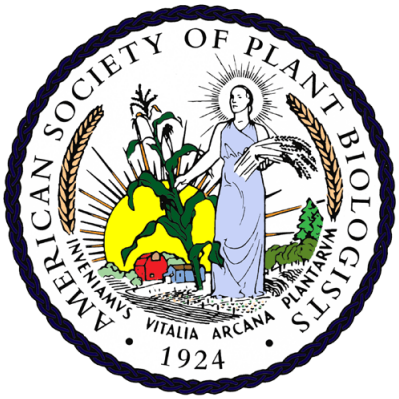
The

Western Regional Meeting

of the

American Society of Plant Biologists



Friday, April 30 – Sunday, May 2, 2010

organized by the

Molecular Plant Sciences Program

at

Pullman, Washington USA

Contents

Acknowledgments

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Conference Organizer: Camille M. Steber.

Organizing Committee and Session Chairs: Asaph Cousins, Kulvinder Gill, Hanjo Hellmann, Patricia Okubara, Michael Neff.

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Schedule

The meeting will take place in two buildings: the Compton Union Building (CUB) and the Smith Center for Undergraduate Education (CUE).

Friday, April 30

5:00 pm - 7:00 pm	Registration, poster set-up, CUB Senior Ballroom
6:30 pm - 8:30 pm	Banquet, CUB Senior Ballroom
7:00 pm - 7:45 pm Abstract 16 Abstract 8	Banquet Lectures: Gerry Edwards *How to be C_4: Different Means to an End* Sascha Offermann *Isolation and Characterization of the Dimorphic Chloroplasts in the Single Cell C_4 species Bienertia sinuspersici*
7:45 pm - 10:00 pm	Poster session, CUB Senior Ballroom

Saturday, May 1

8:00 am - 12:00 pm	Registration and breakfast, CUE 203 Atrium
9:00 am - 9:15 am	Opening remarks in CUE 203
	Development Session Session chair: Hanjo Hellmann, CUE 203
9:15 am - 9:50 am Abstract 21	Keynote Speaker: Bob Sharrock *From Photon to Phenotype – the Phytochrome Family*
9:50 am - 10:10 am Abstract 4	Tie Liu *Interplay of Class II and Class III HD-ZIP Transcription Factors in Establishment of Leaf Polarity*
10:10 am - 10:30 am Abstract 22	Cristina Walcher *$1 + 1 = 3$: When Two Hormones Are Better Than One*
10:30 am - 10:50 am Abstract 43	Jianfei Zhao *Functional Analysis of the AT-Hook Motif Nuclear Localization (AHL) Gene Family in Arabidopsis thaliana*
10:50 am - 11:10 am Abstract 24	Richard Sharpe *Maize Bundle Sheath & Mesophyll Chloroplasts: Same Genes Different BS*
11:10 am - 2:10 pm	Poster session in CUB Senior Ballroom Odd numbered posters: 11:10 am - 12:10 pm Even numbered posters: 1:00 am - 2:00 pm

11:30 am - 1:30 pm	Buffet lunch served in CUB Senior Ballroom

2:10 am - 2:30 pm	Take down posters CUB Senior Ballroom

Physiology and Interactions with the Environment Session	
Session chair: Asaph Cousins, CUE 203	
2:30 pm - 2:50 pm Abstract 56	Emily Palm *The Serpentine Syndrome: Investigating Potential Mechanisms of Tolerance in Mimulus guttatus*
2:50 pm - 3:10 pm Abstract 17	Qiumin Tan *Understanding the Physiological Importance of Amino Acid Transport Processes in Legumes*
3:10 pm - 3:30 pm Abstract 1	Muhammad Arshad *Identification of Genes that Contribute to Drought Stress Tolerance in Poplar*
3:30 pm - 3:50 pm Abstract 27	Deserah Strand *The Effects of Photoinhibition on the Proton Circuit of Photosynthesis*
3:50 pm - 4:10 pm Abstract 34	Berkley Walker *Altered Photorespiratory Carbon Metabolism in Arabidopsis thaliana: Prevalence and Consequences of Increases to the Stoichiometric Release of* CO_2

4:10 pm - 4:30 pm	Coffee break, CUE 203 Atrium

Biochemistry Session	
Session chair: Patricia Okubara, CUE 203	
4:30 pm - 5:05 pm Abstract 29	Judy Callis *The RING-type Ubiquitin E3 Ligase Family in Arabidopsis thaliana*
5:05 pm - 5:25 pm Abstract 23	Ling Meng *A Membrane-associated Thioredoxin Required for Plant Growth Moves from Cell to Cell, Suggestive of a Role in Intercellular Communication*
5:25 pm - 5:45 pm Abstract 9	Jeremy Dahmen *Increasing Polyunsaturated Fatty Acid Production in the Heterologous Host, Saccharomyces cerevisiae, by Entering the Realm of Fatty Acid Desaturation in Tetrahymena thermophila*
5:45 pm - 6:05 pm Abstract 12	Yongil Yang *Analysis of RNA Binding Protein Complexes Involved in Prolamine mRNA Localization in Rice Seeds*
6:05 pm - 6:25 pm Abstract 46	Hanjo Hellmann *The DDB1a Interacting Proteins ATCSA-1 and DDB2 are Critical Factors for UV-B Tolerance and Genomic Integrity in Arabidopsis thaliana*

6:25 pm - 9:30 pm	Pizza, Butch's Den in CUB

Sunday, May 2

8:00 am - 9:00 am	Breakfast, CUE 203 Atrium

Connections to Agriculture Session Session chair: Kulvinder S. Gill, CUE 203	
9:00 am - 9:35 am Abstract 19	John Harada *Dissection of Seed Development*
9:35 am - 9:55 am Abstract 37	Alexander Karasev *Identification of an Unusual Recombinant Potato Virus Y Strain in Potato in North-central Mexico*
9:55 am - 10:15 am Abstract 33	Keum Young Lee *Three Approaches to Enhancing Phytoremediation of Chlorpyrifos*
10:15 am - 10:35 am Abstract 51	Jasdeep Mutti *Comprehensive Analysis of Homoeologue Gene Expression in Polyploid Wheat*
10:35 am - 10:55 am Abstract 6	Jayaveeramuthu Nirmala *Stem Rust Spores Elicit Rapid RPG1 Phosphorylation in Barley*
10:55 am - 11:15 am Abstract 3	Patricia Okubara *Cultivar-Dependent Rhizosphere Colonization, Antifungal Metabolite Accumulation and Gene Expression in a Wheat-Pseudomonas Interaction*

11:15 am - 11:35 am	Announcement of poster and talk prizes in CUE 203

11:35 am - 11:50 am	Coffee break and adjourn, CUE 203 Atrium

Abstracts

Abstract 1

Muhammad Arshad, maa44@sfu.ca, SPEAKER

Identification of Genes that Contribute to Drought Stress Tolerance in Poplar

Muhammad Arshad[1], Kamal Biswas[1] and Aine Plant[1]. [1]Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada, V5A 1S6.

Poplars (*Populus* spp.) are economically important and are favored for forest plantation and reclamation purposes all over the northern hemisphere due to their capacity for rapid juvenile growth, which enables harvesting in short time spans. However, poplars are generally regarded as drought sensitive since they require large amounts of water to support their rapid growth. Given that the Intergovernmental Panel on Climate Change report predicts that drought frequencies are expected to increase globally over the next 50 years, the large amount of water needed by poplar is of great concern to forest industries. We screened several *Populus* hybrid genotypes for their response to drought stress. Based on physiological trials conducted in a greenhouse, Walker (*Populus deltoides* x *Populus petrowskyana*) was identified as drought tolerant whereas WP86 (*Populus deltoides* x *Populus petrowskyana*) was identified as drought sensitive genotype. These 2 genotypes were used to analyze, using quantitative PCR, the expression of 30 candidate genes identified during a *Populus* drought transcriptome analysis. Among the candidate genes tested those encoding predicted proteins related to a PP2C (protein phosphatase 2C), an ABF2 (ABA responsive element binding protein 2), an ethylene responsive protein, a hydrophobic protein, a cysteine protease and a DC1 domain containing protein were differentially expressed in drought stressed leaves of drought-tolerant versus sensitive genotypes. This research will permit the identification of genes that contribute to drought stress tolerance in poplar. Furthermore, these genes may serve as molecular markers, which have importance in breeding and selection programs for development of robust drought tolerant *Populus* genotypes adapted to predicted climate change.

Abstract 2

Camille Steber, csteber@wsu.edu

Relieving DELLA Repression of Stem Elongation, Evidence for a Proteolysis-independent Mechanism for GA signaling

Camille M. Steber[1,2,3], Tohru Ariizumi[1], and Amber Hauvermale[2]. [1]Department of Crop and Soil Sciences, Washington State University. [2]Graduate Program in Molecular Plant Sciences, Washington State University. [3]USDA-ARS Wheat Genetics, Quality, Physiology, and Disease Research Unit, Pullman, WA

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 *gid1a gid1b gid1c* 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 interaction with GID1 with increasing GA levels in vivo, it appears that GA-bound GID1 can block DELLA repressor activity by direct protein-protein interaction with the DELLA domain. Thus, a *SLY1*-independent mechanism for GA signaling may function without DELLA degradation.

Abstract 3

Patricia Okubara, pokubara@wsu.edu, SPEAKER

Cultivar-Dependent Rhizosphere Colonization, Antifungal Metabolite Accumulation and Gene Expression in a Wheat-*Pseudomonas* Interaction

Patricia A. Okubara[1,4,5], Nathalie Walter[1], Robert Bonsall[1], Douglas Call[2], and Daniel Skinner[3]. [1]USDA-ARS Root Disease and Biological Control Research Unit, Pullman, WA. [2]Department of Veterinary Microbiology and Pathology, and School for Global Animal Health, Washington State University. [3]USDA-ARS Wheat Genetics, Quality, Physiology, and Disease Research Unit, Pullman, WA. [4]Department of Plant Pathology, Washington State University. [5]Graduate Program in Molecular Plant Sciences, Washington State University.

We explored the role of host genotype in three aspects of the wheat-*Pseudomonas* biocontrol interaction: rhizosphere population density, accumulation of rhizosphere 2,4-diacetylphloroglucinol (DAPG), and *Pseudomonas*-mediated changes in root gene expression. Wheat cultivars varied in ability to support *P. fluorescens* strain Q8r1-96, an aggressive and persistent colonizer, compared to strain Q2-87, a moderately aggressive, less persistent colonizer. Of 27 cultivars tested, Finley and six others supported significantly ($P<0.05$) higher rhizosphere populations of Q8r1-96 than Q2-87 after 14 d in a non-pasteurized, non-agricultural soil. Cultivar Tara supported relatively high population densities of both bacterial strains, whereas Buchanan supported low population densities of both strains. In a soil-free system, roots of cultivars Tara and Finley accumulated more DAPG when colonized by Q8r1-96 compared to Q2-87. In contrast, Buchanan accumulated the same amounts of DAPG during colonization by both strains, even though Q8r1-96 produced about 100-fold more metabolite than Q2-87 in King's Medium B. These findings demonstrated that rhizoplane DAPG accumulation, like rhizosphere population density, is dependent upon a cultivar-bacterial strain interaction. Cultivar-dependent responses to rhizobacteria were also noted at the gene expression level. In microarray experiments, genes were differentially up- or down-regulated in wheat near-isogenic lines 442, 443 and in cv. Finley in response to root colonization by Q8r1-96. Real-time PCR was used to validate transcriptional changes in 16 genes involved in general stress response and basal resistance, including *COI1, PR-10a, Avr9-2, HRin1* and *HRin2*.

Abstract 4

Tie Liu, tieliu@stanford.edu, SPEAKER

Interplay of Class II and Class III HD-ZIP Transcription Factors in Establishment of Leaf Polarity

Tie Liu[1], Brenda Reinhardt[1], Enrico Magnani[1], and Kathryn Barton[1]. [1]Department of Plant Biology, Carnegie Institution, Stanford University, Stanford, California 94305.

The establishment of adaxial-abaxial polarity is determined by differential gene expression. Among these regulator genes, members of class III homeodomain leucine zipper, *REVOLUTA* (*REV*), *PHABULOSA* (*PHB*) and *PHAVOLUTA* (*PHV*)) family and GARP transcription factor *KANADI* (*KAN1-4*) family play important roles in influencing the activity of the meristem from which the lateral organs formed. To identify the genes whose expression is regulated by these plant-specific gene families (class III HD-ZIPs and KANADIs), microarray analyses were conducted on mRNA from inducible over-expression gene fusions of *REV* and *KAN1* under glucocorticoid control (GR-REV and GR-KAN). Among the oppositely regulated genes, three of them were HD-ZIP class II transcription factors. The expression analysis by in situ hybridization and promoter GUS reporter fusions revealed their polarity-specific expression patterns. Furthermore, in yeast hybrid assay, we confirmed the physical interaction between these class II and class III genes. Interestingly, over-expression of those genes caused a range of developmental abnormality including enlarged meristem, narrow leaf shape, fasciated flower organs. These results suggested that these HD-ZIP class II genes are potential targets of class III genes that are necessary for the fine determination of leaf polarity pattern.

