

**2018 Annual Meeting  
Midwestern Section  
American Society of Plant Biologists**

**March 3 – 4, 2018  
Howe Hall  
Iowa State University  
Ames, Iowa**



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Stinziano JR, Morgan PB, Lynch DJ, Saathoff AJ, McDermitt DK, and Hanson DT. (2017) The rapid A-C<sub>i</sub> response: photosynthesis in the phenomic era. Plant, Cell & Environment, 40:1256-1262. doi: 10.1111/pce.12911



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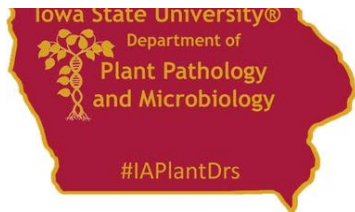
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## **American Society of Plant Biologists Midwestern Section**

### **Section Officers 2017 - 2018**

Chair	David Rosenthal (rosentha@ohio.edu) Ohio University
Vice Chair	Kathrin Schrick (kschrick@ksu.edu) Kansas State University
Secretary-Treasurer	Sen Subramanian (senthil.subramanian@sdsu.edu) South Dakota State University
Meeting Organizer	Olga Zabolina (zabolina@iastate.edu) Iowa State University
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Past Chair, ex officio	Gustavo MacIntosh (gustavo@iastate.edu) Iowa State University
Publications Manager	Jennifer Robison (jenrobis@uic.edu ) Indiana University Purdue University Indianapolis

### **Short Program**

#### **Saturday, March 3**

7:00 – 8:00 .....	Registration/Check-in/Poster Set-up/Breakfast
8:00 – 8:30 .....	Featured Speaker Dr. Patrick Schnable
8:30 – 10:00.....	Oral Session I (T1-T6)
10:00 – 11:30.....	Poster Session I (Even #s)/ Morning Refreshments
11:30 – 1:00.....	Oral Session II (T7-T12)
1:00 – 2:00.....	Lunch
2:00 – 2:30.....	Featured Speaker Dr. Harry Klee
2:30 – 4:00.....	Oral Session III (T13-T18)
4:00 – 4:15.....	Coffee Break
4:15 – 5:15.....	Keynote Speaker Dr. Ruth Welti
5:15 – 6:45.....	Poster Session II (Odd #s) / Appetizers
6:45 – 9:00.....	Banquet Dinner

#### **Sunday, March 4**

7:15 – 8:15 .....	Registration/Check-in/Career Panel Discussion/Breakfast
8:15 – 10:00.....	Oral Session IV (T19-T25)
10:00 – 10:30.....	Morning Refreshments/ Remove Posters
10:30 – 11:00.....	Featured Speaker Dr. Reuben Peters
11:00 – 12:15.....	Oral Session V (T26-T29)
12:15 – 1:00.....	Business Meeting, Announcements, Awards

#### **Meeting Locations..... (Howe Hall)**

Registration .....	<b>Howe Hall Atrium</b>
Saturday Featured and Oral Presentations .....	<b>Alliant Lee Auditorium</b>
Saturday Keynote Address .....	<b>Alliant Lee Auditorium</b>
Sunday Career Panel Discussion .....	<b>Alliant Lee Auditorium</b>
Sunday Featured, and Oral Presentations .....	<b>Alliant Lee Auditorium</b>

Poster Viewing .....	<b>Howe Hall Atrium</b>
Saturday Continental Breakfast .....	<b>Howe Hall Atrium</b>
Saturday, Lunch, Dinner Banquet .....	<b>Howe Hall Atrium</b>
Sunday Continental Breakfast .....	<b>Howe Hall Atrium</b>

*Vegetarian options will be available as part of all meals provided; however, preference will be given to individuals who requested vegetarian meals during meeting registration*

**Posters:** Posters need to be placed on poster boards between 7:00am and 9:30am on Saturday, March 3. Due to space limitations, poster measurements should be NO larger than 42 in. wide X 36 in. tall. Please bring a sufficient supply of pushpins to affix your poster to the poster board. Pins will be available on-site but in limited quantity. Posters need to be removed from poster boards on Sunday, March 4 between 10am and 12pm (noon). Any items remaining on display after 12pm on Sunday will be discarded.

Photographing, videotaping, or recording of any kind is prohibited (including but not limited to camera phones and digital devices).

**Notice to Oral Presenters:** The preferred format for presentations is PowerPoint. As oral presenters check in at registration, they will be directed to an area to upload a new version of presentation. Presenters are expected to provide their presentation a minimum of 1 hour prior to their oral session. This will help keep the session on schedule and allow each oral presenter the full amount of time to present. The presentation room will have a pc, projector, screen and remote mouse for your use.

# Schedule of Sessions and Speakers

Saturday, March 3

## Morning

- 7:00 – 8:00**    **Registration/Check-in/Poster Set-up/Continental Breakfast .....** Howe Hall Atrium
- 8:00**            **Welcoming Remarks .....** Alliant Lee Auditorium
- 8:05 – 8:30**    **Featured Speaker – Dr. Patrick Schnable, Iowa State University**  
*Community building for transformation science: A case study in predictive plant phenomics.*
- 8:30 – 10:00**   **Oral Session I..... Moderator – Dr. Diane Bassham, Iowa State University**
- 8:30**    **T1.**    Dissecting the metabolic and transcriptomic networks underlying the surface lipid metabolome on maize silks: The impact of genotype, environment, and silk development  
*Keting Chen, Roy J. Carver Dept. of Biochemistry, Biophysics and Molecular Biology, Iowa State University*
- 8:45**    **T2.**    Defining the functions of ORM proteins as regulators of sphingolipid metabolism  
*Ariadna Gonzalez-Solis, Dept. of Biochemistry, University of Nebraska, Lincoln*
- 9:00**    **T3.**    Interaction between brassinosteroids and TOR signaling regulates growth and stress responses in *Arabidopsis*  
*Ching-Yi Liao, Dept. of Genetics, Development and Cell Biology, Iowa State University*
- 9:15**    **T4.**    Novel roles of clathrin-coat components in plant immune signaling and development  
*Gayani Ekanayake, Dept. of Biochemistry, University of Missouri, Columbia*
- 9:30**    **T5.**    An insect inhibitor of apoptosis (SfIAP) interacts with SQUAMOSA promoter binding protein (SBP) transcription factors that exhibit pro-cell death characteristics  
*Ryan Kessens, Dept. of Plant Pathology, University of Wisconsin, Madison*
- 9:45**    **T6.**    Crystal structure of xyloglucan xylosyltransferase 1 reveals a mechanism for biologically observed patterns of xylosyl transfer during xyloglucan biosynthesis  
*Alan Culbertson, Roy J. Carver Dept. of Biochemistry, Biophysics and Molecular Biology, Iowa State University*
- 10:00 – 11:30**   **Poster Session I (Even Numbered Posters) and Refreshments .....** Howe Hall Atrium
- 11:30 – 1:00**    **Oral Session II..... Moderator – Dr. William Serson, Ave Maria University**
- 11:30**    **T7.**    Defining the circadian clock interactome using tandem affinity purification coupled with mass spectrometry  
*Maria Sorkin, Dept. of Biology, Washington University, St. Louis*
- 11:45**    **T8.**    The GmNAC42-1 transcription factor regulates the biosynthesis of glyceollin phytoalexins in soybean in response to abiotic and fungal elicitors  
*Md Asraf Jahan, Div. of Plant and Soil Sciences, West Virginia University*
- 12:00**    **T9.**    Transcriptome analysis of a very short root phenotype in wheat  
*Ghana Challa, Dept. of Biology and Microbiology, South Dakota State University*

- 12:15 T10.** Maize carbohydrate partitioning *defective33* functions in sucrose export from leaves  
*Thu Tran, Div. of Biological Sciences, Interdisciplinary Plant Group, University of Missouri, Columbia*
- 12:30 T11.** Using CRISPR/Cas9 genome editing technology to discover biological functions of plant cell wall proteins  
*Yuan Zhang, Dept. of Environmental and Plant Biology, Ohio University*
- 12:45 T12.** Utilizing CRISPR-Cas9 genome editing to improve agronomic traits of the oilseed-producing winter cover crop pennycress (*Thlaspi arvense*)  
*Malihe Esfahanian, School of Biological Sciences, Illinois State University*

