosynthetic pigments, including 86 tropical plant species from 64 families, to examine whether occurrence of a-Car and Lx represents convergent evolution, i.e. adaptive changes in unrelated species under similar ecological constraints. Further, relative investment in different carotenoids was compared between sun and shade leaves/species to infer their importance under contrasting light environments and to identify a general acclimatory pattern in leaf carotenoid biosynthesis in tropical plants. The results will be discussed in the context of trade-off between investment in light harvesting and leaf structural components, differential functions of b,b- and b,e-carotenoids, and enzyme evolution in the carotenoid biosynthetic pathway."
(a) Phytosphaere (ICG-3), Forschungszentrum Juelich (b) Heinrich-Heine-Universitaet Duesseldorf (c) Smithsonian Tropical Research Institute

P65002 "Changes of some Antioxidants, MDA and H_2O_2 in Hot Water Treated 'Hom Thong' Banana Fruits During Storage"
Ummarat, Nittaya-presenter nittaya_um@hotmail.com(a)
"'Hom Thong' banana (*Musa acuminata*, AAA group) fruits were immersed in hot water (50°C) for 10 min, before storage at 25°C until ripening. In order to estimate the effect of hot water treatment on some antioxidants during banana ripening, ascorbic acid (AA), carotenoids, reduced glutathione (GSH) and oxidized glutathione (GSSH) were measured. In addition, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) were analyzed in order to determine membrane peroxidation or membrane damage during storage. Comparing with control banana immersed in water at room temperature, hot water treated banana showed the delay of ripening. The content of carotenoids, GSH, and the ratio of GSH to GSSH (GSH:GSSH) in hot water treatment tended to be higher than that of control. The higher of AA were found in hot water treated banana during the beginning of storage. MDA and H_2O_2 content in hot water treated banana were lower than that of control. These results suggest the effects of hot water treatment on activation of some antioxidants and reduce of membrane damage in 'Hom Thang' banana fruits during storage."
(a) Chulalongkorn University

P65003 An extended AE-rich N-terminal trunk in secreted pineapple cystatin enhances inhibition of bromelain and is post-translationally removed during fruit ripening
Matsumoto, Kristie O.-presenter kokazaki@hawaii.edu(a) Neuteboom, Leon W. (a) Christopher, David A. (a)
"Phytocystatins are potent inhibitors of cysteine proteinases that participate in senescence, seed biogenesis, organ development and, defense against pests and pathogens in plants. Besides kiwi fruit cystatin, no other cystatin has been found to effectively inhibit the cysteine proteinase, bromelain, of pineapple (*Ananas comosus*). Here we demonstrate that a novel pineapple cystatin, *Ac*CYS1, completely inhibits bromelain. *Ac*CYS1 is unique from other cystatins in that it contains an extended N-terminal trunk (NTT) of 63 residues rich in Ala and Glu. A signal peptide that precedes the NTT is processed *in vitro* by canine microsomal membranes giving rise to a 27 kDa species, which is also immunodetected *in vivo*. *Ac*CYS1 mRNA was ubiquitously present in roots, leaves and fruit, with highest abundance in fruit. Immunofluorescence and immunoelectron microscopy indicate that *Ac*CYS1 was present in the apoplasm. Immunoblot analysis identified a distinct 27 kDa protein in fruit, roots, leaves, and a 15 kDa species in mature ripe fruit. We show that ripe fruit extracts proteolytically remove the NTT of *Ac*CYS1 *in vitro* to produce the 15 kDa species. The presence of the AE-rich NTT completely inhibited stem bromelain, whereas its deletion decreased inhibition to 80%. Surface plasmon resonance assays determined that the NTT increased the affinity of *Ac*CYS1 with bromelain. The calculated K_i values with and without the NTT were 0.69 nM and 1.3 nM, respectively. We propose that removal of the NTT enhances the proteolytic activity of bromelain during fruit ripening and senescence. "
(a) University of Hawaii

P65004 Novel thigmomorphogenetic responses in *Carica papaya*: touch decreases anthocyanin levels and stimulates petiole cork outgrowths
Porter, Brad W.-presenter bporter@hawaii.edu(a) Zhu, Yun J. (b) Webb, David T. (a) Christopher, David A. (a)
"Because of its rapid growth rate and genetic amenability afforded by a sequenced genome with relative low gene number, the tropical fruit tree, *Carica papaya*, can serve as a complementary genetic model for complex traits. In this study, we report novel touch-regulated phenotypes and gene homologs that provide further insight into thigmomorphogenesis, a multigenic response involving mechanoreception and morphological change. Touch-treated seedlings were found to have novel, hypertrophic outgrowths on the abaxial side of petioles associated with periderm and suberin. These plants also had higher lignin, dramatically less hypocotyl anthocyanins and chlorophyll, increased hypocotyl diameter, and decreased leaf width, stem length and root fresh weight. In addition, relative to *Arabidopsis*, papaya was found to have fewer *M*echano *s*ensitive *c*hannel of *S*mall conductance-like genes (*MscS*-like genes) and four missing touch-regulated genes. Water-spray treatment was found to enhance the expression of two papaya *TCH1* homologs whereas induction following touch was only slightly correlated. The novel petiole outgrowths caused by non-wounding-mechanical perturbation may be the result of hardening mechanisms, including added lignin providing resistance against petiole movement. Enhanced lignin production may also monopolize *p*-coumaroyl CoA and deprive chalcone synthase of substrate required for anthocyanin biosynthesis. Other factors, however, such as touch regulated ethylene production, may also play a role in regulating anthyocyanin biosynthesis. The phenotypes identified in this study may now be used as markers to aid functional characterization of touch regulated genes."
(a) University of Hawaii (b) Hawaii Agriculture Research Center

P65005 Ginger and turmeric expressed sequence tags identify signature genes for rhizome identity and development and the biosynthesis of curcuminoids and gingerols
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http://ag.arizona.edu/research/ganglab/ArREST.htm
"Ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) accumulate important pharmacologically active metabolites at high levels in their rhizomes. In order to identify rhizome-enriched genes and genes encoding specialized metabolism enzymes and pathway regulators, we evaluated an assembled collection of expressed sequence tags (ESTs) from eight different ginger and turmeric tissues. Comparisons to publicly available sorghum rhizome ESTs revealed a total of 777 contigs common to ginger, turmeric and sorghum rhizomes but absent from other tissues. The list of rhizome-specific contigs was enriched for genes associated with regulation of tissue growth, development, and regulation of transcription. In particular, transcripts for ethylene response factors and AUX/IAA proteins appeared to accumulate in patterns mirroring results from previous studies regarding

rhizome growth responses to exogenous applications of auxin and ethylene. Thus, these genes may play important roles in defining rhizome growth and development. Additional associations were made for ginger and turmeric rhizome-enriched MADS box transcription factors, their putative rhizome-enriched homologs in sorghum, and rhizomatous QTLs in rice. Additionally, analysis of both primary and specialized metabolism genes indicates that ginger and turmeric rhizomes are primarily devoted to the utilization of leaf supplied sucrose for the production and/or storage of specialized metabolites associated with the phenylpropanoid pathway and putative type III polyketide synthase gene products. This finding reinforces earlier hypotheses predicting roles of this enzyme class in the production of curcuminoids and gingerols."
(a) University Of Arizona (b) University of Louisville (c) Chungnam National University, Korea

P65006 Gender Differences in *Pimenta dioca* (L.) Merr.
Boyd, Frederick A. H.-presenter fredahboyd@yahoo.com(a) Benkeblia, Noureddine (a)
http://www.mona.uwi.edu/lifesciences/
Pimenta dioica (L.) Merr. aka. Allspice is an obligate outcrossing neotropical Myrtaceae endemic to the West Indies. These plants exhibit cryptic dioecy with both genders expressing complete flowers yet functionally differentiated. This investigation was carried out to investigate the differences in floral characteristics as well as the genomic and metabolomic profile. The results reflect definitive phenetic as well as physiological differentiation.
(a) University of the West Indies, Department of Life Sciences

P65007 Classification of water lotuses (*Nelumbo* spp.) based on genomic and EST-derived SSR markers
Kubo, Nakao-presenter nk0103@kab.seika.kyoto.jp(a,b) Hirai, Masashi (a,b) Kaneko, Akio (c) Tanaka, Daizo (d) Kasumi, Kumaji (d)
"The water lotus, genus *Nelumbo*, contains two species: Asian (*N. nucifera*) and American lotuses (*N. lutea*). Though hundreds of flowering lotus cultivars are currently known, their classification is unclear. We have developed simple sequence repeat (SSR) markers to classify the *Nelumbo* cultivars. Of 68 SSRs developed here and recently published SSRs, 52 showed clear polymorphisms in 98 samples. Total 300 alleles were observed, ranging from 2 to 11 alleles per locus (average = 5.8). Specific alleles, useful to distinguish American lotus-derived cultivars, were detected. An NJ tree based on the SSRs revealed that eight American lotus-derived cultivars were separated from the rest of cultivars. This indicates genetic differences between *N. lutea* and *N. nucifera*. There was no apparent correlation between flower characters and genetic relationships. Because of site-specificity and codominat manner of SSR, the present data would provide useful information for the classification and identification of *Nelumbo* cultivars."
(a) Kyoto Prefectural University (b) Kyoto Prefectural Institute of Agricultural Biotechnology (c) Kyoto Botanical Garden (d) Kyoto Flower Center

P65008 Physical assignment of papaya linkage groups to chromosomes by fluorescence *in situ* hybridization
Wai, Ching Man-presenter cmwai@hawaii.edu(a) Robert, Paull e. (a) Paul, Moore H. (b) Ming, Ray (c) Yu, Qingyi (d)
"An accurate papaya chromosome map is an essential resource for genome sequence assembly, genetic and physical mapping of targeted genes, and germplasm improvement via genetic engineering and gene manipulation. Papaya genomic resources are available to construct a sequence-tagged genetic map and linking this map to chromosomes by fluorescence *in situ* hybridization (FISH). The current high density genetic map consists of nine major linkage groups (LG 1 to 9) and three minor LGs (LG 10 to 12). To assign the minor papaya LGs to the nine papaya chromosome pairs, a set of papaya chromosome-specific markers based on a sequence-tagged papaya genetic map were developed. By simultaneously labeling markers from the minor LGs with papaya chromosome-specific markers, minor LG 10, LG 11 and LG 12 were assigned to the distal end of major LG 8, LG 9 and LG 7, respectively. The relative order of DNA markers on pachytene chromosome revealed that LG 10 and LG 11 were inverted at top of LG 8 and LG 9, respectively. In addition, we characterized structural features of papaya chromosomes by localization of 45S and 5S rDNAs. Multicolor FISH results showed that only one pair of chromosomes contains 45S rDNA located in the interstitial position of LG 2. Two major loci of 5S rDNA were located on LG 4 and LG 5. The integrated genetic, physical and chromosome map will be a very useful tool for the papaya research community and ultimately benefit papaya growers with improved cultivars and products."
(a) Department of Tropical Plant and Soil Sciences, University of Hawaii at Manoa (b) U. S. Department of Agriculture-Agricultural Research Service (c) Department of plant biology , University of Illinois, Urbana-Champagne (d) Hawaii agriculture research center