Abstract 5

Josh Rosnow, jrosnow@wsu.edu

In Vitro Cultures of *Bienertia sinuspersici* (*chenopodiaceae*) Under Increasing Concentrations of Sodium Chloride and Carbon Dioxide

Josh Rosnow[1,2] and Gerry Edwards[1,2]. [1]Graduate Program in Molecular Plant Sciences, Washington State University. [2]School of Biological Sciences, Washington State University.

The recent discovery of single-cell C_4 photosynthesis in terrestrial plants provided evidence that Kranz anatomy is not required for a C_4 cycle. Although the majority of C_4 plants have an anatomy that separates carboxylation and decarboxylation activities between two cell types (Kranz anatomy), aquatic macrophytes such as *Hydrilla verticulata*, and *Egeria densa*, and terrestrial plants *Bienertia cycloptera*, *B. sinuspersici* and *Suaeda aralocaspica*, all can conduct C_4 photosynthesis within individual chlorenchyma cells. A goal of research on terrestrial single-cell C_4 species is to better understand the chloroplast's developmental transition from C_3 photosynthesis to single-cell C_4 photosynthesis. Physiological investigations into the development of C_4 photosynthesis in response to environmental and chemical factors was investigated using callus cells generated from leaf material and using young shoots developed from callus tissue. The conclusions from these experiments are that callus and shoot growth is enhanced under elevated CO_2 concentrations, increasing the yield of photo system II, overall surface area, and protein and chlorophyll content. The effect of increasing sodium chloride in the media corresponded to an increase in tissue osmolality and overall tissue size, while having a minimal effect on C_4 protein expression and regeneration. There is minimal expression of C_4 biochemical enzymes in photoautotrophic callus, while there is expression in regenerating shoots. These results provide insight into developmental events that are involved in the formation of single cell C_4 species, and provide information for future research on what is required for C_4 photosynthesis.

Abstract 6

Jayaveeramuthu Nirmala, nirmala@wsu.edu, SPEAKER

Stem Rust Spores Elicit Rapid RPG1 Phosphorylation in Barley

Jayaveeramuthu Nirmala[1], Tom Drader[2], Xianming Chen[3,4], Brian Steffenson[5] and Andris Kleinhofs[1,2,6]. [1]Department of Crop and Soil Sciences, Washington State University. [2]School of Molecular Biosciences, Washington State University. [3]Department of Plant Pathology, Washington State University. [4]USDA-ARS Wheat Genetics, Quality, Physiology, and Disease Research Unit, Pullman, WA. [5]Dept of Plant Pathology, University of Minnesota, St. Paul, MN-55108, USA. [6]Graduate Program in Molecular Plant Sciences, Washington State University.

Stem rust threatens cereal production worldwide. Understanding the mechanism by which durable resistance genes such as *Rpg1* function is critical. We show that the RPG1 protein is phosphorylated within 5 minutes by elicitor(s) from avirulent, but not virulent, races of stem rust. Transgenic mutants that contain a gene encoding an inactive protein kinase domain pK2 are susceptible, fail to phosphorylate RPG1 in vivo demonstrating that auto phosphorylation is a pre-requisite for disease resistance. Protein kinase inhibitors prevent RPG1 phosphorylation and result in susceptibility to stem rust, further confirming the importance of RPG1 phosphorylation in disease resistance. We conclude that the RPG1 protein may be the initial recipient of the pathogen elicitor and the kinase activity of the pK2 domain is required for autophosphorylation. The pseudokinase pK1 domain is required for disease resistance, but not autophosphorylation. The very rapid phosphorylation of RPG1 suggests that an elicitor is already present when stem rust urediniospores are placed on the leaf surface. However spores must be alive, as determined by their ability to germinate, in order to elicit RPG1 phosphorylation.

Abstract 7

Lee Hadwiger, chitosan@wsu.edu

The Activation of Defense Genes is Associated with Changes in the Chromatin of Peas

Lee A. Hadwiger[1,2] and Keri Druffel[1]. [1]Department of Plant Pathology, Washington State University. [2]Graduate Program in Molecular Plant Sciences, Washington State University.

In non-host resistance (innate immunity) the activation of an assortment of pathogenesis-related (PR) genes is responsible for slowing the growth of the invading pathogen. In the interaction, between pea endocarp and *Fusarium solani* f. sp. phaseoli, total resistance develops within 6 h and is associated with PR gene activations being initiated within 2 h. The mechanisms involved mirror those recently observed in animal systems and may result from alterations or remodeling of chromatin. The PR gene DRR206 activation occurs as there is an ubiquitination/reduction of histones H2A/H2B and a reduction in the architectural transcription factor, HMG A. The pea RNA polymerase and these nuclear proteins are located within the region of the DRR206 promoter and subsequently within its open reading frame at 2 and 4 h pi, detected by chromatin immunoprecipitation (ChIP) analyses. Also there is a simultaneous reduction in the HMG A associated with this region. The RNA polymerase complex appears to be in place but "paused" at the promoter and subsequent movement through nucleosomes is likely reinitiated by the transient disassembly of histones H2A/H2B from DNA and in its wake a reformation of these and other nuclear components – all occurring within sensitive regions of the pea chromosome.

Abstract 8

Sascha Offermann, soffermann@wsu.edu, SPEAKER

Isolation and Characterization of the Dimorphic Chloroplasts in the Single Cell C_4 species *Bienertia sinuspersici*

Sascha Offermann[1], Kelly A. Doroshenk[2], Thomas W. Okita[2,3], Giulia Friso[4], Klaas J.v. Wijk[4] and Gerald E. Edwards[1,3]. [1]School of Biological Sciences, Washington State University. [2]Institute of Biological Chemistry, Washington State University. [3]Graduate Program in Molecular Plant Sciences, Washington State University. [4]Department of Plant Biology, Cornell University, Ithaca, NY.

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 localization 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 that the dimorphic chloroplasts differentially accumulate proteins with pyruvate, Pi dikinase of the C_4 cycle located in peripheral chloroplasts (PCs) and Rubisco of the C_4 cycle in the central cytoplasmic compartment chloroplasts (CCCPs). To characterize the molecular and physiological basis for single-cell C_4 photosynthesis, a purification protocol for the two different chloroplast types based on cell fractionation, and differential and density centrifugation methods has been developed. Western blots, photosynthetic oxygen evolution and enzyme activity assays show a high degree of differentiation with a fully operational Calvin cycle found only in the CCCPs. Purified chloroplasts are currently being analyzed in a "shotgun" approach via reverse phase nanoLC-ESI-LTQ-Orbitrap MS/MS as well as two-dimensional differential gel electrophoresis (2D-DIGE) followed by LC-MS/MS, which shows a unique pattern for each chloroplast type. Both methods are 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.

Abstract 9

Jeremy Dahmen, jeremydahmen@yahoo.com, SPEAKER

Increasing Polyunsaturated Fatty Acid Production in the Heterologous Host, *Saccharomyces cerevisiae*, by Entering the Realm of Fatty Acid Desaturation in *Tetrahymena thermophila*

Jeremy L. Dahmen[1,2], Becky Olsen[1], Jim Wallis[1], and John Browse[1,2]. [1]Institute of Biological Chemistry, Washington State University. [2]Graduate Program in Molecular Plant Sciences, Washington State University.

Model organisms including yeast are often used in biotechnology to produce novel or unusual fatty acids (FA). Delta 6 desaturases are vital in the production of these nutritional polyunsaturated lipids such as linolenic acid (18:3). The delta 6 desaturase from the ciliated protist, *Tetrahymena thermophila* (TtDes6) was characterized by expression in yeast. When yeast cells were fed 18:2(9,12), 4% is desaturated into 18:3(6, 9, 12). However, when coexpressed with the cytochrome b5 protein from *Arabidopsis thaliana*, desaturation increased to 40%. We have recently identified three putative cytochrome b5s in *T. thermophila* (TtCB5-A,B,and C). Two of the *T. thermophila* cytochrome b5s (TtCB5-A and TtCB5-B) when coexpressed with TtDes6 also shows an increase in desaturation to 40% and 25% respectively. The increases in desaturation observed with the addition of cytochrome b5s from *Arabidopsis* and *T. thermophila* illustrates the importance of the interaction between cytochrome b5s and desaturases in the synthesis of polyunsaturated fatty acids. Current studies are focused on the understanding of the protein-protein interactions between cytochrome b5s from *T. thermophila* and *S. cerevisiae* and the *T. thermophila* delta 6 desaturase. Elucidation of these interactions will aid in identifying factors required to produce an abundance of polyunsaturated fatty acids in heterologous systems.

Abstract 10

Laura Wayne, lwayne@wsu.edu

Investigating Electron Supply for the *Arabidopsis* Desaturases

Laura Wayne[1,2] and John Browse[1,2]. [1]Institute of Biological Chemistry, Washington State University. [2]Graduate Program in Molecular Plant Sciences, Washington State University.

Vegetable oils are one of the most important commodities derived from plants; they are used in industrial products and have a growing role in meeting our demand for fuel, as well as vital for human nutrition. Our research focuses on polyunsaturated fatty acids synthesized by *Arabidopsis* fatty acid desaturases (FAD2 and FAD3). Synthesis of the these fatty acids within the endoplasmic reticulum (ER) membrane requires an electron supply, passing from NADH through a cytochrome b5 reductase (CBR) and a cytochrome b5 (CYB5). Previously, our laboratory has found a single nucleotide mutation in *CBR1* (*cbr1-1*) that caused a reduction in the desaturation of 18:2 to 18:3 (by FAD3) and a decrease in desaturation of 18:1 to 18:2 (by FAD2). A T-DNA knockout of *CBR1* (*cbr1-2*) also has a dramatic reduction in 18:3 accumulation. This mutant has a more severe visual phenotype than *cbr1-1*, including a range of growth sizes, shorter siliques compared to wild-type, and shrunken and shriveled seeds. Potentially, other ER membrane electron transport systems may also aid in reductant supply to the desaturases, namely NADPH:cytochrome P450 reductases (ATR). We have begun characterization of T-DNA *CBR* and *ATR* mutants as well as double mutants. Double mutants of *cbr1-2* and *atr2-1* are currently being analyzed to determine if ATR plays a role in reductant supply for FAD2 or FAD3. Results obtained from these experiments will be presented.

Abstract 11

Valerie Collin, valeriec.collin@gmail.com

Regulation of ATP Synthase Activity by 14-3-3 Proteins in *Arabidopsis*

Collin VC[1], Cruz J[1], Wood M[1], Dhingra A[2,3] and Kramer DM[1,3]. [1]Institute of Biological Chemistry, Washington State University. [2]Department of Horticulture and Landscape Architecture, Washington State University. [3]Graduate Program in Molecular Plant Sciences, Washington State University.

During photosynthesis, the photosynthetic pathway generates a proton gradient between the lumen and the stoma of chloroplasts. This proton gradient is used by ATP synthase for ATP synthesis. In barley, ATP synthase activity is regulated by a direct interaction between 14-3-3 protein and the phosphorylated beta-subunit. In *Arabidopsis*, 14-3-3 proteins constitute a multigenic family of at least 15 expressed genes. This raises the question of which 14-3-3 protein is/are regulating ATP synthase activity in *Arabidopsis*. The ATP synthase activity in knock-out mutant lines for 14-3-3 proteins have been analyzed in different light intensities and CO_2 concentrations. In the upsilon knock-out mutant, the ATP synthase activity was strongly affected, whereas no phenotype was observed for other chloroplast isoforms. The upsilon 14-3-3 is implicated in the enhancement of the ATP synthase activity during low CO_2 treatment. This phenotype could be observed only during light intensity adaptation. So upsilon is temporarily involved in light adaptation. In the Kappa mutant, the ATP synthase conductivity, as the proton flux, are lower for ambient CO2 concentration. The electron flux is becoming cyclic at lower light intensities. This mutant is also insensitive to high CO_2 treatment. Kappa is permanently involved, directly or not, in the regulation of ATP synthase activity. As barley 14-3-3 could link to the beta sub-unit of the ATP synthase only if PP1A and PP2A inhibitor was used, the implication of these phosphatases during regulation of the ATP synthase by 14-3-3 proteins has also been investigated.

Abstract 12

Yongil Yang, yi_yang@wsu.edu, SPEAKER

Analysis of RNA Binding Protein Complexes Involved in Prolamine mRNA Localization in Rice Seeds

Yongil Yang[1], Andrew J. Crofts[2], Naoko Crofts[2], and Thomas W. Okita[1,3]. [1]Institute of Biological Chemistry, Washington State University. [2]Akita International University, Yuwa, Akita-city, 010-1292, Japan. [3]Graduate Program in Molecular Plant Sciences, Washington State University.