***Lunch and Afternoon, March 3***

**1:00 – 2:00 Lunch ..... Howe Hall Atrium**

**2:00 Featured Speaker Dr. Harry Klee, Horticultural Sciences Dept., University of Florida  
The State of the ASPB Union**

**2:30 – 4:00 Oral Session III ..... Moderator – Dr. Emily Heaton, Iowa State University**

**2:30 T13.** The morphological and transcriptomic impact of silver quantum dots in *Arabidopsis thaliana* during early development  
*Natalie Smith, Dept. of Biology, Missouri State University*

**2:45 T14.** Induction of stress response, cell wall damage and cell death in determination of silver nanoparticle toxicity threshold of the heavy-metal accumulating fern, *Azolla caroliniana*  
*Shayla Gunn, Dept. of Plant Biology, Southern Illinois University*

**3:00 T15.** Sex-dependent variation of pumpkin (*Cucurbita maxima* cv Big Max) nectar and nectaries as determined by proteomics and metabolomics  
*Elizabeth Chatt, Roy J. Carver Dept. of Biochemistry, Biophysics and Molecular Biology, Iowa State University*

**3:15 T16.** The role of rolling leaves: Phenomic dissection of the interplay among drought responses across a diverse panel of 501 maize lines  
*Colton McNinch, Dept. of Molecular, Cellular and Developmental Biology, Iowa State University*

**3:30 T17.** Glandular trichome-derived terpenes of wild tomato accessions affect aphid performance and feeding behavior  
*Fumin Wang, Division of Plant and Soil Sciences, West Virginia University*

**3:45 T18.** Not another *Miscanthus × giganteus* nitrogen trial. It is a REPLAY  
*Mauricio Tejera, Dept. of Agronomy, Iowa State University*

**4:00 – 4:15 Break**

**4:15 – 5:15 KEYNOTE SPEAKER**

**Dr. Ruth Welti, Div. of Biology, Kansas State University**  
Using lipidomics to understand plant response to the environment

5:15 – 6:45 Poster Session II (Odd Numbered Posters) and Appetizers..... Howe Hall Atrium

Evening, March 3

6:45 – 9:00 Dinner Banquet and Networking.....Howe Hall Atrium

Sunday, March 4

Morning

7:15 – 8:15 Registration/Check-In/Continental Breakfast..... Howe Hall Atrium

7:15 – 8:15 Career Panel Discussion .....Alliant Lee Auditorium

8:15 – 9:45 Oral Session IV..... Moderator – Dr. Anthony Schmitt, *University of Minnesota*

8:15 T19. Drought stress activates NAC transcription factor RD26 through gsk3-like kinase BIN2 and protein phosphatase 2c ABI1 in *Arabidopsis*  
*Hao Jiang, Dept. of Genetics, Development and Cell Biology, Iowa State University*

8:30 T20. Antimicrobial lipid transfer proteins are a common feature of floral nectar  
*Anthony Schmitt, Dept. of Plant and Microbial Biology, University of Minnesota*

8:45 T21. Enhanced MALDI-Mass Spectrometry imaging of amino acids in maize root tissue through chemical derivatization  
*Kelly O'Neill, Dept. of Chemistry, Iowa State University*

9:00 T22. Functional characterization of compromised hydrolysis of triacylglycerol 7 (CHT7) protein and its CXC domain in *Chlamydomonas reinhardtii*  
*Nick Fekaris, Dept. of Biochemistry and Molecular Biology, Michigan State University*

9:15 T23. Characterization and mapping of the *suppressor of sessile spikelet 3 (sos3)* mutant which functions in paired spikelet development in maize  
*Amanda Blythe, Dept. of Biological Sciences, University of Missouri, Columbia*

9:30 T24. Understanding the metabolic flux of rhamnose in plant cells  
*Kyler Weingartner, Dept. of Biochemistry and Molecular Biophysics, Kansas State University*

9:45 T25. An acyl carrier protein mutant links auxin signaling and fatty acid biosynthesis  
*Clarissa Lewis, Dept. of Genetics, Development and Cell Biology, Iowa State University*

10:00 – 10:30 Refreshment Break ..... Howe Hall Atrium

10:30 – 11:00 Featured Speaker, Dr. Reuben Peters, *Iowa State University*  
From angströms to agriculture with diterpenoid natural products

11:00 – 12:15 Oral Session V ... .....Moderator – Dr. Raimund Nagel, *Iowa State University*

11:00 T26. A homeodomain transcription factor, its START domain and epidermal development in plants  
*Thiya Mukherjee, Div. of Plant Biology, Kansas State University*

11:15 T27. NEMATE, a Nectary-Enriched Multidrug and Toxic Extrusion protein involved in nectar



production in *Arabidopsis*

**Rahul Roy**, Dept. of Plant and Microbial Biology, University of Minnesota

**11:30 T28.** Type-I MADS-box transcription factors play a critical role during early seed development in Rice

**Puneet Paul**, Dept. of Agronomy and Horticulture, University of Nebraska

**11:45 T29.** An engineered novel plasma membrane protein provides broad-spectrum pathogen and pest immunity in soybean

**Micheline Ngaki**, Dept. of Agronomy, Iowa State University

**12:00 T30.** Traveling two diverging roads, cytochrome-P450 catalyzed demethylation and lactone formation in bacterial gibberellin biosynthesis

**Raimund Nagel**, Roy J. Carver Dept. of Biochemistry, Biophysics and Molecular Biology, Iowa State University

*Afternoon, March 4*

**12:15 – 1:00 Business Meeting, Announcements, Award Presentations .....Alliant Lee Auditorium**

**1:00 pm Meeting ends. Safe travels.**

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## Poster Titles

**P1.** Identification of Dynamic Transcriptional Regulators of Plant Disease Resistance, using the Barley-Powdery Mildew Pathosystem