P65009 Isolation and characterization of farnesyl diphosphate synthase cDNA clones from *Polygonum sp*.
Othman, Roohaida-presenter roohaida@ukm.my(a,b) Nuraziyan, Azimi (a,b)
"The enzyme farnesyl diphosphate (FPP) synthase (FPS; EC 2.51.1/EC 2.5.1.10) catalyzes the biosynthesis of farnesyl diphosphate from isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) which is considered to be a rate-limiting step in isoprenoid biosynthesis. A cDNA encoding FPS was isolated from *Polygonum sp*. using the reverse transcriptase polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends (RACE) techniques. Total RNA was extracted from leave tissues as a template in the first strand cDNA synthesis of RT-PCR as well as for 5'- and 3'-RACE. A pair of degenerate primers was designed based on two highly conserved regions of several plant species and was used in RT-PCR to obtain a partial cDNA sequence. Upon ligation and transformation, several clones were screened and the positive transformants were sequenced. Sequence analysis revealed that two partial cDNA clones encoding FPS were obtained. Using RACE-PCR approach, two full length cDNA clones were obtained designated as FPS1 and FPS2 with a size of 1430 and 1245 bp respectively encoding predicted proteins of 342 and 341 amino acid residues each with expected molecular mass of 39.7 kDa for both. The deduced amino acid sequences of the cDNAs were highly similar to FPS from other plants and contain all the conserved regions of trans-prenyl chain-elongating enzymes found in polyprenyl synthetases including FPPS, geranylgeranyl diphosphate synthases (GGPPS) and hexaprenyl diphosphate synthases. "
(a) Institute of Systems Biology, Universiti Kebangsaan Malaysia (b) School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebansaan Malaysia

P65010 Isolation and characterization of a senescence activated promoter from *Anthurium andreanum*
Perez, Pierriden A.-presenter paperez@hawaii.edu(a) Christopher, David A. (a)
"An autoregulated senescence inhibition system has been extensively used in a number of crops and has been shown to delay senescence and aging and increase flower number and seed yields in three other plant species. This system is based on a senescence-induced promoter controlling the expression of a cytokinin biosynthesis gene, an anti-aging plant hormone. Our lab has previously shown that the *sag12* promoter from *Arabidopsis thaliana* is induced in senescent Anthurium leaves, showing the similarity of senescence regulation in both plants. A senescence-induced gene *anth17*, a homolog of *sag12*, has also been isolated from Anthurium as a result of these earlier experiments. The goal of this research is to isolate the *anth17* promoter. This promoter will be used for genetic engineering decreased senescence and to demonstrate further the relationship between the senescence programs in the two plant species. GFP expression under the control of the *anth17* promoter will also be analyzed in Arabidopsis. An Anthurium genomic library was constructed and screened for the *anth17* gene using a cDNA probe. Restriction mapping was performed and the promoter sequence upstream of the *anth17* coding region was cloned. An *Agrobacterium*-based gene construct containing the GFP gene under the

control of the *anth17* promoter was developed and used to transform Arabidopsis."
(a) Molecular Biosciences & Bioengineering - University of Hawaii at Manoa

P65011 "Contrasting profiles in heliconia and bromeliad phytotelmic communities in costa rica: the role of physical, chemical, and trophic cues in controlling community structure"
Hardy, Thomas J-presenter thomasjhardy@gmail.com(a)
"The invertebrate communities of water-filled plant parts constitute a unique world of ecological relationships. The modified plant parts in which they occur are referred to as phytotelmata. The discrete nature of the invertebrate communities within phytotelmata has implied their usefulness as model organisms for the study of community ecology. Early investigations used assemblages of the pitcher plant, *Sarracenia purpurea* (L.) to investigate the impact of keystone species on the structuring of ecological communities. Much less work has been done on diverse forms of phytotelmata in the tropics. In this study, I investigated the applicability of phytotelmata as model systems by comparing the biology of three tropical phytotelmata, *Heliconia bihai*, *Neoregelia carolinae*, and *Vriesea imperialis*. The community profiles among the heliconia and two bromeliads differed significantly. There was less than 18 percent of overlap between the invertebrate communities of *H. bihai* and *N. Carolinae* and a 10 percent overlap between *H. bihai* and *V. imperialis*. At the same time, there was an 82 percent overlap between the invertebrate communities of the two bromeliads. It was determined that the considerable differences observed between the invertebrate communities of the included heliconias and bromeliads were influenced by a combination of factors, including: phytotelm liquid chemistry, phytotelm body size, and trophic interactions within the aquatic habitat. My field and laboratory studies indicate that phytotelmata are useful tools in characterizing the structuring of ecological communities when plant specific contributions are taken into account."
(a) Howard University, Department of Biology

P65012 Molecular cloning and characterization of *GIGANTEA* gene in *Phalaenopsis bellina*
Chen, Emily Chin-Fun-presenter argentina@cyut.edu.tw(a) Tsay, Hsin-Sheng (a) Chen, Pei-Tsu (a)
"Although *Phalaenopsis* is the most prized orchids and internationally known for its attractive flowers, the molecular mechanism of flowering in *Phalaenopsis* spp. remain unclear. Possible environmental cues such as photoperiod, light quality and temperature were demonstrated to have effects on the flowering time. To have a better understanding on the photoperiodic flowering of this major floral crop, we started our studies with a circadian controlled gene that regulates photoperiodic flowering in *Arabidopsis* and many crops, *GIGANTEA* (*GI*). *Phalaenopsis bellina*, which blooms from early summer to autumn like *Arabidopsis*, was used as plant material. According to the gene homology, *PbGI* has been cloned. The deduced amino acid sequences of *PbGI* shared more than 70% of similarity with others GI homologues. The multiple sequence analysis as well as the phylogenetic analysis have shown that PbGI is more closed to rice GI (OsGI) and distant to Arabidopsis GI (AtGI). By detecting *PbGI* expression in different parts of the plant and under different light conditions, we come to know that higher gene expressions was detected in old leaves and petals, and the circadian rhythm of *PbGI* is similar to *AtGI*. An ectopic expression of *PbGI* in *gi* mutants will help us to reveal its function. "
(a) Chaoyang University of Technology

P65013 Genetic and morphological diversity of natural populations of *Carica papaya* in Costa Rica
Rieger, Jennifer E.-presenter riegerje@muohio.edu(a) Lawrie, Joseph F. (a) Rocha, Oscar J. (b) Moore, Richard C. (a)
"The common papaya, *Carica papaya*, is known to have originated in Mesoamerica, in an area ranging from southern Mexico to northern Central America. In Costa Rica, dioecious, small-fruiting common papayas have been observed growing in secondary lowland forests, including regions along the sides of roads. Locals commonly refer to these plants as mountain papayas, which are identified by their small, foul-tasting fruits. Very little research has focused upon characterizing the genetic diversity and/or the morphological diversity of wild papaya, such as the mountain papaya, especially those growing within the region of origin. These studies have only analyzed a relatively small number of individuals dispersed throughout Costa Rica. In light of these previous experiments, it the goal of my research is better quantify the overall amount of diversity that exists within the wild papaya of Costa Rica. Morphological data and tissue samples were collected *in situ* from 252 plants that were equally distributed throughout the country. These data are currently being assessed through a Principle Component Analysis. Genetic diversity of these wild plants is currently being measured and compared to 10 cultivars through the use of 20 fluorescently labeled microsatellite markers, equally distributed throughout the 9 chromosomes of papaya. These results will be assessed through the use of Arlequin, POPGENE, and Structure, to measure allele frequency, overall genetic diversity within the samples, and population structure within the individuals, respectively. The results of this research will offer valuable insight into how the common papaya was domesticated, as well as whether mountain papayas may be beneficial to the conservation of genetic diversity of *Carica papaya*."
(a) Miami University Botany Department (b) Kent State University Department of Biological Sciences

P65014 Characterization of Coffee Prolyl Oligopeptidase Genes
Singh, Ratnesh (a) Irikura, Beth (b) Nagai, Chifumi (c) Albert, Henrik H (d) Kumagai, Monto (e) Paull, Robert E (b) Wang, Ming-Li-presenter mwang@harc-hspa.com(c)
"Coffee (*Coffea arabica* L.) cultivars Tall Mokka (MA2) and Kona-Typica (KO34) produce high quality beans with distinct flavors. MA2 differs from KO34 in having smaller organs including leaves, fruits, and seeds, resulting in lower yield. In an attempt to identify genes responsible for organ size control, the genes encoding prolyl oligopeptidase (*CaPOP*) were identified to be upregulated in the early developmental stages of MA2 in comparison to KO34. The function of POP gene has been widely studied in animals but largely ignored in plants and thus its significance in plants is not known. In order to understand its role in organ size control, the POP genes from MA2 and KO34 were cloned and sequenced. Sequence analysis resulted in the identification of two POP variants arbitrarily named *CaPOP1* and *CaPOP2*, each in both cultivars. Primarily, *CaPOP1* differs from *CaPOP2* in containing two deletions (158 nt and 720 nt, respectively) in their promoter regions. For the functional characterization of *CaPOP*, we have constructed expression vectors for the purpose of studying: the effect of overexpression, promoter activities and subcellular localization in transgenic *Arabidopsis thaliana*. The transformants are currently being analyzed. Furthermore, we are also assessing the effects of T-DNA insertion mutation of *AtPOP* in *Arabidopsis*. This study will increase our understanding of the role of *POP* in plant growth and development."
(a) University of Hawaii at Manoa, Department of Molecular Biosciences and Bioengineering (b) University of Hawaii at Manoa, Department of Tropical Plant and Soil Sciences (c) Hawaii Agriculture Research Center (d) Pacific-Basin Agriculture Research Center USDA-ARS (e) Miller Associates

P65015 Characterization of four unknown genes isolated from *Leucaena leucocephala*
Singh, Ratnesh (a) Negi, Vishal S (a) Pal, Archana-presenter apal@hawaii.edu(a) Jube, Sandro (a) Borthakur, Dulal (a)
"The goal of this research was to characterize novel genes from the tree-legume *Leucaena leucocephala* (leucaena), which is a unique plant free from diseases, and highly resistant to environmental stresses. By using inter-species suppression subtractive hybridization with *Acacia confusa*, we have isolated cDNAs for four novel genes from leucaena, namely, Lc5, Lc8, Lc9 and Lc22, which have no sequence homology with any genes in the

database. Since homology-based functional characterization was not feasible for these cDNAs, we performed protein-structure-based characterization using the deduced amino acid sequences through in silico approaches. The secondary structures, folds, secretory peptide, subcellular localization, and transmembrane helices for these putative proteins were analyzed. These unknown proteins do not contain any trans membrane helices suggesting that these are non- membraneous proteins. Lc5 and Lc8 were detected to have chloroplast transit peptides, which were further supported by sub-cellular localization prediction. Lc9 was also predicted to have chloroplast signal peptide but its sub-cellular localization was other than chloroplast, indicating that it is not a chloroplast localized protein. All the proteins were found to be devoid of secretory signal peptide, which indicates that these proteins do not belong to classical secretory pathways. The four novel proteins may be involved in conferring some unique features to leucaena. Further molecular and biochemical analyses of these proteins will reveal their exact roles in the physiology of leucaena."
(a) University of Hawaii at Manoa, MBBE

P65016 Interspecies suppression subtractive hybridization: A molecular tool to study differential gene expression between two different species
Negi, Vishal S-presenter negi@hawaii.edu(a) Jube, Sandro (a) Borthakur, Dulal (a)
"*Leucaena leucocephala* (leucaena) is a tree-legume that has no known diseases and unlike other closely related tree-legumes, it is highly tolerant to various abiotic stresses. Therefore, it is expected that leucaena has high-level expression of many stress-related genes and may also contains some unique genes that are absent in other closely-related species. To identify such highly-expressed and unique genes, interspecies suppression subtractive hybridization (iSSH) was performed using cDNAs from *Acacia confusa* to subtract homologous cDNA fragments from leucaena transcriptomes. A total of 385 iSSH clones from leucaena were sequenced and analyzed using the Blastclust, BLASTX, BLAST2GO, ESTScan and InterProScan programs. Among the iSSH clones, 169 were represented only once, rest of the sequences were represented 2-24 times. A total of 112 sequences were found to have homology to stress-related genes including some pathogenesis-reated genes (PR genes) such as β-1,3-glucanase, chitinase, thaumatin-like protein, trypsin inhibitor, cysteine protease, osmotin-like protein, PR-10 proteins, and expansin-like PR proteins. PR genes are known to have important roles in providing resistance to pathogen and environmental stress conditions. RLM-RACE is being used for obtaining the full-length sequences of these cDNAs. Identification of such a large number of stress-related genes suggests that these genes are either highly expressed or unique to leucaena. Further characterization of these stress-related genes may reveal the molecular basis of high-level tolerance of leucaena against biotic and abiotic steresses. Additionally, introduction of these stress-related genes into susceptible crops may be a useful strategy for developing stress resistance in agriculturally important crops."
(a) University of Hawaii at Manoa, MBBE