RNA binding proteins (RBPs) in eukaryotes play a role in transport, localization, editing, and degradation of RNA in the cytosol, as well as mRNA splicing in the nucleus. In developing rice seeds, the targeted localization of prolamine storage protein mRNAs to a distinct subdomain of the endoplasmic reticulum is dependent upon two cis-localization elements ("zipcodes") that are likely recognized by RBPs involved in this process. Using affinity chromatography and mass spectrometry, 15 RBPs were found to specifically recognize the prolamine zipcode sequences compared to a control. We investigated the relationship of 7 of these RBPs by studying their possible presence in immunoprecipitates as revealed by mass spectrometry and immunoblot analysis. Our data indicates that RBP-A is present in at least two multi-protein complexes. RBP-A is found associated with RBP-J, RBP-Q, and RBP-G and forms a second independent association with RBP-D and RBP-I. Interestingly, both of these RBP-A containing multi-protein complexes contain glyceraldehyde-3-phosphate dehydrogenase and sorbitol dehydrogenase. The existence of multiple multi-protein complexes indicates that they have different roles in prolamine RNA transport and localization to a subdomain of the endoplasmic reticulum.

Abstract 13

Philip Bates, phil_bates@wsu.edu

Deciphering the Relative Flux through Different Oil Synthesis Pathways is Very Important for Engineering of Novel Fatty Acids into Oilseed Crops

Philip Bates[1] and John Browse[1,2]. [1]Graduate Program in Molecular Plant Sciences, Washington State University. [2]Institute of Biological Chemistry, Washington State University.

Plants contain a large repertoire of industrially useful fatty acids (FA) in seed oils. However, most novel FA are produced in plants that have poor agronomic qualities or low oil yields. Production of novel FA in high yielding oilseed crops through biotechnology has great promise, but in most cases heterologous expression of FA modifying enzymes results in low yields of the desired FA. Castor bean plants accumulate ~90% hydroxylated fatty acids (HFA) in seed triacylglycerol (TAG). *Arabidopsis* plants expressing the Castor FA hydroxylase accumulate ~17% HFA in seed TAG. Co-expression with Castor diacylglycerol:acyltransferase increases HFA content of *Arabidopsis* TAG to ~29%. Thus, novel FA synthesis enzymes and FA specific TAG assembly enzymes may be required to generate high yields of modified oil. Plants contain multiple pathways to produce diacylglycerol (DAG), the immediate precursor to TAG. Recently we demonstrated in developing soybean embryos that DAG is predominantly produced by catabolism of the membrane lipid phosphatidylcholine (PC), rather than sequential acylations and dephosphorylation of glycerol-3-phosphate as in the Kennedy pathway (Bates, Plant Physiol. 2009). Thus, determining the pathway in which endogenous and novel FA are incorporated into DAG and then TAG in transgenic plants can suggest pathway specific engineering strategies to increase novel FA accumulation. *Arabidopsis* plants expressing the Castor hydroxylase were analyzed for the stereochemical location of HFA in TAG and the relative flux through DAG synthesis pathways by [^{14}C] glycerol labeling. The major route of HFA incorporation into TAG was through conversion of hydroxylated PC into DAG for TAG synthesis.

Abstract 14

Elena Voznesenskaya, elena-voz@mail.ru

Anatomical and Biochemical Characterization of Photosynthetic Types in Genus *Portulaca* L. (Portulacaceae)

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Portulacaceae is one of 19 families of terrestrial plants in which species having C_4 photosynthesis have been found. Representative species from major clades of the genus Portulaca were studied to characterize structurally and biochemically the forms of photosynthesis. The species *P. amilis*, *P. grandiflora*, *P. molokiniensis*, *P. oleracea*, *P. pilosa* and *P. umbraticola* belong to the subgenus *Portulaca* and are C_4 plants based on leaf carbon isotope values, Kranz anatomy, and expression of key C_4 enzymes. *P. umbraticola*, clade Umbraticola, is NADP-malic enzyme (NADP-ME) type, while *P. oleracea* and *P. molokiniensis* in clade Oleracea are NAD-ME type C_4 species; all having different forms of Atriplicoid type leaf anatomy. In clade Pilosa, *P. amilis*, *P. grandiflora* and *P. pilosa* are NADP-ME type C_4 species. They have Pilosoid type anatomy in which Kranz tissues enclose peripheral vascular bundles with water storage in the center of the leaf. *Portulaca* cf. *bicolor* (subgenus Portulacella) is an NADP-ME C_4 species with Portulacelloid type anatomy in which well-developed Kranz chlorenchyma surrounds lateral veins distributed in one plane under the adaxial epidermis with water storage cells underneath. One species, Portulaca cryptopetala, is identified as a C_3-C_4 intermediate which may provide insight on evolution of C_4 in the genus. The C_4 *Portulaca* species which were examined also have cotyledons with Kranz type anatomy, while the stems of all species have C_3 type photosynthetic cells. The results indicate multiple structural and biochemical forms of C_4 photosynthesis evolved in genus *Portulaca*.

Abstract 15

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Transcriptome Profiling Analysis of Cultivar-specific 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, transcriptome profile analysis, scanning electron microscopic examination and systematic physiological characterization were performed on two apple cultivars, 'Pink Lady' (PL) and 'Honeycrisp' (HC), which have distinct ripening features and texture attributes. Substantial differences of fruit crispness and firmness were observed. SEM images of fruit cortex tissues prepared from fruits with equivalent maturity indicate that the cell wall thickness may contribute to the observed phenotypes of fruit firmness and crispness. A high-density long-oligo apple microarray consisting of duplex 190,135 cross-hybridization-free 50-70-mer isothermal probes, and representing 23,997 unigenes was manufactured on a Nimblegen array platform. Transcriptome profile analysis on three developmental stages during fruit ripening identified 1793 unigene from HC and 1209 unigenes from PL, which were showed differentially expressed patterns during ripening. Unigenes belong to several functional groups, including hormonal metabolism and response, cell wall biosynthesis and modification, transcription factors were further analyzed. Between two cultivars, most of the gene families were regulated similarly, up- or down-regulation as fruit ripening; however, distinct expression patterns were identified between two cultivars for some of the gene family members, likely representing genotype-specific candidates controlling apple fruit ripening and quality.

Abstract 16

Gerald E. Edwards, edwardsg@wsu.edu, SPEAKER

How to be C_4: Different Means to an End

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A common feature of photosynthesis in practically all organisms is assimilation of CO_2 into organic matter via Rubisco in the carbon assimilation cycle. A constraint on the process in terrestrial plants is conditions where CO_2 becomes limiting due to high temperature, drought, or soil salinity. In response to CO_2 limitations, some terrestrial plants evolved mechanisms to concentrate CO_2 around Rubisco through a C_4 cycle. This requires spatial separation of fixation of atmospheric CO_2 into C_4 acids, and the donation of CO_2 from C_4 acids via decarboxylases, to Rubisco. The paradigm for C_4 photosynthesis in terrestrial plants for about 35 years was that a dual cell system, called Kranz anatomy, is required for spatial separation of these functions. C_4 plants with this anatomy have two structurally and biochemically specialized photosynthetic cell types functioning coordinately in carbon assimilation. It has evolved independently many times with great diversity in forms of Kranz and biochemistry of the C_4 cycle. Surprisingly, more recently it was shown that C_4 photosynthesis can occur within individual photosynthetic cells. Two very novel means of accomplishing this evolved in family *Chenopodiaceae* by spatial development of two cytoplasmic domains. These contain dimorphic chloroplasts, as shown by anatomical, histochemical and biochemical studies. One domain is specialized for supporting fixation of atmospheric CO_2 in the C_4 cycle, the other for accepting CO_2 from decarboxylation of C_4 acids and its assimilation by Rubisco in the C_3 cycle. Essential features of its function and questions of how this unexpected form of C_4 develops will be discussed.

Abstract 17

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Understanding the Physiological Importance of Amino Acid Transport Processes in Legumes

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Most plants use amino acids as their predominant nitrogen (N) transport forms, and transporters are predicted to be involved in the movement of amino N. In this work, the role of amino acid transporters in legume metabolism and productivity were addressed. Several putative amino acid transporters were isolated from pea and common bean and characterized. Functional complementation studies in yeast suggest that these transporters recognize a broad spectrum of amino acids. Analyses of transporter expression and localization studies indicate that they are plasma membrane transporters, and that they are important for amino acid partitioning in nodules, long distance transport of N and amino acid uptake into seed cotyledons. The importance of N transport processes was further examined by repressing or overexpressing amino acid transporters in pea plants. Transporter down-regulation in nodules affected amino acid levels in nodules, and the number of nodules formed on roots. Further, overexpression of an organic N and sulfur (S) transporter in the phloem led to changes in N and S metabolism in both roots and leaves, and to an increase in seed and protein yield. These results suggest that manipulation of N transporter expression influences N partitioning, whole plant metabolism and plant productivity. (This work is funded by the National Science Foundation, grant IOS 0448506).

Abstract 18

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Importance of Transport Proteins in Organic Nitrogen Acquisition from the Soil

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Amino acids are the primary transport forms of nitrogen in most plants, and are essential for plant growth and development. They are synthesized in roots and leaves from nitrate or ammonium, or taken up from preexisting pools in the soil. Uptake of organic nitrogen by plant roots occurs via root tips or epidermal and root hair cells, and involves active transport into the symplasm. Amino acids might also move into the root apoplast, but they are eventually imported into the symplasm to bypass the Casparian Strip of the endodermis and to be loaded into the xylem for transport to the shoot. Recent studies in our lab have shown that the *Arabidopsis* AAP1 transporter is involved in import of glutamate and uncharged amino acids into the root symplasm (Lee et al. 2007, Plant J 50, 305). We now investigated if amino acid uptake from the soil can be increased by overexpressing *AAP1* under control of its endogenous promoter in *Arabidopsis*. When grown on media containing high levels of amino acids, transgenic plants showed a reduction of growth on all neutral amino acids and histidine when compared to wild-type. This indicates an increase in uptake of these amino acids resulting in growth inhibition through feedback regulation of nitrogen metabolism. The increased nitrogen acquisition in *AAP1* overexpressors was confirmed by direct uptake studies with transgenic seedlings and radiolabeled amino acids. Using a reverse genetics approach we further discovered that the LHT6 amino acid transporter is also involved in nitrogen uptake from the rhizosphere.

Abstract 19

John Harada, jjharada@ucdavis.edu, SPEAKER

Dissection of Seed Development

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The seed is a complex structure that consists of three major regions, each with a different genetic composition: the diploid, zygotic embryo, the triploid, zygotic endosperm, and the diploid, maternal seed coat. Each of these regions is comprised of distinct compartment, tissues, and cell types. Little is known of the specific cellular processes that occur in each seed compartment and how these compartment-specific processes are coordinated during seed development. We have profiled RNA populations in each compartment, tissue, and/or cell type of *Arabidopsis* seeds throughout development using laser-capture microdissection and Affymetrix GeneChip hybridization experiments. Specifically, we analyzed gene activity globally in the embryo proper and suspensor of the embryo, the micropylar, peripheral, and chalazal domains of the endosperm, and the chalazal and distal regions of the seed coat at the preglobular, globular, heart, linear-cotyledon, and mature-green stages of seed development. Extensive validation experiments show that the datasets accurately depict gene expression patterns within the seed. Computational analyses of the RNA profiles provide new insight into the physiological and regulatory pathways that operate within specific seed compartments. We will discuss how integration of the temporal and spatial patterns of mRNA accumulation is defining functional relationships between compartments of the embryo, endosperm and seed coat. We anticipate that the information may be useful for the design of approaches to improve seeds of crop plants. This work is supported by award DBI-0501720 from the Plant Genome Program of the National Science Foundation.

Abstract 20

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Influences of Protein Arrangement in *Arabidopsis thaliana* Thylakoid Membranes for Energy Conversion by Photosystem II

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Freeze-fracture electron microscopy (EM) has shown that the photosystem II (PSII) complexes in grana thylakoids are non-randomly distributed, i.e. the photosystems are farther apart and align themselves parallel to each other compared to pure random arrangements. Furthermore, under unfavorable environmental conditions (e.g. cold stress, low light, osmotic stress) PSII can rearrange into highly ordered crystalline like arrays. Our long term goal is to understand the functional consequences of PSII array formation of the photosynthetic machinery. We compared wild-type *Arabidopsis* plants to a fatty acid desaturase mutant (*fad5*) which constitutively forms highly ordered PSII arrays. To study the impact of PSII array formation we visualized the protein arrangement by ultrastructural techniques. In this study we present results demonstrating that scanning electron microscopy (SEM) is a novel method to visualize the differences in protein organization in thylakoid membranes. The water splitting apparatus of individual PSII in crowded isolated grana membranes (inside out vesicles - BBY) can be visualized under SEM. Additionally, the light-harvesting efficiency of PSII in wild type and fad5 leaves was probed by Chlorophyll a fluorescence induction kinetics to examine the influence of protein array formation on PSII functionality. With these novel methods, we were able to visualize protein arrangements in thylakoid membranes and gain insight into a possible function.