**Valeria Velasquez-Zapata**, Bioinformatics and Computational Biology, Iowa State University

**P2.** Computational Classification of Phenologs Across Biological Diversity

**Ian Braun**, Bioinformatics and Computational Biology, Iowa State University

**P3.** Maize GO Annotation - Methods, Evaluation, and Review (maize-GAMER)

**Kokulapalan Wimalanathan**, Bioinformatics and Computational Biology, Iowa State University

**P4.** Effector Gene Expression Regulation by Small RNAs in Barley Powdery Mildew

**Mathew Hunt**, Dept. of Plant Pathology and Microbiology, Iowa State University

**P5.** Control of Quiescence and Cell Division by Compromised Hydrolysis of TAG 7 (CHT7) in

*Chlamydomonas reinhardtii*

**Tomomi Takeuchi**, DOE Plant Research Laboratory, Michigan State University

**P6.** Putative Plastid Rhomboid Protease Plays a Role in Phosphatidate Metabolism in *Arabidopsis thaliana*

**Anastasiya Lavell**, Dept. of Biochemistry and Molecular Biology, Michigan State University

**P7.** Exploring the Intracellular and Extracellular Lipid Metabolic Networks at the Level of Individual Cells

**Liza E. Alexander**, Dept. of Biochemistry, Biophysics, and Molecular Biology, Iowa State University

**P8.** Role of the *Agrobacterium* virulence effector protein VirE2 in modulating plant gene expression

**Rachelle Lapham**, Dept. of Biological Sciences, Purdue University

- P9.** Small RNA, DNA Methylation and Gene Expression in Soybeans with Copy Number Variation at Soybean Cyst Nematode Resistance Locus (*Rhg1*)  
*Usawadee Chaiprom, University of Illinois at Urbana-Champaign*
- P10.** The AP2/ERF Transcription Factor TINY Modulates Brassinosteroid-Regulated Plant Growth and Drought Response in *Arabidopsis*  
*Zhouli Xie, Dept. of Genetics, Development and Cell Biology, Iowa State University*
- P11.** Use of Ribosome Profiling to Decipher Translational Control of Gene Expression during the Unfolded Protein Response in Maize Roots  
*Pulkit Kanodia, Dept. of Plant Pathology and Microbiology, Iowa State University*
- P12.** Mechanism of cap-independent translation by maize chlorotic mottle virus: a step toward genome-engineered resistance  
*Elizabeth J. Carino, Dept. of Plant Pathology and Microbiology, Iowa State University*
- P13.** Dissecting the Subcellular Compartmentalization of Acetyl-CoA Metabolism in *Arabidopsis thaliana* Using Integrated Genetic and Metabolomics Approaches  
*Xinyu Fu, Roy J. Carver Dept. of Biochemistry, Biophysics, and Molecular Biology, Iowa State University*
- P14.** Fine Mapping of Metabolite-QTLs for Extracellular Surface Lipid Accumulation on Maize Silks  
*Tes Posekany, Interdepartmental Genetics and Genomics, Iowa State University*
- P15.** Reducing Seed Coat Fiber Content to Improve Seed Meal Nutritional Value of the Oilseed Crop Pennycress (*Thlaspi arvense*)  
*Taylor Suo, School of Biological Sciences, Illinois State University*
- P16.** Bulked Segregant - genotyping-by-sequencing: Cost-effective and background independent genetic mapping of mutants and QTL  
*Kokulapalan Wimalanathan, Dept. of Genetics Development and Cell Biology, Iowa State University*
- P17.** Deacclimation of cold-acclimated canola: GWAS and physiology  
*Jiaping Zhang, Sunflower and Plant Science Research Unit, USDA-ARS Red River Valley Agricultural Research Unit, Fargo, North Dakota*
- P18.** Gene Regulatory Network Analysis of NKD1, NKD2 and OPAQUE2 in Maize Endosperm Development  
*Hao Wu, Dept. of Genetics Development and Cell Biology, Iowa State University*
- P19.** CRISPR-Cas9 Ribonucleoprotein Complex Delivery in Plants for DNA Free Genome Editing  
*Raviraj Banakar, Dept. of Agronomy, Iowa State University*
- P20.** Soybean Aphids Exploit Soybean Abscisic Acid Signaling to Promote Susceptibility  
*Jessica Hohenstein, Roy J. Carver Dept. of Biochemistry, Biophysics, and Molecular Biology, Iowa State University*
- P21.** Increased Transpiration Is Correlated with Reduced Boron Deficiency Symptoms in the Maize *Tassel-less1* Mutant  
*Michaela Matthes, Div. of Biological Sciences, Interdisciplinary Plant Group, University of Missouri*

- P22.** *barren stalk3* is Required for Axillary Branch Development and Maps to the Same Location as *barren stalk2*.  
*Norman Best, Div. of Biological Sciences, Interdisciplinary Plant Group, University of Missouri*
- P23.** Utilization of a split-root system for controlled, reproducible imposition of water deficit on maize seedlings  
*Rachel Mertz, Div. of Biological Sciences, Interdisciplinary Plant Group, University of Missouri*
- P24.** Directed Evolutionary Study of Class I Diterpene Synthases  
*Meirong Jia, Roy J. Carver Dept. of Biochemistry, Biophysics, and Molecular Biology, Iowa State University*
- P25.** Biodiversity, Geographical Distribution and Phylogenetic Analysis of Geminivirus Associated Alpha-satellites from Cotton Crop in Pakistan  
*Muhammad Shafiq, Institute of Agricultural Sciences, University of Punjab, Quaid-e-Azam Campus, Lahore*
- P26.** Antifungal Plant Defensins: Mechanisms of Action and Engineering Disease Resistance  
*Dilip Shah, Donald Danforth Plant Sciences Center, St. Louis*
- P27.** Targeted subfield switchgrass integration could improve the farm economy, water quality, and bioenergy feedstock production  
*Emily Heaton, Department of Agronomy, Iowa State University*
- P28.** Pod Indehiscence – a Key Factor of Soybean Geo-climate Adaptation during Domestication  
*Jiaoping Zhang, Department of Agronomy, Iowa State University*
- P29.** CRISPR-based genome editing of grain size regulators in wheat  
*Wanlong Li, Dept. of Biology and Microbiology, South Dakota State University*
- P30.** Sequencing the sea wheatgrass genome and developing genome-specific markers to transfer biotic stress resistance and abiotic stress tolerance into wheat  
*Wanlong Li, Dept. of Biology and Microbiology, South Dakota State University*
- P31.** Uncovering the Genetic Regulation of Seed Amino acid Levels and Composition Using High-throughput Detection Method Combined with GWAS  
*Ruthie Angelovici, Div. of Biological Sciences, University of Missouri*
- P32.** Image-Based Analysis to Dissect Vertical Distribution and Horizontal Asymmetry of Conspecific Root System Interactions in Response to Planting Densities, Nutrients and Root Exudates in *Arabidopsis thaliana*  
*Jane Geisler-Lee, Depts. of Plant Biology and Computer Science, Southern Illinois University, Carbondale*
- P33.** Bidirectional Interaction between Plant Root Circadian Clock and Cyst Nematodes  
*Wei Wang, Dept. of Plant Pathology and Microbiology, Iowa State University*
- P34.** Stress Granules-mediated Translational Control of the Plant Immunity  
*Mian Zhou, Dept. of Plant Pathology and Microbiology, Iowa State University*
- P35.** Analysis of DNA Double Strand Breaks Induced by *Pseudomonas syringae* Virulence Factors  
*Andrew Russell, Northern State University, Aberdeen, SD*

- P36.** Role of Bacterial Volatiles in Plant Defense Signaling and Disease Resistance  
*Muthu Venkateshwaran, School of Agriculture, University of Wisconsin-Platteville*
- P37.** The Role of Hydroxyproline O-Arabinosylation in Flowering Plant Reproduction  
*Cora MacAlister, Cellular and Developmental Biology, University of Michigan, Ann Arbor*
- P38.** Integrated Multispectroscopic *In Situ* Imaging of Plant Metabolism at the Level of Subcellular Compartments  
*Geng Ding, Roy J. Carver Dept. of Biochemistry, Biophysics, and Molecular Biology, Iowa State University*
- P39.** Comparative functional genomics of nectaries and nectars in the dicots  
*Clay Carter, Dept. of Plant Microbial Biology, University of Minnesota*
- P40.** Exploring the synergistic bioactivity of major constituents in plant essential oils against *Aedes aegypti*  
*Maria Archevald-Cansobre, Pesticide Toxicology Laboratory, Dept. of Entomology, Iowa State University*
- P41.** Herbivore Derived Fatty Acid Amide Elicitors Induce a Reactive Oxygen Species Burst in Plants  
*Anna Block, Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, FL*
- P42.** Unveiling the Synergistic Effect of Pyramiding Rag1 and Rag2 Aphid-resistance Genes in Soybean  
*Martha Ibore, Roy J. Carver Dept. of Biochemistry, Biophysics, and Molecular Biology, Iowa State University*
- P43.** A High Oil Soybean Mutant Contains a 300 kb Deletion on Chromosome 14  
*William Serson, Dept. of Biology, Ave Maria University*
- P44.** Evaluating Loss of Motility in Tomato-Infecting Xanthomonas Strains  
*Tanvi Majumdar, Dept. of Natural Resources and Environmental Science, University of Illinois, Urbana-Champaign*
- P45.** Reverse Genetic Approaches to Understanding the Role of Auxin in Maize Development  
*Joseph Struttman, Interdisciplinary Plant Group, Div. of Biological Sciences, University of Missouri, Columbia*
- P46.** Investigating Catalytic Site of a Plastidial Putative Rhomboid Protease  
*Olivia Bayliss, Dept. of Biochemistry and Molecular Biology, Michigan State University*
- P47.** Analysis of COP9 Signalosome Mutants Reveals a Role of the CSN in Ethylene Sensitivity in *Arabidopsis thaliana*  
*Steven McKenzie, Cell and Molecular Biology, Grand Valley State University, Allendale, MI*
- P48.** Probing the Biochemistry of an Unusual Fatty Acid Desaturase  
*Montgomery Smith, Dept. of Biochemistry and Molecular Biology, Michigan State University*
- P49.** Natural variation in boron content in *Zea mays* and its implications on seedling development  
*Skyler Kramer, Dept. of Biochemistry and Div. of Biological Sciences, University of Missouri, Columbia*
- P50.** Modulation of GGPPS11 Phenotypes  
*Toria Trost, Dept. of Biological Sciences, Southern Illinois University, Edwardsville*