P65017 "Genomic analysis of the chromoplast-specific lycopene beta-cyclase gene, *CpCYC-b*, revealed a recombination hotspot in papaya."
Blas, Andrea L-presenter ablas@harc-hspa.com(a) Ming, Ray (b) Moore, Paul H (a) Paull, Robert E (c) Yu, Qingyi (d,a)
"The chromoplast-specific lycopene beta-cyclase gene, *CpCYC-b*, is the major gene determinant for papaya fruit flesh color. Fruit flesh color is a major factor in consumer preference and a trait for which papaya breeders have long desired a genetic marker. In our search for a suitable genetic marker for fruit flesh color, we discovered *CpCYC-b* was located near a putative recombination hotspot, the first reported for papaya, in the telomeric region of chromosome 5. Two *CpCYC-b*-containing BAC clones, SH18O09 from the red-fleshed SunUp hermaphrodite and DM105M02 from the yellow-fleshed AU9 male BAC libraries, were fully sequenced using a shotgun approach. Recombination frequencies between *CpCYC-b* and two insertion-deletion polymorphisms, CPFC and fc.5, located downstream of *CpCYC-b* indicate genetic distances of 0.9cM and 2.3cM over a physical distance of 580bp and 8360bp, respectively. The genetic to physical distance ratio between CPFC and fc.5 is 108-fold higher than the genome average (0.0025cM/kb) while the ratio between *CpCYC-b* and CPFC is 620-fold higher. Analysis of the BAC sequence alignment also revealed gene density two-fold higher than the genome average and a 1558bp Ty1 *copia* type retroelement in the 65kb region encompassing *CpCYC-b*. Ten predicted papaya genes in this region are supported by EST or gene expression data and show homology to Arabidopsis genes spread across all five Arabidopsis chromosomes. Collinearity of four homologous gene sets was detected between papaya, tomato and the predicted ancestral Arabidopsis genome. These data indicate conservation of microsynteny between these three genomes and further illustrate the potential of papaya in comparative genomic studies with agronomic species."
(a) Hawaii Agriculture Research Center (b) University of Illinois, Urbana-Champaign, Department of Plant Biology (c) University of Hawaii, Manoa, Tropical Plant and Soil Sciences Department (d) Texas A&M University, AgriLife Research Center

SESSION P66 - TROPISMS

P66001 New insights into phototropism from experiments in microgravity
Millar, Katherine-presenter millark2@muohio.edu(a) Correll, M J (b) Eldelmann, R E (a) Mullen, J L (c) Hangarter, R P (d) Kiss, John Z (a)
"Gravitropism and phototropism were studied in a series of microgravity experiments on the International Space Station (ISS). Specifically, the role of red-light-absorbing phytochrome pigments in modulating tropisms in seedlings of *Arabidopsis thaliana* was investigated. The response of the WT was compared to *phyA*, *phyB*, and *phyAB* mutants. These studies were performed on the European Modular Cultivation System (EMCS), which provides an incubator with atmospheric control, lighting, and high resolution video. The EMCS has two rotating centrifuge platforms so that experiments were performed at 1g (as a control) as well as in microgravity (centrifuge off). The main advantage of these space experiments is that a 'pure' phototropic response can be studied without the 'complications' of a constant gravity vector as found on Earth. Seed germination (57%) during the space experiments was lower than ground controls (>90%), and this was likely due to extended storage of 8 months in flight hardware. However, the seedlings that germinated exhibited robust growth and tropistic curvature. Both roots and hypocotyls from seedlings that developed in microgravity had a stronger phototropic curvature (in response to the various light qualities tested) compared to seedlings in the 1g control. The broader implications of these results for models of phototropism will be discussed in this presentation."
(a) Miami University (b) University of Florida (c) University of Colorado (d) Indiana University

P66002 Studies in preparation of a spaceflight experiment to investigate tropisms.
Kumar, Prem-presenter kumarp@muohio.edu(a) Edelmann, Richard E. (a) Kiss, John Z. (a)
"Phototropism and gravitropism are two physiological processes, and the interactions between these two tropisms determine the final growth form of plants. Although gravitropism can easily be studied on earth, it is difficult to determine the response of roots to light. Therefore, we have developed a project (TROPI, for tropisms) to determine the phototropic response of Arabidopsis plants in the microgravity conditions of space. European Space Agency (ESA) has developed a research facility termed European Modular Cultivation System (EMCS) for growing plants in microgravity. The EMCS provides an incubator with two centrifuges and provides atmospheric control, lighting, and high-resolution video. Working with NASA, we have

developed experimental unique equipment (EUE) for growing Arabidopsis seedlings during spaceflight. The EUE consists of five seedling cassettes with LED lighting and a water delivery system. We have performed several biocompatibility tests in order to optimize the growth of Arabidopsis. Our initial studies showed poor seed germination and seedling growth during long-term storage in the EUE. We determined that the conformal coating of the electrical components of the EUE resulted in release of toxic gases inhibiting the growth of seedlings. We added activated carbon filters to the seedling cassettes and to the base of the EUE, and this improved growth of seedlings. We have also initiated additional biocompatibility tests in the space hardware to determine the cause of poor seed germination in some samples. A follow-up experiment has been planned to be performed at intermediate gravity accelerations similar to levels found on the Moon and Mars."
(a) Miami University

P66003 GNOM-mediated vesicular trafficking plays an essential role in hydrotropism of Arabidopsis roots
Miyazawa, Yutaka-presenter miyazawa@ige.tohoku.ac.jp(a) Takahashi, Akiko (a) Kobayashi, Akie (a) Kaneyasu, Tomoko (a) Nobuharu, Fujii (a) Hideyuki, Takahashi (a)
"Plants are sessile in nature and need to respond to various environmental cues to regulate their growth orientation. Of these responses, hydrotropism is a response to moisture gradient, which is thought to help roots to obtain water and nutrients effectively. Roots also display gravitropism, which interferes with hydrotropism on Earth. However, the mechanisms of how roots display hydrotropism and differentiate it from gravitropism, are not understood. We previously reported *MIZU-KUSSEI1* (*MIZ1*) as a gene required for hydrotropism but not for gravitropism, although the molecular function of its product was not known. Here, we identified a gene responsible for ahydrotropic mutant, *miz2*, which appeared to be a new allele of *gnom*. GNOM encodes a guanine-nucleotide exchange factor for ADP-ribosylation factor-type G protein, which is required for endosomal recycling. Unlike other *gnom* alleles, *miz2* showed no apparent morphological defects or reduced gravitropism. Instead, brefeldin A (BFA) treatment inhibited both hydrotropism and gravitropism in Arabidopsis roots. In addition, a BFA-resistant GNOM variant showed normal hydrotropic response in the presence of BFA, confirming the indispensable role of GNOM in root hydrotropism. Moreover, a weak *gnom* allele, which is defective in root gravitropism, also showed defect in hydrotropic response, suggesting a stronger requirement of GNOM for hydrotropism relative to gravitropism. Our results indicate that a unique GNOM-mediated vesicular trafficking plays an essential role in root hydrotropism."
(a) Tohoku University

P66004 "SHOOT GRAVITROPISM 9, a novel RING finger protein, is involved in statolith dynamics by modulating interaction between F-actin and amyloplasts."
Morita, Miyo T-presenter mimorita@bs.naist.jp(a) Nakamura, Moritaka (a) Tasaka, Masao (a)
"Gravitropism is the directed growth forward or opposite direction of gravity. In higher plants, relative directional change of gravity is suscepted in the specialized cells (statocytes), and then the following processes are initiated. We have studied the molecular genetic mechanism of gravitropism of Arabidopsis inflorescence stems and demonstrated that endodermal cells are most likely to be the statocytes in Arabidopsis shoots. We have also reported that amyloplast sedimentation to the direction of gravity in the endodermal cells is important for gravity perception. The *sgr9 (shoot gravitropism 9)* mutant exhibits greatly reduced shoot gravitropism. In endodermal cells of *sgr9*, amyloplasts dynamically move around but did not sediment to the direction of gravity. The *SGR9* encodes a novel RING finger protein, which is expressed mainly in the shoot endodermis. The homology of RING finger domain between SGR9 and known ubiquitin E3 ligase suggests that SGR9 is also putative E3 ligase. Consistent to the expectation, mSGR9, which contains a point mutation at the putative essential site for E3 ligase activity in the RING finger domain, exerted a dominant negative effect on wild-type plant to mimic the *sgr9* mutant. In addition, mSGR9-GFP was localized to amyloplasts. It has been suggested that actin filaments (F-actin) is involved in amyloplast dynamics. To analyze the relationship between SGR9 and F-actin, we observed F-actin surrounding amyloplasts both in wild-type and in *sgr9*. Amyloplast surrounded by F-actin was more frequently observed in *sgr9* than in wild-type. In addition, inhibition of actin polymerization restored gravitropism and amyloplast sedimentation in *sgr9*. Taken together, SGR9 might modulate interaction between amyloplasts and F-actin on amylopalsts."
(a) Nara Institute of Science and Technology

P66005 Basipetal migration of Ca^{2+} and pH waves during the graviresponse of *Arabidopsis* roots
Monshausen, Gabriele B-presenter monshausen@wisc.edu(a) Miller, Nathan D (a) Murphy, Angus S (b) Spalding, Edgar P (a) Gilroy, Simon (a)
"During gravistimulation of roots, a signal perceived in root cap statocytes is likely translated into the generation of an auxin gradient across the root cap. This gradient is then thought to be propagated along the root axis through the concerted action of an array of auxin transporters. Using a combination of confocal vertical-stage and inverted-stage microscopy we show that gravistimulation also triggers the migration of an asymmetric wave of Ca^{2+} and pH along the Arabidopsis root axis. Furthermore, we provide evidence suggesting that the generation of these Ca^{2+} and pH waves is dependent on auxin fluxes: (i) auxin transport mutants show altered surface pH dynamics compared to wild type; (ii) adding auxin to the medium bathing a root results in an instantaneous increase in both cytosolic Ca^{2+} levels and in surface pH; (iii) localized application of auxin to the cap of vertically growing roots triggers a basipetally migrating wave of elevated Ca^{2+} and surface pH. Previous work has suggested a model where gravistimulation causes the differential accumulation of auxin on the lower flank of the root cap. These high auxin levels are then transported from the root tip to the root elongation zone to effect growth control. Our analyses suggest that such movement of auxin proceeds at a rate of ca 200-300 μ;m min^{-1} and triggers an increase in Ca^{2+} in cells on the lower flank of the root. This elevation in cytosolic Ca^{2+} in turn gives rise to rapid extracellular alkalinization which likely modulates growth and thus promotes root gravitropic curvature. Our observations indicate that the spatial and temporal dynamics of Ca^{2+}-dependent auxin signaling play a central role in coordinating growth during such tropic responses. This work is supported by NSF (MCB- 0641288)."
(a) University of Wisconsin-Madison (b) Purdue University