Abstract 21

Bob Sharrock, sharrock@montana.edu, SPEAKER

From Photon to Phenotype – the Phytochrome Family

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Phytochromes (phy) are R/FR-sensing photoreceptors found in bacteria, cyanobacteria, fungi, and plants. Through phy photo-activation and signaling, organisms can assess their location in space and time and adjust their growth, development, and metabolism to critical aspects of their environment, including diurnal and seasonal rhythms and the presence of competitors for photo-resources. In flowering plants, evolution of three PHY clades – PHYA, PHYB, and PHYC – predated the origins and diversification of dicots and monocots. More recent gene duplications have resulted in evolution of additional forms in various taxa, such as PHYD and PHYE in *Arabidopsis*. The complementary and, in some cases, antagonistic functions of these phy forms in regulating R/FR- controlled plant responses have been extensively studied. In addition, many components of downstream phy signaling mechanisms have been identified, although the complex nature of these pathways is only just beginning to be understood. We have recently shown that phy quaternary structure, including the formation of heterodimeric phys, contributes to plant photoreceptor diversity and differential function. Further efforts target the dependence of phy activity on subunit interactions and the roles of putative dimerization domains in phy structure and function.

Abstract 22

Cristina Walcher, clw6@u.washington.edu, SPEAKER

1+1 = 3: When Two Hormones Are Better Than One

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Auxin and brassinosteroids are two plant hormones that work synergistically and interdependently to promote growth and proper development in many plant species. While several co-regulated early response genes have been identified and there are well-characterized transcription factors from each pathway, it remains an open question how the auxin and brassinosteroid signals are integrated at the transcriptional level. We are using the auxin- and brassinosteroid-responsive gene *SAUR-15* as a tool to dissect this problem. Using transgenic plants expressing reporter genes under the control of different portions of the upstream regulatory region of *SAUR-15*, we are working to determine the minimal region that retains response to each hormone separately and to both together. We are also disrupting known cis-regulatory elements to identify critical elements. We are also taking a comparative approach using previous work on auxin-responsive promoters in various plant species including soybean and pea. Together, these results will help us determine the molecular mechanism for how auxin and brassinosteroids work synergistically to induce expression of shared genes as well as increase our understanding about hormone interactions and how the regulation of hormones modifies plant development.

Abstract 23

Ling Meng, mengling@berkeley.edu, SPEAKER

A Membrane-associated Thioredoxin Required for Plant Growth Moves from Cell to Cell, Suggestive of a Role in Intercellular Communication

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Thioredoxins (Trxs) are small ubiquitous regulatory disulfide proteins. Plants have an unusually complex complement of Trxs composed of six well-defined types (Trxs *h, f, m, x, y,* and *o*) that reside in different cell compartments and function in an array of processes. The extraplastidic *h* type consists of multiple members that in general have resisted isolation of a specific phenotype. In analyzing mutant lines in *Arabidopsis thaliana*, we identified a phenotype of dwarf plants with short roots and small yellowish leaves for AtTrx *h9* (henceforth, Trx *h9*), a member of the *Arabidopsis* Trx *h* family. Trx *h9* was found to be associated with the plasma membrane and to move from cell to cell. Controls conducted in conjunction with the localization of Trx *h9* uncovered another *h*-type Trx in mitochondria (Trx*h2*) and a Trx in plastids earlier described as a cytosolic form in tomato. Analysis of Trx *h9* revealed a 17-amino acid N-terminal extension in which the second Gly (Gly^2) and fourth cysteine (Cys^4) were highly conserved. Mutagenesis experiments demonstrated that Gly^2 was required for membrane binding, possibly via myristoylation. Both Gly^2 and Cys^4 were needed for movement, the latter seemingly for protein structure and palmitoylation. A three-dimensional model was consistent with these predictions as well as with earlier evidence showing that a poplar ortholog is reduced by a glutaredoxin rather than NADP-thioredoxin reductase. In demonstrating the membrane location and intercellular mobility of Trx *h9*, the present results extend the known boundaries of Trx and suggest a role in cell-to-cell communication.

Abstract 24

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Maize Bundle Sheath and Mesophyll Chloroplasts: Same Genes Different BS

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Zea mays is a C_4 plant where mature leaves feature bundle sheath (BS) and mesophyll cells (M) arranged in Kranz anatomy. In this system both BS and M house light dependent portions of photosynthesis while the Calvin cycle occurs within BS. As a result, each cell has morphologically and biochemically distinct chloroplasts. While some key transcript and proteomic components associated with C_4 processes are known, chloroplast transcript abundance during development are lacking in the literature. In this study, we followed the abundance of 23 plastid transcripts in BS and M at two points in development of emerging maize leaves. In addition, a maize chloroplast biogenesis cDNA microarray was utilized to screen juvenile leaves for transcript abundance differences between BS and M. Nine of the differentially regulated nuclear genes found in the array screen were assayed using our developmental leaf system. Differential patterns of transcript abundance were tissue-, cell type-, and gene-specific, suggesting that a single master switch for C_4 development is unlikely. We also noted that many of the nuclear and plastid transcripts were more abundant in the BS vs. M in the least developed tissue. This enrichment diminished and in some cases disappeared as the tissue matured. This suggests that an early set of signals initiates the development of C_4 from the basal developmental state, and that once C_4 photosynthesis is established some effects of those signals subside.

Abstract 25

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Variations in Stable Carbon Isotope Composition and Leaf Traits of *Picea schrenkiana* var. *tianschanica* along an Altitude Gradient in Tianshan Mountains, Northwest China

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Altitudinal gradients provide unique experimental opportunities to study and understand morphological and physiological responses of plants to changes in environmental conditions. *Picea schrenkiana* var. *tianschanica* is the dominant species of alpine forests in Tianshan Mountains in northwest China. We hypothesized that there is an optimum altitudinal zone for *P. schrenkiana* var. *tianschanica* in Tianshan Mountains where growth and metabolism are most vigorous and with increasing distance from this optimum the growth and metabolic rates decrease. We examined morphological and physiological characteristics in *P. schrenkiana* var. *tianschanica* growing along an altitudinal gradient (1420, 1505, 1622, 1757, 1850, 1962, 2045, 2110, 2240 and 2300 m) on the northern slopes of Tianshan Mountains. The maximum values of leaf nitrogen concentration (LNC) and pigment contents (Chla+b) and the minimum values of leaf dry matter content (LDMC) and leaf mass per unit area (LMA) were obtained at an altitude of about 2100 m. Leaf δ^{13}C was positively correlated with LNC, and negatively correlated with stomatal density and leaf projected area per 100 needles (LA). This reinforced that leaf δ^{13}C was indirectly controlled by physiological and morphological adjustments along altitudinal gradient. In their optimum environment, plants have a relatively high photosynthetic rate and growth rate associated with higher LNC and chlorophyll contents or LA, respectively. However, high photosynthetic and relative growth rates are achieved under lower efficiency of resources utilization, which are indicated by low-LDMC and low-LMA. We concluded that the best growing elevation for *P. schrenkiana* var. *tianschanica* in Tianshan Mountains was approximately 2100 m.

Abstract 26

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Impacts of Light Quality on the Photosynthesis of Two C_4 Crops, *Zea mays* and *Sorghum bicolor*, and a C_4 Grass *Miscanthus* x *giganteus*

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Leaf photosynthesis is highly sensitive to changes in light environments, including both light quantity and light quality. Because of differences in frequency and wavelength, red and green light penetrate deeper into leaves than blue light. This unequal distribution of light within the leaf is predicted to have profound impacts on C_4 photosynthesis, which requires coordination of the mesophyll C_4 cycle and bundle sheath C_3 cycle. Understanding how light quality influences photosynthesis of economically important C_4 crops and biofuel grasses is essential for accurately modelling their primary production under future climatic conditions. We measured leaf gas exchange in two C_4 crop species, *Zea mays* and *Sorghum bicolor*, and a C_4 bioenergy grass *Miscanthus* x *giganteus* under white, red, green, and blue light. At a light intensity of 900 μmol $m^{-2}s^{-1}$, net assimilation rates (A) under blue light in the three studied species were significantly lower than under white, red, and green light ($P < 0.01$). The quantum yield for CO_2 assimilation and maximum phosphoenolpyruvate carboxylase activity (Vpmax) were significantly lower ($P < 0.01$) under blue light than under white, red, and green light. The lower Vpmax under blue light suggests that lower A results from a reduced mesophyll C_4 cycle activity. Subsequent tests will assess these results by measuring photosynthetic metabolite pools, online carbon isotope discrimination and rates of cyclic electron flux.

Abstract 27

Deserah Strand, deserah_s@hotmail.com, SPEAKER

The Effects of Photoinhibition on the Proton Circuit of Photosynthesis

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The major mechanisms of photoprotection, the qE response, which regulates light capture, and the cytochrome b6f complex, which regulates electron transfer, are triggered by the thylakoid proton motive force (*pmf*). The *pmf* is generated by proton translocation, driven by linear and cyclic electron transfer, and modulated by the activity of the ATP synthase. The question addressed here is: what happens when a large fraction of photosystem II (PSII) is inhibited by high light? Does the proton circuit of photosynthesis compensate in some way for the loss of linear electron flow? Surprisingly, we show the ATP synthase is not down-regulated under high light. This contradicts our prediction that the ATP synthase would be down-regulated increasing the *pmf* increasing the pH of the lumen and the qE response. We propose that the quenching of excitation energy that accompanies photoinhibition serves to down-regulate light capture and electron transfer under 'excess' light. We also show that cyclic electron flow around photosystem I (CEF1) is not initiated during photoinhibitory light stress. We conclude that, while there are documented cases of downstream regulation of the ATP synthase (e.g. low CO_2, environmental stresses) to modulate quenching mechanisms, there is no obvious modulation by upstream limitations. (This project is supported by the National Research Initiative competitive grant no. 2008-35318-04665 from the USDA National Institute of Food and Agriculture. Additional support came from the Global Plant Science Initiative at Washington State University.)

Abstract 28

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Essential Functions of Ureide Transporters in Soybean Nodules

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Legumes like soybean are able to exploit the large atmospheric nitrogen (N_2) reservoir by assimilating this N_2 through a symbiotic association with bacteria housed in specialized root organs called nodules. In nodulated soybean roots, the major products of N_2 fixation and reduction are organic molecules called ureides. Following synthesis, ureides are transported out of the nodules to provide N to the shoot and ultimately to the seeds. The hypothesis we tested was that specific membrane transport proteins called UPS1 are critical for controlling ureide transport from nodules to other soybean organs. We have isolated and functionally characterized two soybean cDNAs, *UPS1-1* and *UPS1-2*, which encode proteins that transport ureides across cellular membranes. When determining the location of *UPS1* expression in nodules, it was discovered that their cell-specific localization is consistent with their proposed function in ureide export out of the nodules. To further dissect the function of the UPS1 transporters, transgenic nodulated soybean roots were produced, in which *UPS1* expression was reduced or knocked-out. Results showed a reduction in nodule number and size, as well as a decrease in ureide concentrations in the transgenic nodules. This suggests that the UPS1 transporters are important to nodule development and that ureide partitioning processes might affect nodule metabolism. Further studies will be performed with plants overexpressing *UPS1* to more fully understand how UPS1 proteins control transport of ureides and how they impact plant growth and productivity. Funded by NSF IOB0448506 and USDA-NIFA-AFRIC 2010-65115-20382.

Abstract 29

Judy Callis, jcallis@ucdavid.edu, SPEAKER

The RING-type Ubiquitin E3 Ligase Family in *Arabidopsis thaliana*

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The ubiquitin pathway catalyzes covalent attachment of the 76-amino acid ubiquitin, typically to epsilon amino groups of substrate proteins, and includes proteins that recognize and catabolize ubiquitylated proteins. Ubiquitylation can affect the activity, localization and/or longevity of the substrate protein. The ubiquitin E3 ligases play an important role in determining specificity of ubiquitylation by interacting with the E2 carrying activated ubiquitin and the substrate. One type of E3 contains a conserved domain called a RING (for Really Interesting New Gene) domain that serves, in part, to interact with the E2. Bioinformatics searches of the predicted *Arabidopsis* thaliana proteome identified over 470 proteins with RING or RING-like domains. Our major goal is to identify the in vivo functions for selected RING and RING-like type E3 ligases. Currently our approaches have been phenotypic analysis of loss of function mutants and interaction assays. Publicly available T-DNA insertion lines in over 100 RING domain genes were propagated to isolate homozygous individuals that were subsequently subjected to phenotypic analyses. We have focused detailed studies on three different RING genes that are seedling lethal when the insertion is homozygous. Interacting partners for another group of RING proteins were identified by Y2H analyses and results verified by in vitro interaction assays. Altogether, these studies will aid in our understanding of how ubiquitylation by RING E3 ligases is regulated and in what processes regulated ubiquitylation plays a role. Supported by NSF 2010 program.