- P51.** Differential Expression of the Alfin Gene Family Members Among Maize Inbred Lines  
*Diane Janick-Buckner, Dept. of Biology, Truman State University, Kirksville, MO*
- P52.** Evolutionary History and Expression of the LONESOME HIGHWAY Transcription Factor Gene Family in Maize  
*Terra Willard, Dept. of Biology, Truman State University, Kirksville, MO*
- P53.** The Evolutionary History and Transcriptomic Analysis of the Phytoene Synthase Gene Family and Related Paralogs In Maize  
*Megan Neveau, Dept. of Biology, Truman State University, Kirksville, MO*
- P54.** Syn-tasiRNA: One more step forward to unraveling the biogenesis of 22nt tasiRNAs  
*Jennifer Probst, Missouri State University, Donald Danforth Plant Science Center, St. Louis*
- P55.** How does Early Kernel Growth and Development Relate to Mature Kernel Size: A Case Study using Krug Divergently Selected Maize  
*Devon Leroux, Department of Biology, Central Michigan University*
- P56.** The role of maize mutant Suppressor of sessile spikelets 2 (Sos2) in meristem maintenance  
*Katherine Guthrie, Div. of Biological Sciences, Interdisciplinary Plant Group, University of Missouri, Columbia*
- P57.** Discovering Freezing Tolerance Genes from the Native Orchid *Aplectrum hyemale*  
*Rasika Mudalige-Jayawickrama, Dept. of Natural and Applied Sciences, University of Dubuque, Dubuque, IA*
- P58.** Identifying Carbohydrate Partitioning Defective 28/47  
*Kyle Conner, Div. of Biological Sciences, Interdisciplinary Plant Group, University of Missouri, Columbia*
- P59.** Data Analysis of Maize Edited by CRISPR Systems  
*Jonah Miller, College of Agriculture and Life Sciences, Iowa State University*
- P60.** *Arabidopsis* Plants Expressing a Fungal Pectin Methyltransferase Exhibit Dwarfism and Resistance to Stresses  
*Lauran Chambers, Roy J. Carver Dept. of Biochemistry, Biophysics, and Molecular Biology, Iowa State University*
- P61.** Salicylic Acid-Induced Freezing Tolerance in Spinach (*Spinacia oleracea* L.) Leaves Explored through Metabolite Profiling  
*Kyungwon Min, Dept. of Horticulture, Iowa State University*
- P62.** Analyses of Berberine Bridge Enzyme-like Family Genes Potentially Involved in Leaf Development  
*Allison Newton, Dept. of Biological Sciences, Southern Illinois University, Edwardsville*
- P63.** Identification of *BBE*-like Double Mutants in *Arabidopsis thaliana*  
*Peyton Robinson, Dept. of Biological Sciences, Southern Illinois University, Edwardsville*
- P64.** Identification of the Causative Mutant Locus in *Gravity Persistent Signal 5*  
*Erica Periandri, Dept. of Biological Sciences, Southern Illinois University, Edwardsville*
- P65.** Meristem-specific Expression of *Geranylgeranyl Diphosphate Synthase 11 (GGPPS11)* to Rescue the *ggpps11-1* Mutant

*Tessa England, Dept. of Biological Sciences, Southern Illinois University, Edwardsville*

- P66.** Transformation rescue of *ggpps11-1* using *GGPPS8*  
*Lauren Davis, Dept. of Biological Sciences, Southern Illinois University, Edwardsville*
- P67.** Quantifying the Impact of Changes in Atmospheric Vapor Pressure Deficit on Maize and Soybean Yields  
*Kelsie Ferin, Dept. of Agronomy Iowa State University*
- P68.** Role of Lipid-Binding Proteins Involved in Lipid-Mediated Signaling of Abiotic Stress  
*Amanda Koenig, Genetics Program, Michigan State University*
- P69.** Nitrogen metabolism and nectar secretion in *Cucurbita pepo*  
*Erik Solhaug, College of Biological Sciences, University of Minnesota*
- P70.** Metabolite profiling of the terpenoid indole alkaloids in engineered *Catharanthus roseus* hairy root lines  
*Le Zhao, Chemical and Biological Engineering, Iowa State University*
- P71.** Plant Essential Oils Differentially Control Two-Spotted Spider Mites on Distinct Host Plant Species  
*Jacob Johnson, Dept. of Entomology, Iowa State University*
- P72.** Comparative Metabolite Profiling of Commercially Edible Peppers – An Insight into what Makes Peppers “Hot” or “Sweet”  
*Mark Heggen, W. M. Keck Metabolomics Research Laboratory, Iowa State University*
- P73.** Mapping loci that modify the efficacy of Teosinte crossing barrier 1  
*Merritt Burch, Dept. of Biology and Microbiology, South Dakota State University*
- P74.** Distinct Cell Type-Specific Auxin/Cytokinin Ratios in Soybean (*Glycine max*) Nodules  
*Paul Gaillard, Dept. of Agronomy, Horticulture and Plant Science, South Dakota State University*
- P75.** Seed Mass is More Influential than Simulated Climate Change on Biomass of American Chestnuts and Hybrids After One Season  
*Brett Fredericksen, Dept. of Environmental and Plant Biology, Ohio University, Athens*
- P76.** Structure, Function, and Protein-Protein Interactions of Xyloglucan Xylosyltransferase  
*Jacqueline Ehrlich, Roy J. Carver Dept. of Biochemistry, Biophysics and Molecular Biology, Iowa State University*
- P77.** Plant science learning activities for biomedical students.  
*Jessica Lucas, Dept. of Plant Biology, Southern Illinois University, Carbondale*
- P78.** Genetic Analysis of 5’-3’ Exoribonuclease (Xrn) Mutants in Alga *Chlamydomonas reinhardtii*  
*David Higgs, Biological Sciences, University of Wisconsin-Parkside, Kenosha*
- P79.** Optimization of the Enzymatic Synthesis of UDP-Xylose for the Study of Hemicelloses  
*Matt Cook, Roy J. Carver Dept. of Biochemistry, Biophysics and Molecular Biology, Iowa State University*
- P80.** Maize (*Zea mays*) Standing Variation Affects Sensitivity to Auxin Treatment  
*Jenna Bohler, Dept. of Biological Sciences, University of Missouri, Columbia*

- P81.** Hydrotropic Responses in Maize Primary Roots  
*Yafang Wang, Dept. of Biology and Microbiology, Southern Dakota State University*
- P82.** The silk surface lipid metabolome responds to abiotic stress and offers protection against desiccation  
*Bri Vidrine, Roy J. Carver Dept. of Biochemistry, Biophysics and Molecular Biology, Iowa State University*
- P83.** Study of the Xyloglucan Synthesizing Complex Formation  
*Kayla Uthe, Roy J. Carver Dept. of Biochemistry, Biophysics and Molecular Biology, Iowa State University*
- P84.** Identification of *Arabidopsis* Candidate Genes for Cold Stress Response Using a High Throughput Phenotyping System  
*Dipak Kumar Sahoo, Dept. of Agronomy, Iowa State University*
- P85.** Functional Characterization of Three Mitochondrial Acyl Carrier Protein Isoforms in *Arabidopsis thaliana*  
*Rachel Garlock, Roy J. Carver Dept. of Biochemistry, Biophysics and Molecular Biology, Iowa State University*
- P86.** Genome-Wide Association Study of Twelve Inflorescence Traits in the Sorghum Association Panel  
*Jacob Givens, Dept. of Agronomy and Horticulture, University of Nebraska, Lincoln*
- P87.** Determining the Effect of the *sbe1* Allele from *Z. mays parviglumis* on Maize Endosperm Starch Composition in an *ae1* Background  
*Prameela Awale, Dept. of Biology and Microbiology, South Dakota State University*
- P88.** Characterization and Genetic Mapping of the *carbohydrate partitioning defective60* mutant in maize  
*Singha Dhungana, Division of Biological Sciences, Interdisciplinary Plant Group, University of Missouri-Columbia*
- P89.** *bottomless* is a Novel Short Root Mutant Involved in Auxin and Nutrient Signaling  
*Yunting Pu, Dept. of Genetics, Development and Cell Biology, Iowa State University*
- P90.** *Arabidopsis* Aminotransferase ALD1 Site of Action in Plant Systemic Acquired Resistance  
*Shang-Chuan Jiang, Dept. of Molecular Genetics and Cell Biology, University of Chicago*
- P91.** Nodule Zone-Specific Gene Expression in Soybean  
*Sadikshya Aryal, Dept. of Agronomy, Horticulture and Plant Science, South Dakota State University*
- P92.** *Required for Mla Resistance 3*, Revisiting Our Old Friend *Sgt1*  
*Antony Chapman, Interdepartmental Genetics and Genomics Graduate Program, Iowa State University*
- P93.** Elucidating the roles of repressor ARFs by probing ARF and AuxRE interactions  
*Pratiksha KC, Dept. of Agronomy, Horticulture and Plant Science, South Dakota State University*
- P94.** Endoplasmic Reticulum Stress Tolerance Genes in *Arabidopsis*  
*Savannah Jones, Dept. of Genetics, Development and Cell Biology, Iowa State University*
- P95.** A start on understanding boron resistance in salt cedar  
*Lawrence Davis, Dept. of Biochemistry and Molecular Biophysics, Kansas State University*