P66006 Effects of Magnetic Fields on Plant Growth and Development
Frederick, Cory M-presenter mm353498@ohio.edu(a) Wyatt, Sarah (a)
"What effect does magnetism have on plant growth and development? The aquatic bacterium Magnetospirillum magnetotacticum biomineralize magnetite for migration to preferred oxygen gradients (magnetotaxis). The bacterial gene COG3536 is responsible for magnetosome development, iron oxide and iron sulfide nucleation, as well as nanocrystal synthesis. At3g27340, an Arabidopsis thaliana gene that encodes a protein of unknown function, has a similar protein sequence to COG3536. At3g27340 is located on the third chromosome of Arabidopsis thaliana, and two T-DNA insertion mutants, SALK_011817 and SALK_062099, have been selected for study. In initial experiments, two magnets (each 6.4mm x 24mm) were affixed to MS plates to create a negative field. Seeds of WT and mutants were plated either between the magnets in the low-G negative field or on plates without magnets, and the plates were clino-rotated. Clinorotation was employed because it eliminates the effects of gravity. After seven days, total seedling length was recorded along with hypocotyl bending. There was no significant difference among the control plants. In the clino-rotated groups, however SALK_011817 total seedling length averaged 8.96 mm, SALK_062099 averaged 4.55 mm, and WT averaged 4.95 mm. Reasons for

the significant difference in growth rate for SALK_011817 are still being examined, and may be attributed in part to ferrous sulfate in the MS. Continued research includes the characterization and rescue of the phenotype of the mutants and a high-altitude flight to test the effect of solar radiation, gravity, response to the magnetic field and PT. Partial funding provided by ASPB SURF award and the Jeanette Graselli-Brown Undergraduate Research award. "
(a) Department of Environmental and Plant Biology, Ohio University

P66007 *LAZY1* belongs to a novel class of genes involved in gravitropic signal transduction in monocot and dicot plants
Yoshihara, Takeshi-presenter yoshihara@wisc.edu(a,b) Spalding, Edgar P. (a) Iino, Moritoshi (b)
"Adult *lazy* mutants of rice (*Oryza sativa*) display a prostate growth habit. Coleoptiles of seedlings display defective circumnutation and gravitropism due to defective lateral auxin transport. Thus, LAZY1 appears to function at a step upstream of lateral auxin redistribution in gravity-directed processes that rely on differential growth. The *LAZY1* gene encodes a shoot-specific, possibly nuclear-localized protein lacking recognizable functional domains. The closest relative in *Arabidopsis thaliana* is a *LAZY1-like* gene (21% sequence identity) of unknown function. A question addressed by this work is whether the auxin distribution and growth control functions of *LAZY* genes are unique to monocots or if these roles evolved before monocot-dicot divergence. Arabidopsis plants expressing a *ProAtLAZY1-like:GUS* construct showed strong expression in adult shoots, especially in vascular tissues in rosette leaf blades and petioles, and where lateral inflorescence branches originate. In seedlings, the vasculature of cotyledons and upper hypocotyl showed strong expression. To identify the function of the *AtLAZY1-like* gene in Arabidopsis, RNA interference (RNAi) was used to suppress its expression. So far these RNAi lines revealed the following: Firstly, the inflorescence-stem gravitropism is weaker compared with the wild type (WT). Secondly, the angle between the main inflorescence shoot and the lateral shoots is much greater than that of WT. Gravitropism and nutation, which are affected in rice lazy mutants, are being examined in the Arabidopsis RNAi seedlings using custom image-processing tools. Such studies may demonstrate that the *AtLAZY1-like* gene is an ortholog of *OsLAZY1*, performing a similar role in monocot and dicot gravity signal transduction."
(a) University of Wisconsin (b) Osaka City University

P66008 Root cap angle and gravitropic response rate are uncoupled in the Arabidopsis pgm-1 mutant
Paya, Alex (a) Toska, Jonida (a) Wolverton, Chris-presenter scwolver@owu.edu(a)
"The sedimentation of starch-filled plastids is thought to be the primary mechanism by which gravity is perceived in roots. Following gravity perception, auxin redistribution toward the lower flank of roots, initiated in the root cap, is believed to play a role in regulation of the gravity response. These two processes, however, have never been directly linked. The overall aim of this study was to investigate the relationship between plastid sedimentation and auxin flux. Our data show that pgm-1 roots respond to gravity at one-third the rate of WT. Maintaining the root cap at a constant angle using image analysis coupled to a rotating stage showed a dose-independent gravity response in pgm-1 mutants, which contrasts with the response of wild-type and that of pin3-1 mutants, both of which were dose-dependent. The DR5::GFP reporter gene was used to indirectly visualize auxin flux following gravistimulation. In WT roots a GFP gradient was observed along the lower flank as previously reported, whereas pgm1 roots formed a GFP maximum in the central columella but lacked any observable gradient. Our study suggests that there is a correlation between the physical sedimentation of starch filled plastids and auxin flux in the root cap."
(a) Ohio Wesleyan University

P66009 Complex regulation of differential growth by light and gravity involving auxin transport in roots
Mayers, Elizabeth B-presenter ebmayers@owu.edu(a) Evans, Megan (a) Wolverton, Chris (a)
"Although non-motile, plants have highly evolved sensory systems that allow them to grow towards resources such as nutrients, water, and light. In particular, light and gravity are both sensed in the root cap and produce differential growth in the elongation zone of the root. This overlap between photo- and gravitropic signal transduction pathways has not yet been fully characterized and provides a model system to study growth regulation in roots. In order to tease apart responses to gravity and light in roots, we made use of several mutants in Arabidopsis. To analyze the influence of gravity stimulation on the attenuation of phototropism, we compared the wild-type phototropic response to that of pgm-1, a mutant in starch biosynthesis with reduced gravity sensing. We found that while wild-type roots respond to a directional blue light cue sooner than roots of pgm-1, the mutant showed greater overall curvature. Because both gravi- and phototropism are mediated in part by an auxin asymmetry, we studied the phototropic response of the pin3 mutant, which is disrupted in a root cap-specific auxin efflux carrier. We found that this mutant shows a reduced response in roots, suggesting a role for auxin efflux in modulating root phototropism. We tested this possibility using confocal microscopy on roots expressing an auxin-responsive promoter fused to GFP. While this reporter line has previously been used to demonstrate an auxin asymmetry following gravistimulation, we found no evidence of such a gradient following phototropic stimulation. Finally, we are in the process of analyzing root phototropism in the absence of ensuing gravity stimulation by using a feedback system. Preliminary results indicate that roots are capable of long-term growth away from directional light."
(a) Ohio Wesleyan University

P66010 Investigation of mechanism for gravity sensing in Arabidopsis inflorescence stems using a novel centrifuge microscope
Toyota, Masatsugu-presenter toyota@bs.naist.jp(a) Tasaka, Masao (a) Morita, Miyo T (a,b)
"Sedimentation of amyloplasts in endodermal cells has appeared to be involved in gravity sensing in Arabidopsis shoots. Recent live-cell imaging studies, however, suggested that amyloplasts exhibit dynamic and complicated movements toward the direction of gravity after 90^{o}-reorientation probably due to interaction with organelle and/or cytoskeleton. Therefore, it remains unclear what kind of amyloplast movement is required for gravity sensing. To address this issue, we analyzed gravitropism and amyloplast dynamics under hypergravity condition in which sedimentation by gravity is more dominant than other movements. Segments of Arabidopsis inflorescence stem exhibited obvious gravitropism in response to transversely applied hypergravity (10*g*) for 30 sec in a conventional centrifuge, suggesting that amyloplast dynamics during this short time period is indeed involved in gravity sensing. Real-time imaging of amyloplasts during hypergravity was performed using a centrifuge microscope that was recently developed in cooperation with NSK Ltd (Japan). In this system, all optical devices including objective lens, light source (LED) and CCD camera are mounted on a mega torque motor (TM), enabling bright-field imaging with a temporal resolution of 30 frames/sec during rotation. Almost all amyloplasts started to move toward the direction of 10*g* and some of them reached the bottom of endodermal cell within 30 sec. These results support the idea that amyloplast sedimentation toward the bottom region of endodermal cell is involved in gravity sensing."
(a) Graduate School of Biological Sciences, Nara Institute of Science and Technology (b) Precursory Research for Embryonic Science and Technology, JST

P66011 Contribution of a mechanism sensing apical-basal component of gravity and Aux/IAA signaling to plagiotropism of lateral roots in *Arabidopsis thaliana*
Matsuzaki, Jun-presenter jm@sci.hokudai.ac.jp(a) Watahiki, Masaaki K (a) Yamamoto, Kotaro T (a)

"Growth orientation of lateral organs is a major determinant of architecture of a plant body. We analyze molecular mechanism of plagiotropism in lateral roots of Arabidopsis, which temporary grow obliquely (plagiotropism) after the initiation and then grow downward (orthotropism). When rotated by 90 degrees, most lateral roots reorient toward oblique growth direction relative to gravity vector (plagiogravitropism). Thus, plagiotropism in lateral roots may result from plagiogravitropism rather than incomplete orthogravitropism. We built a mathematical model which described bending rate as a linear combination of sine and cosine of root-tip deviation angle from gravity vector. This model fitted well to time course of the root tip reorientation. This implies that a mechanism sensing apical-basal component of gravity, which equals cosine of the deviation angle, such as amyloplast sedimentation toward apical walls of columella cells, contributes to the plagiogravitropism. We are also analyzing auxin-related factors as possible determinants of plagiotropism in lateral roots. The transition from plagiotropism to orthotropism is retarded in mutant *hy5*, which shows plagiotropism in longer lateral roots than wild-type. Lateral roots of *hy5* grown on a medium with antiauxin α-tert-butoxycarbonylaminohexyl-indole-3-acetic acid (BH-IAA) showed orthotropic growth direction more frequently than controls. Because BH-IAA inhibits degradation of Aux/IAA proteins, Aux/IAA may function to maintain the plagiotropism. We also screened for revertants of *hy5* with transition from plagiotropism to orthotropism in shorter lateral roots than *hy5*. We have obtained several candidate lines with a single-locus mutation and are carrying out mapping of these loci. "
(a) Hokkaido University

P66012 Mechanostimulation affects gravitropism and signal persistence in flax roots.
Hasenstein, Karl H-presenter hasenstein@louisiana.edu(a) John, Susan P (a)
http://www.ucs.louisiana.edu/~khh6430/
"In higher plants, root gravitropism results from displacement of amyloplasts but the persistence of the gravistimulus is unknown. Clinorotation is commonly used to negate the effect of gravity and has been used to determine the time necessary to induce curvature (presentation time). However, constant rotation represents a form of mechanostimulation that interferes with root development. Especially sensitive are columella cells where clinorotation can lead to programmed cell death. To investigate how speed and duration of clinorotation affect the graviresponse, we clinorotated roots (0.5 to 5 rpm) in darkness after horizontal reorientation for 0 to 15 min. Clinorotation was performed either parallel or perpendicular to the root axis and roots were imaged after each rotation by an infrared video camera. Vertical clinorotation did not affect root growth rate (0.6 mm/h) but horizontal clinorotation (rotation parallel to root axis) promoted root growth by 150%. The rate of clinorotation did not affect growth for either condition. Maximal curvature decreased as the rate of rotation increased and amounted to 30 degrees for 0.5 rpm but produced straight roots at 5 rpm. Horizontal clinorotation increased curvature with increasing reorientation time. Vertical clinorotation resulted in greater variability of root curvature. These data indicate that clinorotation masks the gravity response but does not negate gravity effects. Although clinorotation minimizes gravity effects, it introduces secondary responses that depend on rate and angle of rotation. Thus, any randomization affects the graviresponse and simulation of weightlessness but allows the assessment of the persistence of mechanostimulation."
(a) Univ. Louisiana at Lafayette