Abstract 30

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The Effect of Wheat ABA Response Mutants on Grain Dormancy and Drought Tolerance

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Mutants with altered responses to the plant hormone abscisic acid (ABA) have been isolated in wheat (*Triticum aestivum* L.). Mutants have been isolated based on ABA hypersensitivity in germination in three spring wheat backgrounds: Chinese Spring (referred to as *Wheat ABA Responsive Mutants*, W*arm*), Scarlet, and Zak. Mutants isolated based on ABA insensitivity in germination have been isolated in the Scarlet background. Because ABA is required for the induction of seed dormancy, stomatal closure, and drought, cold and salt tolerance, wheat ABA response mutants were expected to show altered seed dormancy and leaf transpiration in drying soils. Lack of wheat grain dormancy is associated with a propensity for preharvest sprouting (PHS), the germination of seed on the mother plant when moist conditions persist before harvest. PHS tolerance correlates with higher seed dormancy resulting from red grain color, higher ABA accumulation and sensitivity. Wheat grain loses dormancy and sensitivity to ABA inhibition of seed germination with after-ripening. ABA hypersensitive lines maintained higher ABA sensitivity when partially after-ripened. The *Warm1* and *Warm4* mutants showed the strongest and most reproducible increase in ABA sensitivity, accompanied by a requirement for more prolonged after-ripening to break dormancy. Four Scarlet ABA insensitive lines showed reproducible reductions in ABA sensitivity in germination. A preliminary test of PHS resistance suggested that three Warm mutants and two Zak ABA hypersensitive mutants may have increased resistance to PHS. The *Warm4* mutant showed decreased whole plant transpiration in drying soil, consistent with the role of ABA in inhibiting vegetative leaf transpiration.

Abstract 31

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TILLING and EcoTILLING the Tomato *hp-1* and *hp-2* Genes

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TILLING (Targeting Induced Local Lesions IN Genomes) has been used to successfully isolate novel alleles of known genes from chemically mutagenized populations. TILLING accelerates plant breeding by introducing genetic diversity to domesticated crops, and facilitating the discovery of novel functional alleles in genes of interest. Induced mutations can then be used as molecular markers to rapidly introgress the trait into any cultivar. TILLING technology can also be used to uncover polymorphisms in natural populations, a process that is referred to as EcoTILLING (Comai et al., 2004). EcoTILLING can reveal potentially useful mutations for molecular breeding, inferring introgression lineage or identifying haplotypes. Arcadia is interested in developing products that benefit human health. We focused on two tomato high pigment loci: *hp-1* and *hp-2*, which have been implicated in nutritional benefits such as a higher fruit flavonoid content and increased lycopene (Bino et al, 2004). At Arcadia we conducted both TILLING and EcoTILLING screens over portions of these two tomato genes. Our EcoTILLING screen revealed both known and previously unreported mutations. Our TILLING screen of 5,760 individuals uncovered 7 mutations in *hp-1*, and 13 mutations in *hp-2*. TILLING provides a non-transgenic means of uncovering novel variation in genes of interest.

Abstract 32

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Structural and Functional Characterization of Photosynthesis in *Oryza Lines*

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The genus *Oryza* is composed of 24 species, including *O. sativa* (cultivated rice) which is know to have C_3 type photosynthesis. Comparisons of physiological, anatomical (by light and electron microscopy) and biochemical traits among species in the genus are being studied. The aim is to assess whether different genotypes have differences in level of photorespiration, and any C_4 traits or C_3-C_4 intermediate features. Low levels of PEP carboxylase, pyruvate, Pi dikinase, NAD-ME, and PEP carboxykinase orthologs are present in rice and wild relatives (analyzed by western blots). Gas exchange measurements were conducted on leaves of 30-40 day-old potted plants over a range of temperatures, CO_2 and O_2 levels. A portable Li-Cor system equipped with a leaf chamber measured the CO_2 compensation points (Γ which includes photorespiration and dark type respiration, and $\Gamma*$ which is only dependent on photorespiration and associated with Rubisco function), carboxylation efficiency and CO_2 saturated rates of photosynthesis, and dark type respiratory rates. Measured values of Γ in some species are compared with modelled values of Γ based on Rubisco kinetic properties between 20 and 35 °C and atmospheric levels of O_2 between 2 and 40% O_2. *O. sativa* has lower than predicted values of Γ suggesting reduced expression of photorespiration, possibly by partial refixation of photorespired CO_2. The results will be discussed in relation to structural and photosynthetic features of plants in genus *Oryza* compared to some other species (wheat, tobacco).

Abstract 33

Keum Young Lee, ky1206@uw.edu, SPEAKER

Three Approaches to Enhancing Phytoremediation of *Chlorpyrifos*

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Chlorpyrifos (CPS) is a commonly used organophosphorus insecticide that is implicated in environmental and human health problems. Our hypothesis was that these problems may be partially or thoroughly solved by the emerging phytoremediation technology. To evaluate plant potential for degradation of CPS, several selected plant species such as aspen, cottonwood, and willow were investigated. Analysis of the percent removal of CPS from solution showed that CPS can be taken up and significant amounts of CPS were accumulated in plant tissues. CPS did not persist in the plants, suggesting further metabolism of CPS in plant tissue. To our knowledge, this work represents the first report for phytoremediation of CPS using poplar and willow plants. Transgenic expression of genes involved in CPS metabolism is expected to increase removal of CPS. Cytochrome P450s (CYP) activate CPS in mammals, forming CPS-oxon, which *paraoxonase 1* (*PON1*) detoxifies. Human CYP 2B6 and recombinant rabbit *PON1* have been cloned into separate plant expression vectors and used to simultaneously transform the poplar hybrid clone. We confirmed the presence of *PON1* in putative transgenic lines using polymerase chain reaction (PCR). The confirmation of CYP2B6 presence is in progress. The transformed poplars will be investigated for transgene expression and CPS uptake and degradation. In order to express the PON1 protein at high levels for inexpensive, large-scale production of this remediation tool, we proposed to use the chloroplast genome transformation method. To this end, rabbit *PON1* have been cloned into the chloroplast transformation vector, and we are now in the process of transforming tobacco leaf tissue by particle bombardment. The transgene will be confirmed and investigated for PON1 activity and degradation of CPS.

Abstract 34

Berkley Walker, berkley@wsu.edu, SPEAKER

Altered Photorespiratory Carbon Metabolism in *Arabidopsis thaliana*: Prevalence and Consequences of Increases to the Stoichiometric Release of CO_2

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Plants sustain life by harnessing light energy to convert CO_2 into sugars. Rubisco is the initial enzyme fixing CO_2 to reduced carbon. This enzyme also reacts with O_2 producing phosphoglycolate, a wasteful compound not readily metabolized. To minimize this loss, photorespiration recycles phosphoglycolate to phosphoglycerate at the cost of photorespired CO_2, ATP and NADPH. The stoichiometric release of CO_2 per Rubisco oxygenation is usually assumed to be 0.5 in leaf models of gas exchange and energy use. Here we present gas exchange and modeling data that confirm an increase in the stoichiometric photorespiratory release of CO_2 in a mutant lacking photorespiratory enzymes (peroxisomal malate dehydrogenase and hydroxypyruvate reductase *pmdh1pmdh1hpr1-2*) under ambient conditions. Further modeling suggests a similar increase in wild-type plants under high photorespiratory fluxes. Increases to the release of CO_2 during photorespiration have been ascribed to non enzymatic reactions which should decrease glycolate recycling and NADPH required per Rubisco oxygenation. *pmdh1pmdh1hpr1-2* had lower pools of Calvin-Benson Cycle intermediates, including glycerate, under photorespiring conditions. These results are consistent with the mutants loosing glycerate non-enzymatically during photorespiration. The impacts of this reaction to the electron requirement of photorespiration were then explored by comparing modeled rates of photosynthetic electron transport with rates measured through chlorophyll fluorescence. This work has implications for biochemical models of leaf photosynthesis under high photorespiratory fluxes and for calculating leaf energy metabolism.

Abstract 35

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Selecting an Improved Core Subset of the USDA Wheat Collection

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The National Small Grains Collection maintains a collection of *Triticum aestivum* L. subsp. *aestivum* accessions which is too large to regularly be of use to researchers. Phenotypic data are available but limited for accessions in this collection. A core subset (10% of the total) that attempted to capture the diversity of the complete collection, was selected previously using only country of origin. Our objective was to compare alternate methods for selecting core subsets to the existing core to determine if a more diverse subset can be selected using phenotypic data. Accessions were stratified based on region of origin and growth habit, followed by cluster analysis (two methods), and then followed by selection from within each cluster (four selection intensities). Core subsets were compared with metrics that compared their diversity with the complete collection. Methods of constructing a focused core subset were evaluated by filtering the complete collection for resistance to *Puccinia striiformis* prior to selecting a core, or filtering following the selection of a core. This study demonstrated that using phenotypic data allowed the selection of a core subset that was more diverse than the existing core subset, whereas the approach of selecting for resistance to stripe rust prior to the selection of a core subset resulted in limited gains in diversity. When selecting accessions with resistance to stripe rust, selecting resistant accessions from a high quality core provided a more diverse set than the resistant accessions in the existing core.

Abstract 36

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Efficiency of the CO_2-Concentrating Mechanism in Single-Cell C_4 Photosynthesis

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The availability of atmospheric CO_2 limits rates of photosynthesis and leads to increased photorespiration, particularly when stress induces stomatal closure. In response, some plants 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 prevent diffusional CO_2 loss, or "leakiness", from the leaf after concentration around Rubisco. However, two C_4 photosynthetic pathways using dimorphic chloroplasts within a *single* cell were recently discovered in the Chenopodiaceae family. Combining the increased CO_2 assimilating efficiency of C_4 photosynthesis in an anatomically simpler package both raises ecophysiological questions and has potential for engineering C_4 traits into C_3 crops. This project aims to characterize the CO_2-concentrating mechanism efficiency in two subtypes of single-celled C_4. Bienertioid anatomy concentrates CO_2 across central and peripheral cytoplasmic compartments (*Bienertia sinuspersici*), while Borszczowoid anatomy utilizes distal and proximal compartments (*Borszczowia aralocaspica*). The efficiency of the CO_2-concentrating mechanism was estimated from leaf level measurements of photosynthetic isotope discrimination under various temperature and light conditions. Here we present data showing similar levels of total photosynthetic isotope discrimination, and thus rates of CO_2 leakiness in both forms of single-cell versus Kranz type C_4 species. Temperature manipulations did not significantly alter this result, while decreasing light availability increased photosynthetic isotope discrimination, and thus leakiness, across all C_4 types. Our data demonstrate that the rates of photosynthesis and efficiency of the CO_2-concentrating mechanism in single-cell C_4 is similar to the dual-cell, Kranz type.

Abstract 37

Alexander Karasev, akarasev@uidaho.edu, SPEAKER

Identification of an Unusual Recombinant Potato Virus Y Strain in Potato in North-central Mexico

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Potato virus Y has been reported to occur in potato crops in Mexico. Currently, many states of this country are declared PVY-free by the Mexican government; nevertheless, distribution of individual strains of PVY in potato in different states of Mexico and in different solanaceous crops had not yet been studied. More than 900 random potato leaf samples were collected in August 2009, from cultivars Snowden, Atlantic, FL1867, Felsina, Fianna, Gigant, and Alpha, in a field survey in the State of Chihuahua. Seven were found PVY-positive, collected from cultivars Fianna, Snowden, and FL1867. The PVY status of the collected samples was initially determined with the PVY-specific Immunostrips (Bioreba), and by DAS-ELISA using the polyclonal PVY detection kit (Agdia). In order to determine the strain specificity of these PVY isolates, original potato leaf samples were homogenized and inoculated onto tobacco plants (*Nicotiana tabacum* cv Burley), and symptom appearance and development were observed for 8 weeks, followed by the standard typing by RT-PCR. Of the original seven PVY-positive samples, one induced systemic PVY infection in tobacco producing stunting, mosaic, and vein clearing; no systemic vein necrosis was observed. This isolate, PVY-M3, was typed as recombinant PVY^{NTN} isolate by RT-PCR. It was further analyzed by TAS-ELISA using four PVY^{O} and PVY^{N} strain-specific monoclonal antibodies, and confirmed to have N-specific serology, characteristic of other PVY^{NTN} recombinants. Based on the combination of biological, serological, and molecular characteristics, this recombinant strain from Mexico may belong to the same PVY strain group represented by the isolate PVY-L26.

Abstract 38

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Profiling Orphan Species' Transcriptome (POST): A New Biological and Computational Framework

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Though we are in the heart of the genomics era, a majority of species with unique and specialized physiology lack a genome sequence that enables many research opportunities. Transcriptome profiling via next-generation sequencing platforms is a quicker method to identify the gene(s) responsible for a trait than classical breeding, especially in perennial crops. Advantages of profiling by sequencing over methods like microarrays include the ability to identify novel transcripts; however, a reference sequence is needed to extract the most pertinent information from the wealth of data these new options produce. Current data analysis tools are typically inefficient and time-intensive since they were not designed for next-generation sequencing data. Here, we present an optimized wet-lab and computational framework, POST, for quantitative transcriptome profiling and data analysis. Though the POST framework was developed for 3' untranslated region (UTR) sequencing data from the 454 system, it can be adapted to other current and future technologies. To test this framework and address physiological questions, samples from apple, cherry and pear were obtained from varieties exhibiting contrasting phenotypes. Using the POST framework, we have established the applicability of ESTs, predicted genes and same tissue transcriptomes as a reference for quantitative transcriptome sequencing. Differentially expressed genes have been identified using a custom, seeded-clustering program. These genes are being screened based on the predicted gene function and how it correlates with the physiological question being examined. These related and differentially expressed genes will be tested for biological validation.