- P96.** Role of Lipid Signaling in Plant Development  
*Briaunna Murray, Dept. of Biochemistry and Molecular Biology, Michigan State University*
- P97.** Characterization of Ammonium Transporters from the Model Liverwort *Marchantia polymorpha*  
*Tami McDonald, St. Catherine University, St. Paul, MN*
- P98.** Higher Order Knock-out Mutants of *Arabidopsis* Galactosyl-transferases: A Reverse Genetic Approach to Reveal Functional Roles of O-galactosylation of Arabinogalactan-Proteins (AGPs)  
*Dasmeet Kaur, Dept. of Environmental and Plant Biology, Ohio University*
- P99.** Auxin Mediates Cell-Cell Communication in Unicellular Microalgae, *Chlorella sorokiniana*  
*Jithesh Vijayan, Department of Agronomy and Horticulture, University of Nebraska, Lincoln*
- P100.** Impact of Future Climate on Soybean Breeding Objectives  
*Theodore Hartman, Dept. of Agronomy, Iowa State University*
- P101.** Unraveling the Metabolic and Biological Importance of Sphingolipid <sup>[L]</sup><sub>SEP</sub> Long-Chain Base  $\Delta 4$  Unsaturation in Plants  
*Dongdong Zhang, Dept. of Biochemistry, University of Nebraska, Lincoln*
- P102.** RhizoDive: A education cum research pipeline on rhizobial biodiversity  
*Jesus Loya, Dept. of Agronomy, Horticulture and Plant Science, South Dakota State University*
- P103** Investigating a chromatin-based model for cell cycle regulation by the retinoblastoma complex in *Chlamydomonas*  
*Yi-Hsiang Chou, Donald Danforth Plant Science Center*
- P104** Characterization of xylan synthase complexes (XSCs) in rice cultivar (*Oryza sativa ssp japonica*)  
*Tasleem Javaid, Dept. of Environmental and Plant Biology, Ohio University*
- P105** A Novel Maize Glycosyltransferase is Required for Carbon Export from Source Tissues  
*Tyler McCubbin, Division of Plant Sciences, University of Missouri*
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# Abstracts of Presentations

## Oral Presentations

### **T1. Dissecting the metabolic and transcriptomic networks underlying the surface lipid metabolome on maize silks: The impact of genotype, environment, and silk development.**

**Keting Chen**, *Iowa State University, Ames, IA, USA*

The plant cuticle is infused with and coated by non-polar and amphipathic lipids that form a hydrophobic layer that is protective against environmental stresses. These extracellular surface lipids (SLs) are comprised primarily of long-chain saturated and unsaturated fatty acids, aldehydes, and hydrocarbons, which are metabolically linked by enzymatic reactions as the hypothesized precursors, intermediates, and end products in hydrocarbon biosynthesis. To investigate this biosynthetic pathway, we employed a systems approach to query the metabolomes and transcriptomes of silks from four genotypes (B73, Mo17 and their reciprocal hybrids) across a spatio-temporal gradient that captures acropetal silk development and the environmental transition as silks emerge from the husks. Supervised and un-supervised network analyses were pursued to address key questions: 1) Which metabolites explain the dynamic variations in SL composition? 2) Which enzymatic processes lead to variation in these metabolites? and 3) What genes explain the differential metabolome compositions? Our results show that silk SL composition is dynamic and significantly impacted by encasement status, genotype, and development. Discriminant analysis revealed that differential utilization of fatty acid precursors likely contributes to the observed variation in hydrocarbon composition among genotypes. Product-precursor ratio investigations showed that hydrocarbon abundances are elevated relative to their associated fatty acid precursors at longer chain-lengths, suggesting increased recruitment of longer-chain fatty acid precursors into the biosynthetic pathway. Metabolome-transcriptome associations impacting hydrocarbon production under varied conditions were identified from a partial least squares regression model built from a set of informative metabolites. Preliminary analysis identified candidate genes associated with genotype-based variation in the metabolic network, including 3-ketoacyl-CoA synthases involved in generating fatty acid precursors, and acyl desaturases involved in production of unsaturated SLs. Analyses are being conducted to interrogate the transcriptome in the context of product-precursor, product-intermediate and intermediate-precursor relationships to identify candidate genes associated with specific biochemical reactions in the network.

### **T2. Defining the Functions of ORM Proteins as Regulators of Sphingolipid Metabolism**

**Ariadna Gonzalez-Solis**, *University of Nebraska-Lincoln, Lincoln, NE, USA*

Orosomucoid-like proteins or ORMs contribute to sphingolipid homeostatic maintenance in eukaryotic cells by functioning as negative regulators of serine palmitoyltransferase (SPT), the first enzyme in sphingolipid long-chain base (LCB) biosynthesis. The model plant *Arabidopsis thaliana* (*Arabidopsis*) provides an excellent system for characterization of ORM function in multicellular eukaryotes due to its ease of genetic manipulation. CRISPR-Cas9 knockout mutants were generated for each of the two *Arabidopsis* ORM genes (ORM1, ORM2) as well as knockouts of both genes. Mutants of ORM1 or ORM2 displayed no obvious phenotypes, suggesting that these genes are functionally redundant. However, homozygous double mutants (*orm1orm2*) recovered on tissue culture media were strongly impaired in development and organ differentiation and did not advance beyond the seedling stage of growth. Sphingolipid profiling revealed 100-fold increases in ceramide accumulation in *orm1orm2* mutants relative to wild-type seedlings, including ceramides with C16 fatty acids, principally derived from the LOH2 ceramide synthase. Consistent with ceramide accumulation, expression of genes for the three functionally

distinct ceramide synthases were upregulated in *orm1orm2* seedlings. These findings suggest that ORMs are essential for growth and differentiation of multi-organellar eukaryotes, and that SPT and ceramide synthases are coordinately regulated for maintenance of sphingolipid homeostasis.

### **T3. Interaction between Brassinosteroids and TOR Signaling Regulates Growth and Stress Responses in *Arabidopsis***

**Ching-Yi**, *Iowa State University, Ames, IA, USA*

Brassinosteroids (BRs) are plant steroid hormones that regulate plant growth, development and stress responses. BRs signal through receptors and several intermediates to inhibit the activity of BRASSINOSTEROID-INSENSITIVE 2 (BIN2), a GSK3-like kinase that negatively regulates transcription factors to control growth and stress responses. Autophagy is a major pathway for degradation and recycling of cellular components. Autophagy is active at very low levels under normal growth conditions and is highly upregulated in response to a variety of stress conditions. The Target of Rapamycin (TOR) protein kinase is an essential positive regulator of growth and a negative regulator of autophagy. In this study, we found that the activity of the TOR signaling pathway is regulated by BRs, and that TOR is phosphorylated by BIN2, revealing a previously unknown mechanism of interaction between the BR and TOR signaling pathways in the control of plant growth and stress responses. We are now analyzing potential autophagy phenotypes of BIN2 mutants, investigating the effect of BIN2 phosphorylation on TOR function, and studying global TOR-regulated phosphorylation in regulating growth and autophagy.