P66013 "CYP705A5, a novel cytochrome p450, involved in the gravitropic response in roots. "
Withers, John E (a) Justus, Betsy (a) Wyatt, Sarah E-presenter wyatts@ohio.edu(a)
"Gravity is an important environmental factor that affects the growth and development of plants. In response to changes in gravity, directional growth occurs along the major axes and lateral branches of both shoots and roots. The gravity persistent signal (gps) mutants of Arabidopsis thaliana were previously identified as having an altered response to gravity; in particular, *gps1* showed a no response phenotype when subjected to cold gravity stimulation. Cloning and characterization of the GPS1 gene has revealed that GPS1 encodes CYP705A22 a cytochrome P450 monooxygenase that is expressed in the inflorescence stems. Mining of microarray expression data collected from gravistimulated root tips of Arabidopsis indicated that although CYP705A22 was not expressed in roots; however, a family member CYP705A5 was up-regulated within 3 minutes after gravistimulation. Expression profiling of CYP705A5, using real-time quantitative PCR, showed that CYP705A5 was up-regulated nearly five fold within minutes of gravity stimulation. Reporter gene fusions showed that CYP705A5 was expressed in the root zones of elongation and maturation. A mutation affecting CYP705A5 expression resulted in a delayed gravity response and decreased basipetal auxin transport. DPBA staining in the CYP705A5 mutant indicated an accumulation of quercetin in mutant roots as compared to WT. These data, taken together, may indicate that we have identified a flavonoid pathway that regulates auxin distribution and thus is involved in gravitropic signal transduction. (Partially support by NSF: 0618506 to SEW)"
(a) Ohio University

SESSION P67 – VASCULAR BIOLOGY

P67001 Downregulating the sucrose transporter VpSUT1 in *Verbascum phoeniceum* L. does not inhibit phloem loading
Zhang, Cankui-presenter cz46@cornell.edu(a) Turgeon, Robert (a)
"Photoassimilate must be loaded into the phloem in the minor veins of leaves before long-distance transport to sink organs occurs. Two species-specific phloem loading pathways, apoplastic and symplastic, have been proposed. Several model plants have been shown to be apoplastic loaders by molecular genetic approaches. Downregulation of the sucrose transporter (SUT1) has been especially compelling in this regard as it interferes directly with the loading mechanism, leading to pronounced accumulation of photoassimilate in leaves and leaf chlorosis. In this study we took a similar approach using the putative symplastic loader, *Verbascum phoeniceum*. We cloned *VpSUT1* and downregulated it in transgenic plants by the RNAi technique. To test the effectiveness of downregulation we measured *VpSUT1* mRNA levels and sucrose-H+ symport in leaf discs. Similar experiments were conducted on *Nicotiana tabacum*, a known apoplastic loader. Mild *NtSUT1* downregulation in *N. tabacum* resulted in the pronounced phenotype associated with loading inhibition. In contrast, no such phenotype developed when *VpSUT1* was downregulated in *V. phoeniceum*. Although the leaves of plants with the most severe VpSUT1 downregulation accumulated more carbohydrate than usual, these plants cleared starch during the night period, did not become chlorotic, and the plants grew normally. The results provide direct evidence that the mechanism of phloem loading in *V. phoeniceum* does not require active sucrose uptake from the apoplast and indicates that the loading pathway is symplastic in this species. "
(a) Cornell University, Department of Plant Biology

P67002 Cell wall-degrading enzymes enlarge the pore size of intervessel pit membranes in healthy and *Xylella fastidiosa*-infected grapevines
Perez Donoso, Alonso G.-presenter agperez@uc.cl(a) Greve, L. Carl (b) Sun, Qiang (c) Roper, Caroline (d) Labavitch, John M (b)
"The pit membrane is a primary cell wall barrier that separates adjacent xylem water conduits, limiting the spread of pathogens and air embolisms from one conduit to the next. This work provides a characterization of the size of the pores in the pit membranes of grapevine (*Vitis vinifera*). The pit membrane porosity (PMP) of stems infected with the bacterium *Xylella fastidiosa (Xf)* was compared with the PMP of healthy stems. Stems explants were infused with pressurized water and flow rates were determined, then gold particles of known size were introduced with the water and their

passage through the explants was assessed to assist in determining the size of pit membrane pores. The possibility that cell wall-degrading enzymes could alter the pore sizes, thus facilitating the ability of *Xf* to cross the pit membranes was tested. Two cell wall-degrading enzymes known to be produced by *Xf* polygalactuoronase and endo-1,4-β-glucanase), were infused into stems and particle passage tests were performed to check for changes in PMP. The effects of introducing the pectin-solubilizing extractant CDTA, oligogalacturonides and polygalacturonic acid into stems were also evaluated."
(a) Departamento de Fruticultura y Enologia. Pontificia Universidad Catolica de Chile (b) Department of Plant Sciences. University of California at Davis (c) Department of Biology. University of Wisconsin-Stevens Point (d) Department of Plant Pathology and Microbiology. University of California at Riverside

P67003 *PEAPOD* limits and coordinates vascular procambium activity and stomatal density in *Arabidopsis*.
White, Derek W. R.-presenter derek.white@agresearch.co.nz(a)
"Vascular plants can vary their size in response to different environmental conditions by regulating the growth of leaves, stems and roots. Although this is a common observation there is only limited information about the genetic mechanisms controlling and coordinating plant secondary growth. Much of higher plant secondary growth is determined by the activity of populations of dispersed stem cells (DSC), most notably the vascular procambium/cambium and the shoot epidermal meristemoid mother cells (MMC) that initiate the stomatal lineage. The densities of both the vascular tissue and stomata play significant roles in the water transpiration stream that is critical to the growth of higher plants. In *Arabidopsis* deletion of the *PEAPOD* (*PPD*) locus results in enlarged domed-shaped leaves (1). This excess leaf growth in *ppd* mutant plants is due to increased proliferation of the MMCs that develop into the stomata guard and leaf pavement cells of the epidermis. The loss-of-function mutant also has thickened roots, hypocotyls and stems due to higher levels of procambium/cambium activity producing greater vascular growth throughout the plant. The *PPD* locus is composed of two orthologs, *PPD1* and *PPD2*, which encode novel members of the *TIFY* gene family. Over expression of *PPD* reduces procambium/cambium activity and MMC activity resulting in reductions in both vascular growth and stomatal density. The *PPD* genes therefore act as repressors of dispersed stem cell activity during organ development, coordinating tissue growth by limiting vascular and stomatal density. *PPD* homologs are present in a wide range of eudicot plants, conifers and lycophytes, all plants that have a vascular cambium,but absent from plants without vascular cambium. White, D.W.R. (2006) PNAS 103:13238-13243 "
(a) AgResearch, Grasslands Research Centre

P67004 Sieve Element Occlusion (SEO) proteins are involved in forisome formation
Pelissier, Helene C-presenter hpelissi@mail.wsu.edu(a) Winfried, Peters S (b) Knoblauch@wsu.edu, Michael (a)
"Forisomes are Ca^{2+} ATP-independent contractile protein bodies that reversibly occlude sieve elements in faboid legumes. They apparently consist of at least three proteins. We isolated three genes from *Medicago truncatula* that correspond to the putative forisome proteins and expressed their GFP-fusion products in *Vicia faba* and *Glycine max* using the composite plant methodology. In both species, expression of any of the constructs resulted in homogenously fluorescent forisomes that formed sieve tube plugs upon stimulation. Isolated fluorescent forisomes reacted to Ca^{2+} and chelators by contraction and expansion, respectively, and did not lose fluorescence in the process. The three proteins shared numerous conserved motifs between themselves and with hypothetical proteins derived from the genomes of *M. truncatula*, *Vitis vinifera*, and *Arabidopsis thaliana*. However, they showed neither significant similarities to proteins of known function nor canonical metal binding motifs. We conclude that the proteins are components of forisomes that are encoded by a well-defined gene family with relatives in taxa that lack forisomes. The family was named SEO (Sieve Element Occlusion). We are currently doing live cell imaging with tobacco expressing *pMtSEO2-GFP* in sieve element compartments to investigate sieve element dynamics."
(a) Washington State University (b) Indiana/Purdue University Fort Wayne

P67005 Dissection of the *SHORT ROOT- PHABULOSA* pathway in the vascular tissue patterning of *Arabidopsis* roots
Zhou, Jing-presenter jz253@cornell.edu(a,b) Lee, Ji-Young (a,b)
http://bti.cornell.edu/JiYoungLeeLab/JingZhou.htm
"In plants, organ growth is achieved by the precise temporal and spatial regulation of tissue patterning. This process requires the coordination of transcriptional regulatory networks and cell-cell communication. To understand how this is achieved, we investigate the regulatory processes of vascular tissue patterning in the Arabidopsis root. Recently, we found that GRAS family transcription factor *SHORT ROOT* (*SHR*) activates *miRNA165/166* by moving from the stele to the ground tissue. *MiRNA165/166* generated in the endodermis moves back to the stele and modulates the domains and levels Class III HD-ZIP transcription factors including *PHABULOSA* (*PHB*). This transcriptional and posttranscriptional regulatory network controls patterning of xylem and phloem as well as root growth activity and vascular cell proliferation. The question is how these complex developmental processes mediated by *SHR* are related. Our preliminary data indicate that *SHR-miRNA* pathway does not represent all the *SHR* function in the control of vascular developmental processes. Therefore we asked how *SHR* in the stele performs the rest of its function in the vascular development. To address this, we generated a compendium of transgenic plants that express non-mobile but functional SHR and PHB in specific vascular cell types in *shr* and *shr phb* mutants. Our analysis indicates that *SHR* might control the vascular tissue patterning and root growth via both cell autonomous and non-cell autonomous pathways. Unlike the non-cell autonomous regulation of *SHR*, cell autonomous regulatory pathway seems to be independent of class III HD-ZIP transcription factors. We are in the process of identifying the key targets involved in each pathway using genome-wide transcript profiling of these transgenic plants."
(a) Boyce Thompson Institute (b) Plant Biology Cornell University

P67006 "Histidine kinases CKI1, AHK2 and AHK3 control vascular tissue development in *Arabidopsis* shoots"
Hejatko, Jan-presenter hejatko@sci.muni.cz(a,h) Ryu, Hojin (b,h) Hwang, Ildoo (b) Kim, Gyung-Tae (c,d) Dobesova, Romana (a) Choi, Sunhwa (b) Choi, Sang Mi (b) Soucek, Premysl (e,f) Horak, Jakub (a) Palme, Klaus (g) Brzobohaty, Bretislav (e,f)
http://www.sci.muni.cz/FGP/
"The development and activity of the procambium and cambium, which ensure vascular tissue formation, is critical for overall plant architecture and growth. However, little is known about the molecular factors affecting the activity of vascular meristems and vascular tissue formation. Here we show that the sensor histidine kinase CKI1 and the cytokinin receptors AHK2 and AHK3 are important regulators of vascular tissue development in *Arabidopsis* shoots. Genetic modifications of CKI1 activity in *Arabidopsis* causes dysfunction of the two-component signaling pathway and defects in procambial cell maintenance and/or identity. *CKI1* overexpression in protoplasts leads to cytokinin-independent activation of the two-component phosphorelay, and intracellular domains are responsible for cytokinin-independent activity of CKI1. *CKI1* expression is restricted to vascular tissues in inflorescence stems, and CKI1 forms homodimers both *in vitro* and *in planta*. Loss-of-function *ahk2* and *ahk3* mutants and plants with reduced levels of endogenous cytokinins show defects in procambium proliferation and an absence of secondary growth. These results indicate that the cytokinin-independent activity of CKI1 and cytokinin-induced AHK2 and AHK3 are important for vascular bundle formation and biomass production in *Arabidopsis*."