Abstract 39

Michael Neff, mmneff@wsu.edu

Light-Mediated Germination in Lettuce Seeds: Resurrection of a Classic Plant Physiology Lab Exercise

Lori Sanderson[1], Daniel Tedor[1] and Michael M. Neff[1,2]. [1]Department of Crop and Soil Sciences, Washington State University. [2]Graduate Program in Molecular Plant Sciences, Washington State University.

Most students know that plants use light for photosynthesis. However, many do not realize that plants also use light as a source of information to regulate growth and development. In 1952, H.A. Borthwick and colleagues demonstrated that light controls germination in certain varieties of lettuce seeds. They found that red light was most effective at inducing germination of Grand Rapids lettuce seeds whereas far-red light inhibited germination and, when given multiple exposures of one light color followed by the other, it was the last light treatment that determined the germination response. Based on this publication, Grand Rapids lettuce seeds were used in plant physiology laboratory teaching exercises to demonstrate phytochrome's control of seed germination. However, during the past 20 years, most lettuce varieties no long require red light to induce germination. Though having uniform seed germination is ideal for agriculture, these lettuce-breeding efforts have also led to the abandonment of this laboratory exercise. We have now resurrected this lab exercise by identifying which Grand Rapids derived varieties of lettuce maintain light regulation of seed germination. We also show how to use lettuce leaves as a far-red light filter to inhibit germination, an important and inexpensive addition to this exercise demonstrating the biological relevance of red/far-red control on seed germination. This research was supported by an Undergraduate Research Award to L. Sanderson from the College of Agricultural, Human and Natural Resource Sciences at WSU as well as by the NSF Division of Integrative Organismal Systems, Grant # 0758411 to M. Neff.

Abstract 40

Shantel Martinez, shantelmartinez15@hotmail.com

Determining the Location of Genes Contributing to Supersoft

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The end usage of wheat is determined in part by the softness of the flour after the seed is milled. This value is referred to as the Skcs value. Hard wheat is used in making breads and soft wheat is used for cakes. Grain softness is generally determined by the Ha locus, which is found on chromosome 5D. My project is looking at a line of wheat that was found to be softer than the average soft wheat, which was developed by the crossing of bread wheat with a related wheat species called *Agropyron*. This resulted in a chromosomal translocation from the *Agropyron* to the bread wheat on chromosome 6A. We call this line 'Supersoft'. This extremely soft wheat could possibly contribute to better cakes and cookies. I'm investing why this wheat is so soft and whether there is a genetic component, other than the *Ha* locus, that is contributing to the increase in softness. An initial experiment using the two extremes of Skcs values within the population of our experimental lines showed genetic differences, but not at the *Ha* locus. While we are mapping the entire wheat genome, we are attempting to saturate chromosome 6A to determine whether the Supersoft gene(s) is on this translocation. Our objective is to map the gene that is contributing to the "Supersoftness".

Abstract 41

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Optimization of Extraction Protocols and Development of Sensitive LC-MS Techniques for Detection of Bioactive Metabolites in Medicinal Plants

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Plants have a long tradition of use in traditional medicine across the world. Consequentially, natural sources of numerous medicinal plants have been over-exploited and certain very important species are facing extinction. Huperzine A is an alkaloid from the clubmoss *Huperzia squarrosa* currently being investigated for its cholinesterase inhibitory activity and the treatment of Alzheimer's disease; triptolide is a diterpene from the vine *Tripterygium regelii* with potent anti-inflammatory activity presently in clinical trials for the treatment of rheumatoid arthritis. Both plants grow very slowly and have been extensively exploited in their natural habitats in China. Extraction protocols for huperzine A and triptolide have been optimized to microscale and very small amounts of starting material (as low as 10 mg). Rapid resolution of bioactive metabolites from the extracts has been achieved on the LC-QToF-MS system using electrospray ionization and atmospheric pressure chemical ionization source in the positive polarity, and the presence of the compounds of interest confirmed via tandem mass spectrometry in various organs of the plant. Total RNA extractions have been performed from the tissues actively making these metabolites, as a step towards the long-term goal of elucidating their biosynthetic pathways.

Abstract 42

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Passing the Plastocyanin Football

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The passing of electrons through the photosynthetic electron transport chain in the chloroplast's grana is like playing football in a crowded shopping mall. The protein complexes involved are packed very densely into the thylakoid membrane, which may help them capture photons and transfer their energy to the photosystems. However, with approximately 70% of the membrane surface composed of proteins (Kirchhoff et al, unpublished), there is very little space for the lateral diffusion of electron carriers such as Plastocyanin. We used in vivo spectroscopic measurements of the equilibrium balance between cytochrome f and photosystem I in wild-type *Arabidopsis* leaves in the dark and with 15s actinic prepulses. We found that their redox states deviated from thermodynamic equilibrium, suggesting that Plastocyanin diffusion may be limiting the rate of electron flow from the cytochrome b6f complex to photosystem I.

Abstract 43

Jianfei Zhao, jfzhao@wsu.edu, SPEAKER

Functional Analysis of the AT-Hook Motif Nuclear Localization (AHL) Gene Family in *Arabidopsis thaliana*

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The *AT-HOOK MOTIF NUCLEAR LOCALIZED* (*AHL*) gene family in *Arabidopsis thaliana* has 29 members, which are characterized by containing one or two AT-hook DNA binding motif(s) and a Plant and Prokaryote Conserved/Domain of Unknown Function #296 (PPC/DUF296). Through evolution, the *AHL* gene family has evolved into two clades distinguished by the number of AT-hook motif. *SOB3/AHL29*, *SUPPRESSOR OF PHYTOCHROME B-4, #3-DOMINANT*, was identified in an activation tagging screen for extragenic suppressors of the long-hypocotyl phenotype conferred by the *phytochrome B-4* mutation. Over-expression of *SOB3/AHL29* or *ESC/AHL27* (*ESCAROLA*), the closest family member of *SOB3*, confers repressed hypocotyl elongation for seedlings grown in the light but not in darkness. As adults, these gene-over-expression plants develop larger organs including expanded leaves and enlarged flowers and fruits together with delayed flowering and senescence. In contrast, the *sob3-4 esc-8* double-null mutant confers enhanced seedling hypocotyl growth under continuous white, red, far-red and blue light. Our study indicates that *SOB3*, *ESC*, and possibly other *AHL* genes, such as *HERCULES* (*HRC/AHL25*), function in a redundant manner to regulate hypocotyl elongation in response to light at the seedling stage and possibly flowering time and biomass for adult plants (Street *et al*, 2008). A unique missense mutation in *SOB3* as well as a similar mutation in *ESC* confers a more severe dominant-negative long-hypocotyl phenotype. We present data derived from these dominant-negative alleles demonstrating that multiple family members function by physically interacting with themselves and with each other *in vivo*. Street *et al.*, (2008) Plant J. 54 1-14.

Abstract 44

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Genetic Interactions between Brassinosteroid-inactivating Enzymes and Photomorphogenic Photoreceptors

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Brassinosteroids (BRs), a group of plant growth-promoting hormones, are important for seedling growth and development. *BAS1* and *SOB7* encode cytochrome P450's involved in the inactivation of BRs. Both *BAS1* and *SOB7* were identified in an activation-tagging screen for suppressors of the long-hypocotyl phenotype of *phyB-4* (a weak mutant of *PHYB*) seedlings when grown in white light. A study of loss-of-function mutants of *BAS1* and *SOB7* demonstrates their role in light-mediated development. Processes such as hypocotyl growth inhibition by light and flowering are altered in double null *bas1-2 sob7-1* plants, demonstrating redundancy between the two genes and a role of BR inactivation in light-mediated development. Current study of genetic interactions of *BAS1* and *SOB7* with *PHYA*, *PHYB* and *CRY1* revealed their differential regulation by these photoreceptors, particularly with respect to inhibition of hypocotyl elongation and flowering time. *BAS1* and *SOB7* act independently from *CRY1* to modulate hypocotyl growth in response to white and blue light. However, in red and far-red light the hypocotyl growth phenotype conferred by the loss of *BAS1* and *SOB7* requires *PHYB* and *PHYA* respectively, demonstrating a role for these photoreceptors in modulating brassinosteroid inactivation. The *bas1-2* mutation suppresses the *PHYA* late flowering phenotype in both long days and short days whereas *sob7-1* has only a slight enhancement effect, thus suggesting separate roles of these two BR inactivating genes with regard to flowering time. The study also reveals differential expression patterns of BAS1 and SOB7 by using translational GUS fusions with native promoters.

Abstract 45

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AtSOFL1, *AtSOFL2* and Possibly *SOB5* are Part of a Partially Redundant Gene Family that Regulates Cytokinin Levels and Responsiveness in *Arabidopsis*

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sob5-D (*ACTIVATION-TAGGED SUPPRESSOR OF PHYB-4, #5-DOMINANT*) was identified in a gain-of-function mutant screen for extragenic suppressors of the long-hypocotyl phenotype conferred by a weak photoreceptor mutation, *phyB-4*. Over-expression of *SOB5* confers dwarf phenotypes reminiscent of plants with elevated levels of the plant hormones cytokinins (CKs). The *sob5-D* mutation also confers higher levels of specific CKs relative to the wild type. These results suggest a role for SOB5 in CK-mediated development. Loss-of-function mutations in *SOB5* are phenotypically wild-type, suggesting that there are functionally redundant proteins that compensate for a loss of SOB5 activity. Sequence analysis identifies two SOB FIVE LIKE proteins in *Arabidopsis* (AtSOFL1 and AtSOFL2) that may be functional SOB5 homologues. Over-expression of *AtSOFL1* and *AtSOFL2* confer similar phenotypes to the *sob5-D* mutant over-expressing *SOB5*. In contrast, the *sofl1-RNAi62 sofl2-1* double mutant accumulates lower levels of specific CKs and is less sensitive to exogenous CK in a seedling hypocotyl growth assay. Together these gain-of-function and loss-of-function results demonstrate that *AtSOFL1* and *AtSOFL2*, and possibly *SOB5*, act redundantly as positive regulators of specific CK levels and responsiveness. Overexpression of proteins harboring mis-sense mutations in conserved domains results in either recapitulation of the wild type phenotype or dwarf plants depending on the type of mutation. Preliminary results show that some dwarf plants have reduced *GA3ox* transcript levels. To understand more about this small semi-redundant gene family, all three proteins are being used as baits in a yeast two hybrid library screen. A number of putative interactors are currently undergoing further evaluation.

Abstract 46

Hanjo Hellmann, hellmann@wsu.edu, SPEAKER

The DDB1a Interacting Proteins ATCSA-1 and DDB2 are Critical Factors for UV-B Tolerance and Genomic Integrity in *Arabidopsis thaliana*

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The integrity of the genome is a fundamental prerequisite for the well-being of all living organisms. Critical for the genomic integrity are effective DNA damage detection mechanisms that enable the cell to rapidly activate the necessary repair machinery. Here, we describe *Arabidopsis thaliana* ATCSA-1, which is an ortholog of the mammalian Cockayne Syndrome type-A protein involved in transcription-coupled DNA repair processes. ATCSA-1 is a critical component for initiating the repair of UV-B-induced DNA lesions, and, together with the damage-specific DNA binding protein 2 (DDB2), is necessary for light-independent repair processes in *Arabidopsis*. The transcriptional profile of both genes revealed that *ATCSA-1* is strongly expressed in most tissues, whereas *DDB2* is only weakly expressed, predominantly in the root tips and anthers of flowers. In contrast to *ATCSA-1*, *DDB2* expression is rapidly inducible by UV treatment. Like DDB2, ATCSA-1 is localized to the nucleus, and assembles with DDB1 and CUL4 proteins into a complex. ATCSA-1 is an unstable protein that is degraded in a 26S proteasome-dependent manner. Overall, the results presented here form a functional description of a plant Cockayne syndrome factor A (CSA) ortholog, and demonstrate the importance of ATCSA-1 for UV-B tolerance.

Abstract 47

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Functional Characterization of a Flavin Interconverting Enzyme in *Arabidopsis*

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FMN and FAD are important cofactors for a variety of enzymes involved in a multitude of metabolic processes in all organisms. These cofactors, as well as their inactive precursor riboflavin, are known to be interconverted by a network of enzyme catalyzed reactions. While examples of these enzymes have been characterized in several different organisms, in plants most of those interconverting enzymes have yet to be identified. Our lab has identified an *Arabidopsis* homolog to the known yeast FAD synthetase FAD1, an enzyme which facilitates the ATP-dependent conversion of FMN to FAD. In addition to the homologous domain, the *Arabidopsis* homolog possesses an extra 245-residue C-terminal domain with no predicted function, and we have named the protein AtFAD/XD. Our goal is to characterize AtFAD/XD, determining the kinetic properties of the FAD synthetase and identifying the function of the extra domain. Results obtained using refolded recombinant protein have confirmed the FAD synthetase activity. Furthermore, preliminary results using a yeast homolog to the extra domain suggest its function to be an FMN hydrolase.