### **T4. Novel Roles of Clathrin-Coat Components in Plant Immune Signaling and Development**

**Gayani Ekanayake**, *University of Missouri Columbia, Columbia, MO, USA*

Vesicular trafficking proteins have emerged as crucial regulators of plant immunity in model and crop species. To understand the role of vesicular trafficking in plant innate immunity, our lab utilizes the FLS2-flagellin model system, in which the plant receptor Flagellin Sensing 2 (FLS2) perceives bacterial flagellin (or flg22) to induce immune responses. Ligand-induced endocytosis of FLS2 is important to desensitize cells to flg22 and remove the activated receptor from the plasma membrane (PM), likely to attenuate signaling. In eukaryotes, dynamins and a subset of dynamin-related proteins (DRPs) are large GTPases that act as molecular scissors during clathrin-mediated endocytosis. Previously, we identified *Arabidopsis* DRP2B as a non-canonical regulator of flg22-signaling and immunity against the hemibiotrophic bacteria *Pseudomonas syringae* pv. tomato (Pto) DC3000. Loss of DRP2B causes 20% decrease of flg22-induced endocytosis of FLS2; and the resulting delay in removal of FLS2 from the PM correlates with increased early flg22-signaling. To delineate the dynamin-related protein network functioning with DRP2B at the PM, we identified proteins, including Vesicular Trafficking 3 (VES3), that co-immunoprecipitated with DRP2B. Loss of VES3 resulted in similar non-canonical flg22-signaling defects as observed for *drp2b* and an about 70% decrease of flg22-induced endocytosis of FLS2. These results indicate that these two vesicle proteins regulate flg22-signaling and trafficking responses in a similar manner. *drp2b ves3* double mutants showed synergistic defects in both plant immune signaling and development consistent with altered accumulation of various PM proteins with roles in immunity and development. Importantly, we were able to uncouple the requirement of DRP2B and VES3 for immune signaling from development. We are currently utilizing the *drp2b ves3* double mutant in proteomics and live-cell imaging studies to examine the synergistic roles of VES3 and DRP2B in constitutive and/or ligand-induced endocytosis of PM proteins other than FLS2.

### **T5. An insect inhibitor of apoptosis (SfiAP) interacts with SQUAMOSA promoter binding protein (SBP) transcription factors that exhibit pro-cell death characteristics**

**Ryan Kessens**, *University of Wisconsin-Madison, Madison, WI, USA*

Our understanding of programmed cell death (PCD) regulation in plants is limited despite its important role in stress tolerance. This is largely due to the absence of conserved animal PCD regulators in plant genomes. Nevertheless, numerous studies have shown that the ectopic expression of animal anti-PCD regulators in plants can suppress cell death in response to many stresses. Specifically, an insect inhibitor of apoptosis (SfIAP) suppresses cell death induced by the mycotoxin fumonisin B1, the necrotrophic fungal pathogen *Alternaria alternata*, and abiotic stress when expressed in tomato. We hypothesize that SfIAP is inhibiting the activity of endogenous pro-death regulators in tomato. However, a biochemical mechanism by which SfIAP functions in plants is still lacking. To address this deficiency, we sought to identify SfIAP-interacting partners from tomato using a yeast two-hybrid assay. Several transcription factors in the SQUAMOSA promoter binding protein (SBP) family were identified as potential binding partners. We confirmed this interaction in vivo for our top two interactors, SlySBP8b and SlySBP12a, using coimmunoprecipitation. Overexpression of SlySBP8b and -12a in *N. benthamiana* leaves induced tissue death characterized by the accumulation of reactive oxygen species. Additionally, the growth of two necrotrophic fungal pathogens, *Sclerotinia sclerotiorum*; and *A. alternata*; was enhanced in leaves overexpressing SlySBP8a and -12a. Fluorescence microscopy confirmed the nuclear localization of both SlySBP8b and -12a, which we show is required for cell death induction by these transcription factors. SlySBP12a is also present at the ER membrane and deleting a putative transmembrane domain from SlySBP12a resulted in complete nuclear localization. These results support a pro-death role for SlySBP8b and -12a and suggest ER membrane tethering as a means of regulating SlySBP12a activity.

## **T6. Crystal Structure of Xyloglucan Xylosyltransferase 1 Reveals a Mechanism for Biologically Observed Patterns of Xylosyl Transfer During Xyloglucan Biosynthesis**

**Alan Culbertson**, *Iowa State University, Ames, IA, USA*

The plant cell wall is a complex network composed mainly of polysaccharides including cellulose, hemicellulose, and pectins. Biosynthesis of these biopolymers is poorly understood, largely due to difficulties in the structural characterization of glycosyltransferases and to the lack of suitable substrates for in vitro analysis. The dearth of structural information for enzymes involved in plant cell-wall polysaccharide biosynthesis impedes the development of more resilient plants better suited for numerous industrial applications. Xyloglucan is the most abundant hemicellulose in most plants and is composed of a glucan chain with numerous side chain decorations. Xyloglucan xylosyltransferases (XXTs) initiate side-chain extensions from a linear glucan polymer by transferring the xylosyl group from UDP-xylose during xyloglucan biosynthesis. Presented here is the structure of Arabidopsis XXT1 without ligands and in complexes with UDP and celohexaose. XXT1, a homodimer and member of the GT-A fold family of glycosyltransferases, binds UDP analogously to other GT-A fold enzymes. Recognition of the glucan chain by XXT1 involves an extended cleft that is distinct from other systems. Based on steric constraints in the extended cleft of the crystal structure of XXT1 and homology models of other XXTs, we were able to elucidate their specificity for an acceptor substrate and demonstrate the requirement for an assembly of the three XXTs to produce the xylosylation patterns of native xyloglucans.

## **T7. Defining the Circadian Clock Interactome Using Tandem Affinity Purification Coupled with Mass Spectrometry**

**Maria Sorkin**, *University of Nebraska-Lincoln, Lincoln, NE, USA*

The circadian clock is an endogenous timekeeper that integrates daily and seasonal cues to coordinate physiological responses to the environment. In plants, the clock is composed of a complex network of transcriptional and translational feedback loops involving numerous molecular components. Identification of these components is a critical first step for future research projects that seek to manipulate circadian-associated agricultural traits such as flowering time and development. In the model organism Arabidopsis

*thaliana*, research continues to identify the protein components that make up the molecular machinery responsible for 1) sensing environmental inputs, 2) maintaining the core clock mechanism, and 3) coordinating the appropriate output response. We have developed epitope-tagged versions of several core clock proteins for tandem affinity purification coupled with mass spectrometry (TAP-MS) for protein identification. These reagents have allowed us to identify new protein interactions associated with the circadian clock. We have demonstrated the efficacy of this approach by using TAP-MS to define the interactome of the *A. thaliana* Evening Complex (EC), which contains ELF3 (Early Flowering 3), ELF4 (Early Flowering 4), and LUX (LUX Arrhythmo). We identified novel interactions between the EC—a core component of the circadian clock—and proteins involved in both light signaling input pathways and putative output response pathways. Our current efforts are to identify key interacting proteins of the remaining clock genes, beginning with the morning-phased proteins CCA1 (Circadian Clock Associated 1) and LHY (Late Elongated Hypocotyl). Ultimately, we will integrate TAP-MS datasets for all the core clock genes to establish a protein interactome that defines a full 24-hour turn of the clock. We anticipate that these discoveries will provide key insights into the molecular mechanisms that underlie the pervasive regulation of physiology and development by the circadian clock.