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P67007 Overexpression of phloem specific *TRT1* gene leads to etiolation and early flowering

Tsuwamoto, Ryo-presenter rtuwa@cc.hirosaki-u.ac.jp(a) Harada, Takeo (a)

"The phloem contributes to the development and growth in vascular plants as a highway transporting nutrients, defensive compounds, and informational signals. But molecular basis underlying function, differentiation and development for phloem is largely unknown. To understand it, we attempted the isolation of novel phloem specific gene using an *Arabidopsis* GAL4-enhancer trap line N9187 exhibiting specific expression of GFP in phloem. TAIL-PCR and following promoter-GUS analysis disclosed that the phloem specific expression of *GFP* in N9187 reflects the expression pattern of a gene encoding plant specific unknown protein predicted as a precursor of secretory signal peptide. We termed this gene as *TRAVELING TINTER 1* (*TRT1*) because of following characters. The introgression of *TRT1* driven by CaMV35S promoter leaded to pleiotropic defects including etiolation and early flowering. This etiolation was found in whole plant, but in sink organs such as shoot apical meristem and newly emerged lateral bud, remarkably severe etiolation was observed. Since PHYB-mediated red and far-red light signaling contributes de-etiolation and repression of flowering via *FT* gene, we examined whether TRT1 is concerned with this light signaling. The *TRT1*-overexpressed plant showed up-regulation of *FT*, although expression of PHYB-regulated genes, *cabB* and *RbcSA1* was not affected. Furthermore, the expression of *TRT1* was up-regulated in dark period, though scarcely expressed in light period. Taken all results, TRT1 may play a role as signal molecule represses a downstream part of light signaling mediated by PHYB under dark condition, and it may be transported through the phloem. This work was supported by the Program for Promotion of Basic Activities for Innovative Bioscience (PROBRAIN) in Japan."

(a) Faculty of Agriculture and Life Science, Hirosaki University

SESSION P68 – VEGETATIVE DEVELOPMENT

P68001 "Functional characterization of FLP, a MYB protein involved in Arabidopsis stomata development"

Xie, Zidian-presenter xie.44@osu.edu(a) Lucas, Jessica (a) Morohashi, Kengo (a) Li, Dongmei (c) Sack, Fred (b) Grotewold, Erich (a)

"Stomata, having a pore between two identical guard cells, play an essential role during plant development by controlling gas exchange and water vapor. During stomata development lineage, with only one symmetric division, the precursor cell guard mother cell produces two identical guard cells. FOUR LIPS (FLP), as one of R2R-MYB proteins in Arabidopsis, is required for this symmetric division. Loss of FLP function results into extra guard mother cell divisions, which leads to a clustering of guard cells. Therefore, FLP limits guard mother cell division to one followed by terminal guard cell differentiation. Previously, we showed that FLP is a transcription factor. Here, we are presenting the molecular functions for FLP during guard mother cell division. Using chromatin immunoprecipitation coupled with microarray (ChIP-chip) technique, we identified many FLP putative direct targets in vivo, including many cell cycle genes involved in different cell cycle phases. One of cell cycle genes was further studied using a variety of genetic and molecular tools. Based on the data we have, a working model for FLP molecular functions during stomata development was proposed."

(a) Ohio State University (b) University of British Columbia (c) University of Hawaii

P68002 "Arabidopsis orthologs of the Petunia HAM mutant regulate meristem indeterminacy, organ generation and growth in both the shoot and the root."

Engstrom, Eric-presenter emengs@wm.edu(a) Hu, John (a) Orlova, Evguenia (a) Bowman, John (b)

"Maintenance of indeterminacy is fundamental to the generation of plant architecture, and a central component of the plant life-strategy, woody perennials being capable of growing for thousands of years. The Petunia HAIRY MERISTEM (HAM) gene, a member of the GRAS family of transcriptional regulators, promotes meristem indeterminacy by undefined non cell-autonomous signaling mechanisms. ham mutants exhibit arrest of lateral organ formation and differentiation of the meristem as stem. No equivalent phenotypes are have been reported to date in Arabidopsis, nor have phenotypes resulting from loss-of-function alleles of Arabdopsis HAM orthologs (AtHAMs). Here we report that Arabidopsis mutants homozygous for probable null alleles of three AtHAMs exhibit phenotypes of, axillary shoot meristem arrest, abnormal phyllotaxis, meristem identity defects, reduced shoot growth, partial male infertility, and reduced length of the main root, and reduction in the size of the root apex. Gain-of-function mutants, resulting from fusion of a YFP protein to the C-terminal end of the AtHAM At2g45160, exhibit the complementary phenotype of multiple shoots, while gain-of-function mutants resulting from disruption of the microRNA binding site exhibit the phenotypes of reduced shoot and root growth. Phylogenetic analysis of GRAS genes from P. patens, S. moellendorffii, O. sativa and Arabidopsis, suggest that HAM genes are closely related to the DELLA subfamily, and that At4g36710 is a HAM gene that lacks the microRNA binding site conserved among all other members of the HAM subfamily. "

(a) The College of William and Mary (b) Monash University, Clayton Campus

P68003 Regulation of Strawberry Leaf Architecture by a KNOX Ortholog

Chatterjee, Mithu-presenter cmithu@ufl.edu(a) Folta, Kevin M (a)

http://www.strawberrygenomics.com

"Leaves are lateral organs that develop in succession from shoot apical meristem and are specialized for photosynthesis. Leaves are grouped into two form simple (single lamina or blade at a node) or compound (lamina branches to produce leaflets). The compound leaves pursue a less determinate pathway of development than simple leaves. One of the key regulator gene playing a role in the development of simple and compound leaf is *KNOX* (KNOTTED1-like homeobox) genes. KNOX genes are required for initiation and maintenance for shoot apical meristem. In functionally analyzing a series of strawberry genes we identified a transcript with identity to a *KNOX* homeodomain protein. *FaKNOX* showed finite similarity to classI Knotted- like homeodomain with 60% identity with maize *LIGULESS3*. Using a strawberry transgenic system we demonstrated that genetic manipulation of strawberry homeobox gene, *FaKNOX*, expression caused a remarkable change in the leaf morphology. The RNAi strawberry plants of *FaKNOX* gene shows a dwarf phenotype with deeply lobed leaflets and longer petiolules. These plants generate runners and small flowers, but fail to produce fruits. The severely effected RNAi plant shows tremendous change in the leaf anatomy. Expression of this transcript in wild-type plants is most prevalent in flowers and fruits, yet the strong effects of RNAi suggest roles in other tissues as well. Overexpression of the gene in *Arabidopsis thaliana* leads to dwarfed rosettes and infertile plants. The compound-leafed diploid strawberry species (*Fragaria vesca*) has natural variants that

produce a simple leaf, providing a genetic means to describe the mechanism of *FaKNOX1*. These analyses will help unravel the role of *FaKNOX* in leaf evolution and development in these species. "
(a) Horticultural Sciences Department and the Graduate Program in Plant Cellular and Molecular Biology, University of Florida

P68004 Using oppositely acting transcription factors to identify components of the ad/abaxial network of *Arabidopsis*
Barton, Kathryn-presenter kbarton@stanford.edu(a) Reinhart, Brenda J. (a) Kerstetter, Randall (b) Huang, Tengbo (b) Wenkel, Stephan (a)
"The asymmetric distribution of cell and tissue types from the top to the bottom of the leaf is critical to leaf function. The upper domain of the leaf with its tightly packed, chlorophyll rich palisade layer of cells is specialized for light capture while the lower domain with its loosely packed spongy mesophyll is specialized for gas exchange. Asymmetric differences are established along the ad/abaxial dimension of the leaf primordium while the primordium is still closely associated with the meristem. The ad/abaxial regulatory network controls the specification of ad/abaxial polarity within the leaf primordium. In addition to controlling the asymmetric development of the leaf blade this network also controls branching in the shoot and the root and vascular development. The KANADI and REVOLUTA transcription factors work in opposite directions in the ad/abaxial network: KANADI promotes abaxial development while REVOLUTA promotes adaxial development. We have used inducible versions of these transcription factors and time course experiments to identify downstream targets in an effort to complete our understanding of the ad/abaxial network. Because these factors act in opposite directions, it is possible to leverage more information from these studies than would be possible by analyzing each factor in isolation. In this way, we have been able to identify targets regulated by both factors acting in opposite directions. One of the key findings of this work is that the ad/abaxial network patterns hormone signaling. We will discuss aspects of hormone signaling in the brassinolide, auxin and ABA pathways that are patterned by the ad/abaxial network. "
(a) Department of Plant Biology, Carnegie Institution (b) Waksman Institute, Rutgers University

P68005 The genetic architecture of vegetative phase change in maize
Kaeppler, Shawn -presenter smkaeppl@wisc.edu(a) Hansey, Candice (a) Riedeman, Eric (a) Sekhon, Rajan (a) Kaeppler, Heidi (a) Tracy, William (a) de Leon, Natalia (a)
"Juvenile to adult transition, termed vegetative phase change, is a trait with substantial variability in maize. Juvenile vegetative tissues have a thin cuticle, epicuticular wax and aerial roots, lack of epidermal hairs and bulliform cells, and have lateral buds that can form tillers. Adult vegetative tissues, in contrast, lack epicuticular wax and aerial roots, and have a thick cuticle, epidermal hairs, bulliform cells, and lateral buds that are either absent or can form ears. The variable proportion of the plant that is juvenile has implications for traits of commercial interest. For example, juvenile leaves are more digestible by ruminant animals, and more susceptible to certain insect and disease pests. Timing of the juvenile to adult transition is heritable and varies substantially in maize populations and among maize varieties. We have characterized the timing of juvenile to adult transition in approximately 4000 lines of the Buckler Nested Association Mapping population and in a collection of inbred lines. Substantial variation was identified in this material with the earliest transitioning genotype having the fourth leaf as the first adult leaf and the latest transitioning genotype having the 14th leaf as the first adult leaf. While there was some correlation between maturity, leaf number, and transition leaf, there are QTL that could clearly be identified as controlling timing of transition independent of maturity and leaf number. Information on quantitative trait loci and correlations with stover quality will be presented. "
(a) University of Wisconsin

P68006 "EMF1 interacts with a WNK kinase, a B-box zinc-finger or a DnaJ protein to maintain vegetative development in Arabidopsis"
Park, Hee-Yeon-presenter phy820511@hanmail.net(a) Kim, Sun-Ho (a) Park, Ji-Im (a) Sung, Z. Renee (b) Moon, Yong-Hwan (a)
"*EMBRYONIC FLOWER* (*EMF*) *1* gene is necessary for the maintenance of vegetative development and functions as floral repressor. In this study, to gain further insight into the molecular mechanism of *EMF1*-mediated vegetative development, we attempted to identify the proteins that physically interact with EMF1. We selected three EMF1-Interacting Proteins (EMF1IPs), EMF1IP1, 6 and 9, that are predicted to encode a WNK kinase, a B-box zinc-finger protein and a DnaJ-domain protein, respectively. The heterodimers between EMF1 and EMF1IP1, 6 or 9 were localized in the nucleus. Interestingly, EMF1 interacted with EMF2 via EMF1IP9, forming an EMF1-EMF1IP9-EMF2 protein complex. The subcellular localizations of EMF1 protein complexes suggest that EMF1 mediates the transport of EMF1IP9 protein and EMF1IP9-EMF2 complex from the cytoplasm into the nucleus. The expression analysis revealed that *EMF1* and *EMF1IP1*, *6* and *9* showed similar expression patterns during vegetative development. In addition, *emf1ip1*, *emf1ip9-1* and *emf1ip9-2* showed early flowering phenotypes compared to that of wild-type plants. Taken together, our results suggest that EMF1 functions as a scaffold protein interacting with various EMF1IPs to maintain vegetative development in Arabidopsis."
(a) Pusan National University (b) University of California, Berkeley