Abstract 48

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Unraveling the Regulation of Monoterpene Biosynthesis in Specialized Epithelial Cells of Grapefruit Peel

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The essential oil of Citrus peel, which consists primarily of monoterpenes, is synthesized in specialized epithelial cells lining secretory cavities. We have used grapefruit (*Citrus paradisi*) as a model for monoterpenoid essential oil biosynthesis in the genus *Citrus*. In order to obtain a quantitative understanding of essential oil formation, we have determined the distribution and size of secretory cavities at different stages of fruit development, and correlated these data sets with essential oil yields from distillations. Essential oil biosynthesis starts at a very early stage of fruit formation (although at relatively low rates), then enters an exponential phase (fruit size between 30 and 100 mm), before slowing down to a linear increase until the final fruit size is reached. The specialized epithelial cells actively synthesizing essential oils were harvested using laser capture microdissection and RNA was extracted using a newly developed protocol. Global gene expression patterns in these samples were assessed using Affymetrix Citrus GeneChip Genome Arrays. A preliminary correlation analysis of transcripts putatively involved in monoterpene biosynthesis and developmental changes in monoterpene composition will be presented. Our goal is the integration of developmental, biochemical, physiological, and anatomical data sets into a comprehensive mathematical model of Citrus peel essential oil biosynthesis.

Abstract 49

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Compensatory Response of Black Cottonwood to Defoliation by Cottonwood Leaf Beetle

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Plants may buffer the negative effects of herbivory through mechanisms of tolerance. Many studies report *Populus* spp. tolerate herbivory, but fail to report the physiological mechanisms of tolerance or to accurately simulate insect feeding behavior. During the summer of 2009, we measured the physiological and morphological tolerance response of *P. trichocarpa* to natural and simulated defoliation by *Chrysomela scripta*. Two-year old trees were treated with either artificial or natural defoliation and branches from each tree were defoliated 0, 15, 20, or 30% throughout the growing season. Leaf dry weight and total dry weight of branches were not different across defoliation levels. Biomass was not different due to decreased specific leaf area and increased sylleptic branching. Specific leaf area decreased from 55 m^2g^{-1} in control branches to 47 m^2g^{-1} in 30% defoliated branches. Sylleptic branching increased with increased defoliation and contributed to increased leaf area and leaf number. Branches with insect defoliation had 25% higher leaf C:N than branches with artificial treatment due to a 22% lower leaf N content in the insect treated branches. We conclude that 1) *P. trichocarpa* completely compensated for low levels (<30%) of defoliation by *C. scripta* through decreased specific leaf area and increased sylleptic branching, and 2) defoliation by *C. scripta* decreased leaf nitrogen content compared to artificial defoliation.

Abstract 50

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Physiological and Biochemical Characterization of a Mottling Defect in Potato Tubers

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Mottling is an undesirable postharvest disorder of potato tubers characterized by the development of random pockets of translucent tissue containing high concentrations of reducing sugars. Mottled areas turn dark during frying due to the reaction of reducing sugars with free amino acids (Maillard reaction). The newly released cultivar, Premier Russet, is susceptible to mottling; symptoms appear as early as 180 days and get progressively worse with time in storage (4-9 °C, 95% RH). The mechanism of mottling is unknown; however, the disorder appears to have similarities with the irreversible senescent sweetening that normally occurs in tubers during long-term (>12 months) storage. Much of the decline in tuber quality is a consequence of progressive increases in oxidative metabolism associated with aging. Thus, mottling may be the phenotypic manifestation of oxidative stress associated with an accelerated aging genotype. Tissue from mottled and control tubers were compared for their respiration rates and various markers of oxidative metabolism. The respiration rate of mottled tissue was 1.8-fold greater than control tissue. In addition to higher concentrations of glucose, fructose, and sucrose, mottling resulted in lower dry matter, higher specific activities of starch phosphorylase and glu-6-phosphate dehydrogenase, higher protease activity, and loss of protein. Moreover, membrane integrity declined during mottling, likely due to increased peroxidation of membrane lipids. Superoxide dismutase activity and the ratio of oxidized to reduced glutathione were substantially higher in mottled tissue. Mottling thus appears to be the consequence of oxidative stress associated with accelerated aging of Premier Russet tubers.

Abstract 51

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Comprehensive Analysis of Homoeologue Gene Expression in Polyploid Wheat

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Gene duplication by polyploidy (homoeologues) or other means (paralogues) is a prominent feature of angiosperm evolution. We studied gene expression among three homoeologues of hexaploid wheat that evolved from a common progenitor about 3 million years ago (MYA) and came into a common nucleus at different times: ∼0.5 and 0.01 MYA. Gene expression corresponding to each homoeologue was identified by sequence comparison of cultivar 'Chinese spring' ESTs and the results were confirmed by SSCP analysis of RNA using nulli-tetra lines. Of the 2524 genes analyzed, 63% were expressed from all three homoeologues, 30% from two, and only 7% were expressed from one of the three homoeologues. The largest percentage of genes (14%) were expressed in anthers and the least (7%) were expressed in pistils. Whereas, the highest number of homoeologues/gene were expressed in roots (1.72 out of 3 homoeologues) and the lowest number were expressed from anthers (1.03 out of 3 homoeologues). In general, the proportion of expressed copies decreased with the increase in homoeologue copy number. The most significant observation was that homoeologues for 87% of the genes showed different expression patterns in different tissues and thus have likely evolved different gene expression controls. About 30% of the genes showed dosage dependence as the expression of homoeologues changed in response to changes in structural copy number.

Abstract 52

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Is AtSt4a a Brassinosteroid Inactivating Enzyme in Planta?

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Arabidopsis ATST4a belongs to an ancient super family of sulfotransferases. A biochemical analysis of the ATST4a protein has shown that it is capable of catalyzing O-sulfonation of brassinosteroids (BR) (Marsolais *et al.*, 2007). However, no genetic studies involving loss of function or over expression were done to show that BR inactivation is the in vivo function of *ATST4a*. Originally ATST4a was over expressed in a wildtype (Columbia) background, however the T_1 generation had only few lines with an obvious dwarf phenotype. The low number of primary transformants conferring a BR-deficient phenotype is most likely due to silencing of the endogenous gene via the over expression construct. Therefore, to get a stable over expression phenotype, it was essential to over express the gene of interest in its null genetic background. Since the null line of *ATST4a* is kanamycin resistant, the original pCHF3-ATST4a vector cannot be used due to the inability to screen for the primary transformants. Therefore an alternative strategy was designed to clone the *ATST4a* cDNA in a Gateway compatible clone pED15, which provides for basta resistance selection of the primary transformants. These transformants have been created and will be physiologically and biochemically analyzed to further explore the genetic role of *ATST4a* in *Arabidopsis*. Marsolais *et al.*, 2007 Planta 225: 1233-1244.

Abstract 53

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Expression, Purification, and Characterization of *Arabidopsis thaliana* Mitochondrial Serine Hydroxymethyltransferase

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Serine hydroxymethyltransferase (SHMT) is a highly conserved PLP-dependent enzyme in eukaryotes and prokaryote, catalyzing the reversible conversion of L-serine and (6S)-H4PteGlun to glycine and (6S)-5,10-CH2-H4PteGlun. The reaction is the major one-carbon unit source provider and plays a central role in plant photorespiration. In plants, it is now clear that SHMT isoforms of different subcellular localizations have their own specific physiological roles. Although there is evidence for SHMT activity in cytoplasm, mitochondria, plastids and nuclei, little is known about the biochemical properties and physiological significance of each isoform. Lack of such knowledge is limiting our ability to understand and manipulate one-carbon pathway in plants, which is an important target for nutritional enhancement of crops. In *Arabidopsis*, ATSHMT1 and ATSHMT2 are two isoforms in mitochondria. Although located in the same organelle, ATSHMT1 and ATSHMT2 have been confirmed to be functionally different. Based on a previous study and our preliminary results we hypothesize that the difference is caused by their distinct biochemical properties. We plan to express and purify the two proteins, and investigate their biochemical properties in vitro. The biochemical properties we plan to investigate will include enzyme oligomerization state, Michaelis-Menten kinetic parameters, and impact of polyglutamate tetrahydrofolate and of non-substrate folate on the kinetic parameters. Results obtained from this research will significantly advance current knowledge of mitochondrial SHMT and improve the understanding of plant one-carbon metabolism network.

Abstract 54

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An Unusual Photorespiratory-like Phenotype in Plants Expressing 5,10 - Methylenetetrahydrofolate Reductase in Plastids

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Methionine (Met) is an essential amino acid for humans and nonruminant animals, and therefore is the costly supplement to methionine-deficient forage. Thus, there is an interest in creating plants enriched in this amino acid. Met synthase, catalyzing 5- methyltetrahydrofolate (5-CH3-THF)-driven methylation of homocysteine to Met, exists in plastids and the cytosol. 5,10-Methylenetetrahydrofolate reductase (MTHFR), catalyzing reduction of 5,10-methylenetetrahydrofolate (5,10-CH2-THF) to 5-methyltetrahydrofolate, exclusively exists in the cytosol. The goal of this study was to determine if expressing *MTHFR* in plastids would increase 5-CH3-THF availability for Met synthesis in these organelles. To accomplish this goal, the *MTHFR* gene from *E. coli* was introduced into tobacco. Five tobacco transgenic lines with the highest MTHFR activity were selected for metabolite analyses. Preliminary amino acid analysis with leaves of tissue culture-grown transgenic lines showed up to 500-fold Gly accumulation, little increase in Met, and no change in S- methylmethionine (SMM) and Thr. Folate analysis showed a decrease in 5,10-CH=THF and 5-CH3-THF, and an increase in THF + 5,10-CH2-THF. Preliminary analysis of roots from one line showed a 15-fold increase in glycine, no change in methionine, and a decrease in SMM and Thr. Leaves of transgenic plants that were grown in soil in high CO2 (∼2000 ppm) had increased Gly, Met, and SMM. These plants died within 10 days of being transferred to regular air. The decrease in Gly two days after moving plants to air with elevated CO2 was ∼2-fold.

Abstract 55

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FMN Hydrolase: An Enzyme in the Flavin Nucleotides Metabolic Pathway in Plants

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Flavin nucleotides (FMN and FAD) are phosphorylated derivatives of riboflavin. Riboflavin, FMN, and FAD are collectively known as B2 vitamers. A network of enzymes, many of which are not yet identified and fully characterized, mediates the inter-conversion of riboflavin, FMN, and FAD in plants. FMN and FAD are synthesized by the enzymes riboflavin kinase and FAD synthetase, respectively, in the presence of ATP and Mg2+ while the conversion of FMN to riboflavin is catalyzed by FMN hydrolase. The goal of this study is to determine how plants synthesize and maintain the intracellular levels of flavin nucleotides. Towards that goal, one of our primary objectives is to clone a biochemically characterize FMN hydrolase from plastids, and to determine its physiological function using a combination of forward and reverse genetic approaches. We are pursuing a two-prong approach, whereby we are purifying an FMN hydrolase from pea plastids, and also screening recombinant haloacid dehalogenase-like enzymes from *Arabidopsis* for FMN hydrolase activity. We have optimized the steps for the purification of FMN hydrolase from pea plastids using column chromatography techniques. We have also overexpressed in *E. coli* several proteins from *Arabidopsis* having the haloacid dehalogenase domain, and identified one with a high level of FMN hydrolase activity.

Abstract 56

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The Serpentine Syndrome: Investigating Potential Mechanisms of Tolerance in *Mimulus guttatus*

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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 an ideal study system to address this question, with locally adapted populations occurring both on and off serpentine soils. Cuttings are grown hydroponically in aerated solutions mimicking normal (Ca^{++}:Mg^{++} of 4.0) and serpentine soils (Ca^{++}:Mg^{++} of 0.04 – 0.001). Carbon assimilation, total biomass, root number and total root growth were higher in serpentine-adapted plants at low Ca^{++}:Mg^{++} than in non-adapted plants. Chlorophyll concentrations did not vary between populations or treatments. Internal concentrations of Ca^{++} and Mg^{++} reflected the external concentration of the treatment solution in both populations. Root tissue influx rates of Ca^{++} were found to decrease with the concentration of Ca^{++} in solution in both populations, with a higher uptake rate in non-adapted plants when solution Ca^{++}:Mg^{++} ratios were above 0.01. In the extreme Ca^{++}:Mg^{++} ratio of 0.001, all roots were found to efflux Ca^{++}. Serpentine-adapted *M. guttatus* does not appear to be avoiding Mg^{++} toxicity by excluding the ion from its tissues or avoiding Ca^{++} deficiency by increased Ca^{++} uptake in low soil concentrations. Evolution of internal mechanisms may explain the tolerance to low Ca^{++}:Mg^{++} of serpentine-adapted plants and their limited distribution on normal soil.