#### **T8. The GmNAC42-1 Transcription Factor Regulates the Biosynthesis of Glyceollin Phytoalexins in Soybean in Response to Abiotic and Fungal Elicitors**

**Md Asraful Jahan**, *West Virginia University, Morgantown, WV, USA*

Glyceollins phytoalexins are inducible secondary metabolites belong to isoflavonoid family of molecules that accumulate in soybean in response to many pathogenic and abiotic factors like, UV irradiation, metals and jasmonate. Phytophthora root and stem rot caused by *P. sojae* is a destructive disease throughout the soybean-growing regions worldwide causing devastating economic damages (1-2 billion dollars) globally every year and aggression of this disease can be restrain by enhancing the accumulation of glyceollins in soybeans. Transcription factors (TFs) activate the gene expression for the induction of phytoalexins which may play important role in the induction of glyceollins. The NAC proteins belong to plant-specific TFs and play important role in regulating plant growth and development processes, responses to abiotic stresses. A little is known how abiotic elicitors affect elicitation of glyceollins compared to biotic elicitors. We treated soybean seedlings with a panel of abiotic stresses and identified low pH as a novel elicitor and dehydration as a suppressor leading to the inductive and repressive production of glyceollins, respectively. We isolated, identified and functionally characterized an abiotic stress responsive NAC that regulates glyceollins biosynthesis. RNA-seq data showed that NAC is highly upregulated in acidic medium treated seedlings, wall glucan elicitor from *P. sojae* treated hairy roots and seeds, and down-regulated in dehydrated tissues. Acidic medium induced and dehydration suppressed glyceollins and transcripts level of glyceollin genes. Overexpression and RNAi silencing of NAC42-1 in soybean hairy roots resulted in a remarkable increase and decrease of the accumulation of glyceollins, respectively. The qRT-PCR results showed that overexpression and knockdown of NAC42-1 resulted in a significant increase transcript levels of IFS1, IFS2 and decrease level of G4DT, IFS2. These results clearly indicated that NAC42-1 regulates the induction glyceollins in soybean by transcriptional regulation of glyceollins genes and plays a pivotal role in the elicitation of glyceollins.

#### **T9. Transcriptome Analysis of a Very Short Root Phenotype in Wheat**

**Ghama Challa**, *South Dakota State University, Vermillion, ND, USA*

Roots play an important role in plant growth, development and stress perception. Recently we identified a very short root (VSR) phenotype in an F1 hybrid between Chinese spring and synthetic wheat accession TA4152-71. Root growth virtually stops 3 d after germination in the VSR seedlings. Genetic analysis indicated that the VSR locus is located on the chromosome arm 5DL and the phenotype was a result of the

non-additive interaction of the CS (Vsr1a) and synthetic wheat (Vsr1b) alleles in the Vsr1 locus. RNA-seq analysis of the root tip transcriptomes from near-isogenic lines (NILs) of long and short root was carried out using the IWGSC RefSeq 1.0 as the reference genome. Of the up-regulated genes in the VSR root tips, many are involved in defense response, abiotic stress responses, lignin biosynthesis, calcium signaling, and autophagy. Besides these, negative regulators of cell proliferation like an E3 ubiquitin ligase BIG BROTHER and of root proliferation like the receptor-like kinase FERONIA and HERCULES were also up-regulated. Sequencing of small RNA from long and VSR root tips identified miR159, a negative regulator of ABA signaling was up-regulated and members of the miR319 family, involved in positive regulation of cell proliferation were either absent or significantly down-regulated in VSR. DAB staining of the root tips detected the increased accumulation of ROS in VSR, especially in the elongation zone. In conclusion, the VSR phenotype could be a result of repressed cell proliferation by ROS accumulation and reduced the cell elasticity by ectopic lignin deposition

#### **T10. Maize Carbohydrate Partitioning Defective33 Functions in Sucrose Export from Leaves**

**Thu Tran**, *University of Missouri, Columbia, MO, USA*

To sustain plant growth, development, and ultimately crop yield, sucrose must be transported from its site of synthesis in leaves to distant parts of the plant, such as seeds or roots. Yet we know little about the genes controlling carbohydrate distribution in plants. Here we discuss our exciting discovery of a gene impacting sucrose export from maize leaves. Carbohydrate partitioning defective33 (cpd33) is a recessive mutant, which accumulates excess starch and soluble sugars in the mature leaves. Additionally, cpd33 mutants exhibit chlorosis in the leaf blades, greatly diminished plant growth, and reduced fertility. Furthermore, application of radioactively labeled F18-sucrose to cpd33 mutant and wild-type leaves showed that sucrose export was greatly decreased in cpd33 mutant leaves compared with wild type. The Cpd33 gene has been cloned by genetic fine-mapping and whole genome sequencing experiments, and its identity confirmed through characterizing multiple mutant alleles. The Cpd33 gene encodes an evolutionarily conserved plant-specific protein predicted to contain multiple transmembrane domains. In tobacco leaves, a CPD33-yellow fluorescent protein translational fusion protein is associated with the plasma membrane; however, the signal appears discontinuously along the membrane, suggesting that CPD33 is localized at plasmodesmata. Based on these results, we propose that CPD33 functions to control sucrose export from leaves through regulating cell-to-cell transport through plasmodesmata. Our ongoing work utilizes molecular approaches in combination with imaging techniques to test models of CPD33 function. This research reveals a new gene involved in sucrose export and deepens our understanding of the control of carbohydrate partitioning.

#### **T11. Using CRISPR/Cas9 Genome Editing Technology to Discover Biological Functions of Plant Cell Wall Proteins**

**Yuan Zhang**, *Ohio University, Athens, OH, USA*

Arabinogalactan-proteins (AGPs) are a diverse family of hydroxyproline-rich glycoproteins implicated to function in a number of physiological processes including growth, development, cellular signaling, somatic embryogenesis, programmed cell death, and wounding. AGPs are known for the diversity of sugars present on their molecular surface. Addition of the various sugars to AGPs requires the action of a large number of distinct enzymes, called glycosyltransferases (GTs). Due to gene redundancy in the GT families, a comprehensive understanding of functions of the diverse sugars decorating the AGP protein core requires that higher order genetic mutants need to be produced in order to substantially or completely eliminate addition of a particular sugar residue and observe its functional consequences. This study focuses on the generation of higher order mutants for two distinct GT families, the galactosyltransferases (GALTs) and the glucuronic acid transferases (GlcATs), using CRISPR/Cas9. GALTs are enzymes that the first sugar, galactose, onto the AGP protein core in Arabidopsis. To date, eight genes (GALT2-6 and HPGT1-3)

encode GALTs acting on AGPs. Moreover, three genes (GlcAT14A, GlcAT14B, and GlcAT14c) are responsible for adding the sugar glucuronic acid to AGPs in Arabidopsis. Multiplexing guide RNA (gRNA) expressions of GALTs are being achieved by using the glycine tRNA- gRNA (PTG) construct, where each gRNA is flanked by tRNA sequences. When this construct is expressed, eukaryotic RNase P and Z recognize each tRNA sequence, cleave and release the gRNA. Multiple gene knock-outs for GlcAT family are being generated by using the Arabidopsis U6 promoter and U6 terminator for each gRNA. Higher order genetic mutants of GTs generated in this project will determine the functional importance of the galactose sugars (added by these GALTs) and the glucuronic acid sugars (added by the GlcATs), thus elucidating sugar structure/function relationships for the AGPs. This research provides a simpler and faster way to generate higher order mutants for functional characterization. The CRISPR/Cas9 mediated multiplexing technique is especially useful for editing multi-gene families with members having redundant functions such as the GT families and other cell wall gene families.

#### **T12. Utilizing CRISPR-Cas9 genome editing to improve agronomic traits of the oilseed-producing winter cover crop pennycress (*Thlaspi arvense*)**

**Malihe Esfahanian**, *Illinois State University, Normal, IL, USA*

Pennycress (*Thlaspi arvense*) is an emerging oilseed crop closely related to Arabidopsis and rapeseed canola that holds considerable agronomic and economic potential in producing seed oil and meal to be used as food, feed, and as a biofuels feedstock. Pennycress possesses a unique combination of attributes including extreme cold tolerance, rapid growth, over-wintering growth habit, and a natural ability to produce copious amounts of seeds high in oil and protein. Pennycress could generate billions of liters of oil annually throughout temperate regions of the world without displacing food crops or requiring land use changes. For example, pennycress can be grown throughout the 40 million-acre U.S. Midwest Corn Belt during the fall through spring months, double-cropped between corn and soybeans on otherwise fallow farmland thereby providing ecosystem services of erosion and nutrients runoff control. Post oil extraction, the pennycress seed meal can be used as a high protein, nutrient-filled animal feed. Being that current pennycress varieties are not far removed from wild strains, we are working to rapidly improve breeding-line agronomic traits such as seed dormancy, pod shatter, seed oil and meal quality, and time to maturity, by using both forward and reverse genetics approaches. This presentation will highlight our efforts in using CRISPR-Cas9 genome editing tools to rapidly improve pennycress as a profitable oilseed-producing winter cover crop, employing knowledge gained from decades of research on Arabidopsis and other Brassicaceae.