P68007 "*blade expansion defective1 (bed1)* reveals interconnections between circadian clock, translation and leaf development"
Kittiwongwattana, Chokchai-presenter chokchai@waksman.rutgers.edu(a) Bharti, Ritu (a) Kerstetter, Randall (a)
"Optimal photosynthesis in plants relies on the formation of mature leaves with proper shapes, sizes and anatomical structures. The establishment of leaf polarity along the adaxial (dorsal)-abaxial (ventral) axis has been shown to be a primary and crucial step in leaf development. In Arabidopsis, several families of genes have been identified as regulators that determine adaxial and abaxial leaf cell identities. The outcome of this process is the organization of various cell types and tissues along the axis. Interestingly, in extreme leaf polarity mutants, a dramatic reduction of leaf blade expansion is observed indicating a requirement of proper leaf polarity for leaf blade expansion. In contrast to leaf polarity genes, only a few regulators of leaf blade expansion have been identified. In this study, we describe a novel Arabidopsis mutant *bed1* that produces relatively narrow mature leaves compared to wild type. This defect was enhanced in continuous-light conditions resulting in whip-like leaves along with the loss of clear distinction between the petiole and leaf blade. Only additive effects were observed in double mutants between *bed1* and classical leaf polarity mutants. In contrast, epistatic and synergistic interactions were found between *bed1* and translational machinery mutants that have been demonstrated to affect leaf polarity establishment. Using a map-based cloning strategy, we were able to locate a point mutation on the second exon of *SENSITIVE TO RED LIGHT REDUCED1 (SRR1)* previously shown to be required for phytochromeB-mediated light signaling and circadian clock regulation. We present here data obtained from our study and further discuss potential links between leaf development, light and translational machinery. "
(a) Rutgers University

P68008 Function of *KRP1* and *KRP3* genes in shoot apical meristem and leaf development
Kim, Gyung-Tae-presenter kimgt@donga.ac.kr(a,c) Jun, Sang Eun (a) Okushima, Yoko (b) Cho, Kiu-Hyung (a) Park, Sang Chul (a) Umeda, Masaaki (b)

"Cell division plays a key role in proper development and appropriate shape during plant development. Recent study of Kip-related proteins (KRPs), which are inhibitor of cyclin-dependent kinase (CDK), indicated that negative regulation of cell division plays an important role in plant morphogenesis. To investigate how cell division affects the architecture of shoot apical meristem (SAM) and leaf morphogenesis, we have characterized transgenic plants overexpressing *KRP1* and *KRP3* genes which are highly expressed in the vicinity of SAM and leaves. As a result, we have observed the common phenotype, such as reduced sized leaves with serration, reduced fertility, reduced root growth and reduced SAM size in each KRP overexpressing transgenic plants. In addition, overexpression of *KRP1* and *KRP3* resulted in the structural changes of SAM and leaves in cell level. Taken together, we will discuss about the roles of KRP in the regulation of cell division in SAM and leaf development."
(a) Department of Molecular Biotechnology, Dong-A University, Busan 604-714, South Korea (b) Graduate School of Biological Sciences, Nara Institute of Science and Technology, Nara 603-0102, Japan (c) Environmental Biotechnology Research Center, Gyeongsang University, Jinju 660-701, South Korea

P68009 Starting to unravel the relationship between tendril development and LEAFY in the tribe Bignonieae (Bignoniaceae)
Sousa-Baena, Mariane S.-presenter marianesousa@usp.br(a) Rossi, Magdalena (a) Kajihara, Daniela (a) Lohmann, Lucia G. (a)
"The tribe Bignonieae includes all Neotropical lianescent Bignoniaceae. The leaves of Bignonieae are often 2-3 foliolate with the terminal leaflet modified into a tendril. These tendrils present varied morphologies and are thought to have been involved in the diversification of Bignonieae. Despite that, little is known about the biology of tendrils as a whole. Recently, researches have demonstrated that the gene LEAFY has an important role in several compound-leaved species. The prolonged expression of LEAFY during leaf development controls the degree of leaf compoundness. This study aims to analyze the leaf morphogenesis of *Mansoa difficilis* (trifid tendrils) and *Cuspidaria convoluta* (simple tendrils) in order to understand how changes during leaf development led to current differences in tendril types. In addition, this work aims to clone the LEAFY homologue in Bignonieae and investigate its expression pattern during tendril development. Scanning electron microscopy and standard methods in plant anatomy were used to describe the ontogeny of leaves. These analyses revealed that both species present similar developmental characteristics till the third stage of development. In the fourth developmental stage, the tendril primordium of *Mansoa* starts to become trifid through the proliferation of a tissue at the flanks of the tendril primary branch. A fragment of LEAFY homologue gene from *Cuspidaria* (CcLFY) was cloned and sequenced by using a PCR-based approach. A phylogenetic analysis that included the cloned LEAFY sequence from Bignonieae and LEAFY homologues from several angiosperms, placed CcLFY in a clade within Lamiales. Our next step will be to analyze LEAFY expression pattern during tendril development in both species to understand its role in tendril complexity."
(a) Universidade de Sao Paulo, Department of Botany

P68010 "Characterization of MADS-box genes of soybean (*Glycine max*, L. Merr.) and initial functional analysis in whole plant senescence"
Schreier, Leeann-presenter Redbuggy666@hotmail.com(a) Chandlee, Joel M (a)
"Plant MADS-box genes represent a large family of transcription factors that exhibit a wide range of roles in different aspects of growth and development. The genes contain a highly conserved domain, or MADS-box, in the N-terminal region that is involved in DNA binding and dimerization. The gene family is well studied and characterized in many plants, but not the agronomically important soybean (*Glycine max*, L. Merr.). A chromosome based assembly of the soybean genome has recently become available through v3.1.1 of Phytozome (www.phytozome.net) and allows for rapid gene discovery. We will present an initial genome-wide characterization of members of the soybean MADS-box gene family and integrate this information with available EST data in an attempt to accurately portray the organization and functionality of this important gene family in soybean. Our interest in MADS-box genes is due to experimental evidence that suggests at least one member of this gene family being expressed in early stages of whole plant senescence in soybean. Genetic lesions in three genes of soybean (g, D1 and D2) show alterations in the normal progression of whole plant/leaf senescence leading to an evergreen phenotype in double and triple mutant combinations. We will report evidence suggesting that one or more of these genes is a MADS-box gene family member and exhibits an altered expression pattern in the double and triple mutant backgrounds. Finally we will present preliminary expression data that relates to the central goal of our study of senescence, which is to better understand the underlying molecular genetic mechanisms that provide for regulation of this important developmental program. In addition, we hope to identify those genes that play a significant role in its progression."
(a) Department of Cell and Molecular Biology, University Of Rhode Island

P68011 Understanding the function of *Ligulelessnarrow* gene in maize leaf development
Moon, Jihyun-presenter moonj@berkeley.edu(a) Hake, Sarah (a)
"Maize leaves arise from the shoot apical meristem in a distichous pattern. Downregulation of *knox* genes in a subset of cells lead to the formation of a leaf primordia that develop into a phytomer which consists of a leaf, internode and an axillary bud. The maize leaf develop different tissue types along the proximo-distal axis; the distal blade, proximal sheath and the auricle and ligule which form a boundary between the blade and sheath. Here, we introduce a new dominant mutant *Ligulelessnarrow*(*Lgn*) which shows defects in several axis of patterning. *Lgn* heterozygotes have disrupted blade-sheath boundaries and narrower leaves. The mutant is shorter in stature and often fail to develop ears. When homozygote for the mutation, the phenotype gets more severe. To further understand the function of the gene, double mutants were generated with previously known liguleless mutants, *lg1* and *lg2*. Our results show that *Lgn* and *lg1* mutations synergistically affect the boundary development while *Lgn* and *lg2* has additive effects. Addition of the *Lgn/+* mutation to the ectopically expressed *KN1-N* allele leads to complete suppression of the knots in the leaves which are proximal tissue appearing in the distal blade. This result suggests the role of *Lgn* in controlling the proximo-distal patterning via regulation of *KN1* either directly or indirectly. Using map based cloning, a mutation in the kinase domain III of a Ser/Thr receptor-like kinase has been found. "
(a) University of California at Berkeley/ Plant Gene Expression Center

P68012 Determination of compound leaf forms in *Medicago truncatula*
Chen, Jianghua (a) Yu, Jianbin (b) Madueno, Francisco (c) Ratet, Pascal (d) Bai, Guihua (b) Chen, Rujin-presenter rchen@noble.org(a) Wang, Hongliang (a) Berbel, Ana (c) Liu, Yu (a) Ge, Liangfa (a) Wen, Jiangqi (a) Li, Guangming (a) Tadege, Million (a) Mysore, Kirankumar S. (a)
"In seed plants, various leaf forms can be categorized as simple or compound. Simple leaves have a single unit of blade supported by a petiole, whereas compound leaves have several units of blades called leaflets. Legumes (Fabaceae), the third-largest family of flowering plants, have a diversity of simple and compound leaves. Although striking diversity in size, arrangement, and complexity of leaves can sometimes be seen in closely related species of legumes, mechanisms that underlie these various leaf forms remain unknown. A leaf form mutant, *palmate pentafoliolate1* (*palm1*), was identified from a fast neutron deletion population of *Medicago truncatula*. In *palm1*, leaves have a palmate compound leaf form, with an increase in the leaflet number from three to five. Genetic analysis indicates that the leaf phenotype is controlled by a single recessive nuclear gene (*Palmate Pentafoliolate1*, *PALM1*) in *M. truncatula*. Using a map-based cloning approach, we have cloned the *PALM1* gene and show that *PALM1* encodes a novel transcription factor belonging to the zinc-finger family of transcription factors. Using a stable plant transformation approach, we

show that the wild-type *PALM1* gene complemented the *palm1* leaf mutant phenotype. The conversion of the trifoliolate wild-type leaves into palmate pentafoliolate leaves in the mutant suggests that the *M. truncatula* leaf form can be modulated by PALM1 possibly through regulation of the expression of the *SINGLE LEAFLET1* (*SGL1*), which we show previously encodes the *M. truncatula LFY* ortholog. We will discuss these results and a genetic model of compound leaf development in *M. truncatula*"
(a) Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK 73401, USA (b) USDA-ARS-PSERU, Department of Agronomy, Kansas State University, Manhattan, KS 66506, USA (c) IBMCP, Consejo Superior de Investigaciones Cientificas-Universidad Politecnica de Valencia, 46022 Valencia, Spain (d) Institut des Sciences du Vegetale, CNRS, 91198 Gif sur Yvette cedex, France

SESSION P69 – WATER RELATIONS

P69001 Effects of short-term salt stress by the application of the concentrated deep seawater on phloem transport and quality of tomatoes
Araki, Takuya-presenter araki@agr.kyushu-u.ac.jp(a) Wajima, Takahiro (b) Kitano, Masaharu (a)
" Suitable application of the concentrated deep seawater for the high quality production was examined by analyzing phloem transport. Tomato plants (*Lycopersicon esculentum* Mill.) were grown by soil-less culture with non-woven fabric system, where short-term salt stress was induced for just two weeks at the stage of rapid fruit growth by adding the concentrated deep seawater to the standard nutrient solution (electric conductivity (EC), 1.2 dS m^{-1}) to increase EC by 13.5 dS m^{-1}. A heat-ring method was applied to the tomato pedicel to evaluate phloem fluxes of sap and soluble solids and concentrations of soluble solids in the phloem sap, and effect of the short-term application of the concentrated deep seawater was analyzed with special reference to osmoregulation in the phloem transport to tomato fruits. Although fresh weight of the fruits was restricted by salt stress, dry matter ratio of the fruits was increased. Soluble solid concentration and flux in the phloem sap was accelerated not only during the short-term salt stress but also after the removal. Concentrations of Na^+, K^+, and Mg^+ in the phloem sap were also enhanced by the concentrated deep seawater. From these quantitative analyses of the phloem transport to tomato fruits, it was verified that the short-term application of the concentrated deep seawater at the stage of rapid fruit growth can induce the osmoregulation in the phloem transport to fruits and produce the high quality tomatoes enriched with sugar and minerals."
(a) Kyushu University (b) Prefectural University of Kumamoto