Abstract 57

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Ripening in Winter Pear

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Due to a prolonged juvenility period, fruit trees in *Rosaceae* present a unique challenge to plant biologists in understanding the gene-based underpinnings of complex physiological processes. One such intriguing physiological issue is the divergent ripening phenotype within European pear (*Pyrus communis* L.). Despite their genetic similarity with Bartlett cultivar, D'Anjou pear requires a cold-treatment to induce autocatalytic ethylene production. We utilized a quantitative RNAseq approach to understand the differences in genomic response between these contrasting genotypes during a developmental continuum. Utilizing custom computational biology tools and statistical analysis to distill the transcriptome and genome data, we identified a gene with high homology to low-copy cold-induced membrane bound protein previously reported in *Arabidopsis*, potato, tomato, Castor Bean, citrus crops, and *P. trichocarpa*. We hypothesize this protein to be involved in regulation of the autocatalytic ethylene burst characteristic of ripening D'Anjou pears. Induction of this gene upon cold stress is proposed to remove the repression of the ethylene burst. Results obtained from testing of this hypothesis will be presented.

Abstract 58

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Developmental Characterization of *Arabidopsis thaliana* Mutants Affected in DDB1 Proteins

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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.

Abstract 59

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Modeling Oxygen Isotope Exchange Between Atmospheric CO_2 and Leaf H_2O in C_4 Plants

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Understanding the mechanisms controlling isotope exchange between atmospheric CO_2 and leaf H_2O is important for estimating variation in the global carbon cycle. During photosynthesis atmospheric CO_2 diffuses into a leaf, dissolves and exchanges oxygen atoms with leaf H_2O. In C_4 plants the enzyme carbonic anhydrase (CA) catalyses the hydration reaction of CO_2 to HCO_3^- which is then taken up via Phosphoenol Pyruvate Carboxylase (PEPC) for use in photosynthesis. CA also catalyzes the dehydration of HCO_3^- to CO_2. This CO_2 can subsequently diffuse out of the leaf. The extent of isotopic equilibrium between atmospheric CO_2 and leaf H_2O has been estimated with CA activities and the gross flux of CO_2 into a leaf. However, leaf level measurements of atmospheric CO_2 and leaf H_2O isotope exchange indicates that the extend of isotopic equilibrium is significantly lower than predicted from in vitro CA activities in C_4 plants. The current model based on CA activity excludes the consumption of HCO_3^- by PEPC activity and possibly overestimates the predicted equilibrium. Our hypothesis is that the overestimation of isotopic equilibrium in C_4 plants is due to the lack of PEPC activity incorporated into this model. We are currently developing a new model based on the rate constants of CA and PEPC to estimate the extent of isotopic equilibrium during C_4 photosynthesis.

Abstract 60

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Analysis of *Arabidopsis thaliana* PDX Overexpresser and PLP-Synthase Complex Formation

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Vitamin B6 is an important compound for all living organisms that serves, in its phosphorylated form (pyridoxal 5'-phoshate), as an enzymatic cofactor for more than 140 biochemical reactions. It is mostly involved in amino acid metabolism, but also plays a role in storage carbohydrate degradation and fatty acid metabolism . These wide ranging actions have therefore an important impact on plant metabolism and development. In plants the final steps in vitamin B6 biosynthesis require the activity of PDX1/PDX2 proteins. In the present work we analyzed plants which express the different *Arabidopsis* PDX genes (PDX1.1, PDX1.2, PDX1.3 and PDX2) under the control of the 35S cauliflower mosaic virus promoter by metabolic profiling and can detect various changes in PDX1 expression and metabolite levels that further underscore the central role of PDX1 proteins for the cellular well-being. Second we verified that the *Arabidopsis* PDX proteins aggregate together in higher molecular weight complexes as it is described for the functional pyridoxal 5'-phoshate (PLP) synthase in e.g. *Bacillus subtilis*. We can show in planta complex assembly of all PDX1 proteins, based on split-YFP assays. Furthermore we can also demonstrate for all PDX proteins with size exclusion chromatography their presence in higher order complexes of around 750 kDa which resembles the expected size of a PLP synthase. In summary, these findings provide first insights into in planta vitamin B6 synthase complex assembly and new information on how the different PDX proteins affect plant metabolism.

Abstract 61

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The Importance of Leaf Starch in Maize Leaves to Plant Growth

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Photosynthesis produces carbohydrates in source tissues which may be allocated to sink tissues for storage, growth, and development. The enzyme ADP-glucose pyrophosphorylase (AGPase) controls the rate limiting step in starch biosynthesis in both seed and leaf tissue. Previous studies, focused on increasing AGPase in seeds, indicated that yield advantages accrue only when resources are nonlimiting. The objective of this study is to determine the importance of leaf starch on the productivity and growth of maize by measuring plant productivity and photosynthetic rates of an AGPase knockout mutant. Field trials indicate there are significant differences between the AGPase knockout mutant and wild type populations for plant height, days to flowering, starch and protein content, and individual seed weight. Greenhouse studies indicate that there is no difference in photosynthetic rates under high light conditions. Further study regarding starch production in source tissues may provide insight as to how plant productivity may be increased.

Abstract 62

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Reinvestigation of the Roles of Soybean Leaf Vegetative Lipoxygenases

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The paraveinal mesophyll (PVM) of soybean leaves is a layer of laterally expanded cells sandwiched between the palisade and spongy mesophyll chlorenchyma. The vacuoles of PVM cells contain an abundance of a putative vegetative storage protein, VSP (α, β). VSP is is constitutively produced, but is up-regulated during sink limitation experiments involving flower, fruit, or vegetative bud removal. Soybean vegetative lipoxygenases (Vlx), consisting of 5 isozymes (Vlx, A-D), have been identified as potential storage proteins, because they accumulate to high levels with experimental sink limitation, and because at least one Vlx isozyme has been co-localized with VSP to the vacuoles of PVM cells. We reinvestigated the sub-cellular locations of these enzymes with TEM immuno-cytochemistry; we employed laser micro-dissection to compared RNA expression of PVM cells with mesophyll chlorenchyma cells; and we performed a micro-array analysis of soybean leaf samples representing a time-course, sink-limitation, experiment. We found that none of the Vlx isozymes co-localize with putative storage proteins in PVM vacuoles, and that our sink limitation experiment (typical of those used in the past) induced a strong up-regulation of stress response genes, simultaneous with the up-regulation of the Vlx isozymes. Our findings do not support a storage function for soybean Vlx.

Abstract 63

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Growth of the 7B-1 Tomato Mutant Under Water Deficit

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7B-1, a spontaneous mutant of the wild type Rutgers variety of tomato (*Solanum lycopersicum*), has been thought to be drought resistant in part due to robust seed germination in hyper-osmotic environments. In addition to a defective blue light response *7B-1* overproduces abscisic acid (ABA), which has led to the hypothesis that the *7B-1* seed is pre-adapted to germinating in stressful environments. Thus *7B-1* presents an attractive system to improving crop yields in tomato and may better our understanding of the physiological and molecular relationships between the blue light response, ABA and plant growth. A battery of water deficit treatments was used to test the rigor of mature *7B-1* plants and test the hypothesis that *7B-1* would withstand the negative effects of water deficit on retarding plant growth. Three separate experiments were conducted designated as mild, extended and extreme drought. Growth parameters and physiological parameters were measured to quantify the extent *7B-1* maintained its shoot growth, biomass, leaf area, and water potential under stressed conditions. Though studies of the *7B-1* mutation in tomato seedlings made it a candidate for improving drought tolerance in crops, maturing tomato plants exhibit drought sensitivity. Under well-watered conditions *7B-1* showed astounding overall growth compared to the wild type but depressed growth under all water deficit treatments and an inability to maintain turgor pressure in its leaves under mild water deficit.

Abstract 64

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Puroindoline Mediated Changes in Polar Lipids During Wheat Seed Development

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The *Ha* locus controls most of the variation in wheat grain hardness and consists of the tightly linked genes *Puroindoline a* (*Pina*) and *Puroindoline b* (*Pinb*). PINA and PINB act cooperatively to create grain softness and increase seed polar lipid content. Here we examined developing seeds to identify when this change occurs and if individual polar lipids are PINA or PINB associated. The genotypes studied varied only in PINA or PINB content. The first pair was created using the hard wheat cultivar Canadian Red which lacks *PINB* while its soft counterpart contained a *PINB* transgene. The second pair was created in the hard wheat cultivar Falcon which lacks *PINA* while its soft counterpart contained wild type *PINA*. Grain characteristics and polar lipid composition were analyzed using seeds harvested weekly beginning at 7 days after flowering (DAF). Addition of *PINB* to genotypes lacking only *PINB* and the addition of *PINA* to genotypes lacking only *PINA* resulted in soft texture and increased polar lipids. For the genotypes segregating for *PINB*, polar lipids were maximal on a dry weight basis at 7 DAF while for the *PINA* segregating genotypes, total polar lipids were maximal at 14-21 DAF. The effect of both PINA and PINB increased throughout seed development with greater decreases in phospholipids and glycolipids in genotypes lacking either PINA or PINB. The results indicate that both PINA and PINB may act to prevent polar lipid breakdown during seed maturation.

Abstract 65

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Functional Analysis of the Glu-D1 HMW-GS Alleles to Improve Wheat Mixing and Baking Properties

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The high molecular weight glutenin subunits (HMW-GS) are a primary determinant of wheat end product quality. HMW-GS are encoded by the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci. *Glu-D1* is the most important HMW-GS locus to wheat flour quality. Our goal is to better understand the structure/function relationship between *Glu-D1* allelic variation and wheat flour quality. To that end, EMS was used to mutagenize the spring wheat variety 'Alpowa' whose *Glu-D1* locus contains the 1Dx5 and 1Dy10 HMW-GS subunits conferring strong dough mixing properties. A total of 135 new alleles were identified. Of the 69 new *Dx5* alleles, 35 were missense, 13 were nonsense, and 21 were silent at the amino acid level. For *Dy10*, 66 new alleles were identified consisting of 45 missense, 9 nonsense, and 12 silent alleles. A subset of the *Dx5* and *Dy10* missense alleles were analyzed further after backcrossing to Alpowa. The majority of the alleles studied reduced *Dx5* and *Dy10* function based upon measurements of dough mixing properties via SDS sedimentation volume tests. Based on direct comparisons of stop codon mutations in *Dx5* or *Dy10*, *Dx5* is more important to dough mixing properties than *Dy10*. One *Dx5* missense mutation, a polar to positive aa change (G200R), had increased dough mixing strength and may represent a gain of function. The results indicate that the novel alleles may shed light on the regions of the HMW-GS subunits most important to dough quality. The new alleles also may prove useful as a novel source of allelic variation for wheat breeding programs.

Abstract 66

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Comparing CID and RWC in Field vs. Greenhouse Grown Wheat

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Drought is a major factor influencing yield in crop plants. To date, many physiological parameters have been considered as metrics for drought tolerance as a phenotype in wheat, but many consider greenhouse experiments to be insufficient predictors of drought tolerance in the field. In this study, we compared two physiological measures of drought tolerance in two wheat varieties in two field locations (dry vs wet) and under two quantitative greenhouse watering regimes (dry vs wet). The measurements were carbon isotope discrimination (CID, an indicator of transpiration efficiency) and relative water content (RWC). The correspondence between CID values in the field and greenhouse differed based on life stage. However, the dry treatment in the greenhouse was not statistically different at 180 degree days (post-anthesis, Zadock stage 6.5) and at harvest from our dry field site (Lind Dryland Research Station) for either genotype. For RWC, there was no difference across locations at jointing, but at 180 degree days both greenhouse watering regimes showed higher RWC in both genotypes compared to the field. Water stress was apparent earlier in development (at jointing) at Lind compared to the greenhouse experiment, and the moist growing conditions at the Spillman Agronomy Farm were not precisely duplicated by the greenhouse wet treatment. It appears that with further refinement greenhouse drought experiments can be used to predict field performance.

Abstract 67

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Deviation of H_2O Oxygen Isotope Composition between the Leaf Site of Water Evaporation and Source Water

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As CO_2 enters a leaf it exchanges oxygen atoms with water in a hydration/dehydration reaction catalyzed by the enzyme carbonic anhydrase (CA). Due to this exchange, and the relative abundance of CO_2 and H_2O inside a leaf, the oxygen isotope composition ($\delta^{18}O$) of CO_2 is heavily influenced by the $\delta^{18}O$ of H_2O. The $\delta^{18}O$ at the site of water evaporation within the leaf (δ_e) is used as an estimate for the $\delta^{18}O$ of CO_2. However, the $\delta^{18}O$ of CO_2 is actually derived from $\delta^{18}O$ of H_2O at the site of oxygen exchange (δ_{ex}). When δ_e and the $\delta^{18}O$ of source water (δ_{sw}) coming up through the vascular tissue are not equal, an isotopic gradient along the diffusional pathway is assumed to exist. Due to differences in CA distribution and this isotopic gradient within the leaf the values of δ_e and δ_{ex} are not necessarily equivalent, potentially overestimating $\delta^{18}O$ of CO_2. We measure δ_e in several species of the family *Poaceae* and compare to δ_{sw} to observe the variation in δ_e and δ_{sw} under various environmental conditions. Under conditions studied so far we find that δ_e does not equal δ_{sw} even under steady state conditions. Future work will manipulate the parameters influencing δ_e, including temperature, relative humidity, and boundary layer conductance; attempting to minimize the difference between δ_e and δ_{ex} by bringing δ_e close to δ_{sw}.

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