P69002 Estimation of transpiration rates during table grapes rachis development
Varela-Ovalle, Jose I-presenter jivarela@uc.cl(a) Bahamonde-Migueles, Diego I (a) Defilippi, Bruno G (b,d) Campos-Vargas, Reinaldo (c,d) Perez-Donoso, Alonso G (a)
"The rachis is the stem-like structure that holds the berries in a grape cluster (*Vitis vinifera*). The browning of the rachis is a major problem seen during table grape cold storage and transport overseas that has been attributed mainly to water loss. We developed a novel methodology to estimate the epidermal diffusive (i.e., stomatal and cuticular) and aerodynamic resistances for water vapor and to study transpiration rates from the rachis surface during its development. The method is based on establishing the energy budgets of three pieces from the same grape cluster that are treated so as to be under different evapo-transpirative conditions. The grape cluster pieces were placed inside a small wind tunnel and high frequency temperature measurements were taken at the rachis surfaces to characterize sensible heat and latent heat (evapo-transpiration) energy flux densities at different horizontal wind speeds. Relative water contents of the rachis samples were also determined before and after exposing the cluster pieces to wind. Preliminary results indicate that water vapor transference from the rachis of cv. Red Globe is highly dependent on wind speed, and that epidermal resistances increase rapidly as the wind speed reaches values close to 0.6 ms^{-1}, perhaps revealing a biological regulation mechanism similar to the stomatal closure control present in leaves. FONDECYT 1085025."
(a) Pontificia Universidad Catolica de Chile (b) Instituto de Investigaciones Agropecuarias (c) Universidad Nacional Andres Bello (d) Plant Cell Biotechnology Millennium Nucleus

P69003 Temporal variations of Ostreococcus blooms in the surf zone of Valencian Coast (Mediterranean)
Paches, Maria-presenter mapacgi@upvnet.upv.es(a) Gonzalez del Rio, Julio (a) Martinez-Guijarro, Remedios (a) Romero, Inmaculada (a)
"Here are presented the results from phytoplankton counts carried out in the surf zone of Valencian coast for Water Frame Directive. With regard to the Ostreococcus spp. counts were made by epifluorescence after dehydrating the membrane. Results belong to 65 sampling stations that have been established along the coast line. Data are from the sampling campaigns carried out from August 2005 to July 2006. From the results obtained it can be appreciated that: During the sampling period, in most of the stations, blooms of Ostreoccocus spp. occurred. Temporal patterns are quite irregular, although it is worth emphazising between them that in most of the sampling stations a bloom of Ostreococcus spp. was detected in December, periods when Ostreococcus spp. reaches in some of the stations up to 40x10E6 cel/l and dominates the eukaryotic population with percentages higher than 95%. In several stations this peak accompanies another of similar significance in March. This March s bloom, although it is detected in fewer stations, sometimes reaches cellular densities higher than December, with a maximum of 35x10E6 cel/l. Although in most of the stations this picoeukaryotic bloom disappears between the two peaks (December-March) there are a few stations that keep significant values between them and in some, bloom events occur even from November to April. There are stations where blooms are different from the general patterns with significant increases (even peaks) during springtime (April-May) and even in some of them, proliferations have been detected in summer months, especially in August, although significant values have been also detected in June and July. From April to October, despite these peaks, cellular densities are lower and never exceed the value of 10x10E6cel/l. "
(a) Institute for Water Engineering and the Environment-Polytechnic University of Valencia

P69004 Determination of phytoplankton communities composition using visible spectroscopy and their relation with epifluorescence microscopic counts.
Martinez, Remedios-presenter mmarting@hma.upv.es(a) Romero, Inmaculada (a) Paches, Maria (a) Gonzalez del Rio, Julio (a)
"Characterisation of phytoplankton communities in aquatic ecosystems is a costly task in terms of time, material and human resources. The aim of this poster is to fine-tune a technique through spectrophotometry that reduces these costs doing absorption spectra measurements. This new technique would not replace microscopic counts but would complement them. Phytoplankton communities analyses were done by the epifluorescence microscopic count method. Absorption spectra were carried out using a UV-VIS spectrophotometer equipped with an integrating sphere. Each water sample was filtered through a Whatman GF/F membrane, placed on a Petri plate and kept at -20 grade C. On the membrane, absorbance was determined at 1-nm intervals at wavelengths between 400-750nm. After that, the membrane is wet with warm methanol in order to eliminate pigments. Optical density was analysed again on the same membrane as it was done before. A multivariate statistical technique (PLS) between phytoplankton counts and absorption spectra was done finding high correlation between them. Thus, the models obtained (one per each phytoplankton class) could be used in a future to speed up phytoplankton counts. From each water sample, absorption spectra could be done,

introducing data into models, getting results and, depending on these, deciding on the need or not of doing phytoplankton counts. "
(a) Institute for Water Engineering and the Environment-Polytechnic University of Valencia

P69005 Light sensitivity of shoot hydraulic conductance of temperate deciduous tree species
Aasamaa, Kroot-presenter kroot.aasamaa@emu.ee(a) Sober, Anu (b)
"Shoot hydraulic conductance of several temperate deciduous tree species was measured in different light conditions. It was found that the hydraulic conductance is sensitive to light intensity and wavelength. Under high intensities of white or blue light, shoot hydraulic conductance was up to two times higher in slow-growing species and up to 20% higher in fast-growing species, as compared to the conductance in darkness. High red light increased the conductance of all the species about by 1/3 less than white and blue light. The effect of the lights was also significant under low light intensities. And changes in low light intensity were more efficient to the hydraulic conductance than changes in high light. Under half-maximum intensities of the lights, the hydraulic conductance was almost equal to the conductances measured under maximum intensities of the lights. Same results were got by two different methods of hydraulic conductance measurement - by high pressure flow method, and by Scholander pressure chamber method that includes holding the shoot in darkness during the measurement about ten minutes. The species with high sensitivity of shoot hydraulic conductance to light were also characterised with high sensitivity of xylem hydraulic conductance to xylem sap ion concentration, and with high stomatal sensitivity to changes in light intensity, in atmospheric carbon dioxide concentration and in atmospheric humidity. It was concluded that: 1. phytochromes are important in mechanism of the light sensitivity; 2. the methods that include some minutes of darkness periods are also suitable for studying the light effect; 3. high sensitivity of hydraulic conductance to light intensity could be also included to set of characteristics of conservative water use strategy."
(a) Estonian University of Life Sciences, Institute of Forestry and Rural Engineering (b) University of Tartu, Institute of Ecology and Earth Sciences, Department of Botany

P69006 Hydraulic properties of xylem in seasonal stems of hops (*Humulus lupulus* L.)
Gloser, Vit-presenter VitGloser@sci.muni.cz(a) Balaz, Milan (a)
Herbaceous vines represent a group of plants where transport efficiency and cavitation safety need to be particularly well balanced. Long stems of vines usually supply water to large leaf area on shoot but are also highly vulnerable to embolism. We explored properties of xylem in stems of hop plants that are only 8 to 12 mm thin and serve to rapid water transport along shoot axis up to 12 m long. We examined hydraulic conductance along the stem axes and analyzed anatomical traits that are responsible for its variation. The diameter of xylem vessels varied from 20 to 130 micrometers. More than 40% of conduits were shorter than 5 cm but some vessels reached 50 cm. Relatively small decrease of conductance along the stem that we found could be possibly explained by continuous presence of small number of long vessels with big internal diameter that provided high transport capacity. Relationships between the mean dimensions of vessels and their vulnerability to embolism are also presented.
(a) Masaryk University, Institute of Experimental Biology

P69007 The role of stomatal density in governing growth and competitive interactions in relation to water stress: experimental observations with Arabidopsis
Ferrero-Serrano, Angel-presenter anxo@liv.ac.uk(a) Mortimer, Martin (a) Hartwell, James (hartwell@liverpool.ac.uk,a)
"Stomata allow atmospheric carbon dioxide to reach the plant's mesophyll for photosynthetic fixation. Stomatal aperture is a compromise between conservation of water and the optimization of CO_2 fixation. We studied *Arabidopsis thaliana* as a model system to examine the effect of stomatal density on individual plant growth, and within populations. The stomatal density and distribution mutant 1-2 (*sdd1-2*) mutant line which shows increased stomatal density, was compared with wild type under well watered and water limited conditions. We aimed to address two questions: i) does increased stomatal density translates into a differential photosynthetic CO_2 fixation, leading to increased growth (RGR)? And if so: ii) how does this differential growth affect plant-plant interactions? Results suggest, that despite the fact that the relative water content under both water regimes remained constant, CO_2 assimilation rates were similar for both genotypes under high water conditions, but reduced in *sdd1-2* under water limitation. Consequently, water use efficiency was significantly reduced by water stress. As a result, higher vegetative RGR's were recorded under well-watered regimes the wild type exceeding the mutant, whereas both genotypes established a similar pattern of growth under water limitation. Under this water regime, the root:shoot biomass ratio of the wild type was significantly increased, but did not alter in *sdd1-2*. Studies of intra-genotypic competition using a plant biomass-density model, revealed that vegetative biomass was more sensitive to density under the high water regime in both genotypes. Contrastingly in terms of reproductive biomass, *sdd1-2* mutants under well watered conditions showed the lowest yield in comparison to other genotype-water regime combinations. "
(a) University of Liverpool

P69008 The role of ethylene-induced tyloses in canopy hydraulic failure of mature walnut trees afflicted by apoplexy disorder
McElrone, Andrew J-presenter ajmcelrone@ucdavis.edu(a,b) Grant, Joseph A (c) Kluepfel, Daniel (a)
"In the Central Valley of California, mature walnut trees afflicted with apoplexy disorder exhibit rapid and complete canopy defoliation within a few weeks of symptom initiation. Symptoms are typically found throughout the entire canopy and are initially expressed as scorching and chlorosis of leaflet edges. The cause of apoplexy disorder is unknown, so we set out to elucidate the water relations physiology underlying this condition. Leaf water potentials (Ψs) on healthy, asymptomatic trees remained high throughout the growing season while those of afflicted trees decreased significantly with the onset of symptoms. Ψs were significantly reduced in the lower, middle, and upper portions of the symptomatic canopies compared to those from healthy trees. Sap flow velocities measured in the main trunk at three radial depths consistently plummeted with the onset of symptoms. Hydraulic conductivity (K_s) of symptomatic branches was dramatically lower than that of healthy branches, however, shallow root K_s did not differ between trees. This finding suggested that hydraulic failure was isolated to the canopy of these grafted trees. Light and scanning electron microscopy of stem and trunk sapwood xylem revealed significant tylose development in vessels of symptomatic trees, which was later linked to increased ethylene production in the active sapwood. Continued work is planned to determine the cause of increased ethylene and tylose production that lead to apoplexy symptoms."
(a) USDA-ARS, Crops Pathology and Genetics Research Unit Davis, CA 95616 (b) Department of Viticulture and Enology, University of California, Davis, CA 95616 (c) Department of Plant Sciences, University of California, Davis CA 95616

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