#### **T13. The Morphological and Transcriptomic Impact of Silver Quantum Dots in Arabidopsis thaliana during Early Development**

**Natalie Smith**, *Missouri State University, Springfield, MO, USA*

In the past decade, the use of engineered nanomaterials (ENMs) has exponentially increased, but their ecological impact remains poorly understood. The specific impact of ENMs in plants is especially concerning because plants represent a likely route through which ENMs can enter the food chain and accumulate in higher biological systems. A phenology- and gene expression-based bioassay, funded by a government agency, was adopted to determine the phytotoxicity of ENMs in plants. The assay utilizes Arabidopsis thaliana plants exposed to low concentrations of silver quantum dots (AgQDs) from germination through 14 days of growth. Phenology data across a concentration gradient was collected to determine toxicity threshold, so gene expression experiments could be performed at sub-lethal concentrations. Gene expression experiments involved RNA-seq and RT-qPCR validation. Phenology data showed a phenotype that has not before been reported in association with AgQD exposure. The rosette leaves of Arabidopsis grown in the presence of sub-lethal concentrations of AgQDs showed a translucent green phenotype. This phenotype has been previously described by others to have association with the

transcription factor gene Translucent Green (AT1G36060) and aquaporin gene TIP 1-1 (AT2G36830). RNA-seq analysis revealed 674 significantly differently expressed genes in response to AgQDs, but did not include Translucent Green and TIP 1-1. The gene expression profile in AgQD-treated plants was similar to, but distinguishable from a pathogen-triggered transcriptome. RT-qPCR on 14 genes validated the RNA-seq results. Currently, RT-qPCR experiments are attempting to clarify the involvement of these genes with the translucent green phenotype. If Translucent Green and TIP 1-1 are not involved with the translucent green phenotype in AgQD-exposed plants, further experiments will need to be performed to determine its cause.

**T14. Induction of Stress Response, Cell Wall Damage and Cell Death in Determination of Silver Nanoparticle Toxicity Threshold of the Heavy-metal Accumulating Fern *Azolla caroliniana***  
**Shayla Gunn, Southern Illinois University-Carbondale, Carbondale, IL, USA**

The field of nanoecotoxicology has been pioneered in recent years as concern grows in response to the potential environmental hazards of engineered nanoparticle release. Studies have investigated the plausible routes of silver nanoparticles (AgNPs) to reach aquatic systems and their biological impacts, but none have investigated the potential remediation of these waters using the heavy metal accumulating fern *Azolla caroliniana*. This study employed biological staining techniques implemented in fluorescence microscopy to identify various stress responses of *A. caroliniana* roots to assess the capability of this plant to withstand AgNP exposure. Two concentrations series were applied, 0-1.0ppm and 0-10.0ppm for 1, 3, 5 days after transfer (DAT). Oxidative stress, measured in production of ROS, increased in a dose-dependent manner. Callose (1,3- $\beta$ -glucan) is deposited in response to cell wall damage and was observed elevated in a dose-dependent manner. Cell vitality appeared from a general decline in nucleic content to nuclei lysis. Response to AgNPs was observed at 1 DAT but recovery could be seen at 3~5 DAT. In sum, these data were imperative to suggest the toxicity threshold of 1.0ppm at which *A. caroliniana* roots can mediate exposure.

**T15. Sex-dependent Variation of Pumpkin (*Cucurbita maxima* cv Big Max) Nectar and Nectaries as Determined by Proteomics and Metabolomics**  
**Elizabeth Chatt, Iowa State University, Ames, IA, USA**

Nectar is a floral reward that sustains mutualisms with pollinators, which in turn, improves fruit set. While it is known that nectar is a chemically complex solution, extensive identification and quantification of this complexity has been lacking. *Cucurbita maxima* c.v. Big Max, like many cucurbits, is monoecious with separate male and female flowers. Attraction of bees to the flowers through the reward of nectar is essential for reproductive success in this economically valuable crop. In this study, the sex-dependent variation in composition of male and female nectar and the nectary were defined using a combination of GC-MS based metabolomics and LC-MS/MS based proteomics. Metabolomics analysis of nectar detected 88 metabolites, of which 40 were positively identified, and included sugars, sugar alcohols, aromatics, diols, organic acids, and amino acids. There were differences in 29 metabolites between male and female nectar. The nectar proteome consisted of 46 proteins, of which 70% overlapped between nectar types. Only two proteins were unique to female nectar, compared to 11 specific to male nectar. The nectary proteome, defined using iTRAQ labeling, was composed of 339 proteins, 71% of which were descriptively annotatable by homology to Plantae. The abundance of 45 proteins differed significantly between male and female nectaries. This rich dataset significantly expands the known complexity of nectar composition.

**T16. The Role of Rolling Leaves: Phenomic Dissection of the Interplay Among Drought Responses Across a Diverse Panel of 501 Maize Lines**  
**Colton McNinch, Iowa State University, Ames, IA, USA**

Leaf rolling occurs widely in grasses as a response to water-deficit and is particularly dramatic in maize.

The response is mechanically mediated by the contraction of bulliform cells, specialized and longitudinally arrayed cells that act akin to water reservoirs in the adaxial epidermis of the leaf. Rolling protects the plant by reducing water loss from leaves and by allowing reallocation of water from storage and photosynthesis to growth and development. However, a plant with rolled leaves has depleted reserves and diminished productive capacity. Thus, rolling can be viewed alternatively as a measure of resilience or distress. Our broad goal is to reveal the structure-function biology underlying the economization of water during deficits at critical junctures of maize development. Understanding the dynamics of the leaf rolling response in the context of known drought responses such as stunting and male-female flowering asynchrony is a critical first step. Here we report findings from a field-based study of 501 diverse maize isolines under drought versus well-watered conditions. Leaf rolling observations from a 17-day period of water stress were leveraged to identify low-, medium- and high-rolling sets of lines suitable for further phenomic analysis of shoot and root traits. Several key findings have emerged: (1) floral transition timing relative to the onset of drought stress is a strong driver of rolling severity, demonstrating both the prioritization and high water demand imposed by reproductive development; (2) several morphometric shoot and root traits are clear predictors of rolling severity, providing functional insights; (3) leaf rolling can indeed be a sign of resilience or distress, suggesting that further investigation of the capacities and architectures of the reservoir system will be essential for clarifying the interplay between leaf rolling and stress outcomes such as stunting, female flowering delay, and reduced grain yield.

#### **T17. Glandular trichome-derived terpenes of wild tomato accessions affect aphid performance and feeding behavior**

**Fumin Wang**, *West Virginia University, Morgantown, WV, USA*

Piercing-sucking pests such as aphids pose a serious problem in the commercial production of horticultural crops including tomato, since damage is caused by direct feeding, and transmission of viruses for which these herbivores serve as vectors. Current control strategies involving synthetic insecticides are increasingly considered problematic due to emerging resistances, costs for growers, and concerns of consumers, highlighting the need to develop new efficient and sustainable approaches. Recent studies of terpene production in glandular trichomes of tomato, found on leaves and stems, and known to be involved in plant-insect interactions, demonstrated significant differences between cultivated and wild tomato, as well as quantitative and qualitative variation among wild tomato accessions. Since these wild accessions are likely good sources of defensive traits against aphids, we have performed non-choice assays to compare the development of *Macrosiphum euphorbiae* (Hemiptera: Aphidae) on leaves of cultivated tomato (*Solanum lycopersicum*) and multiple *S. habrochaites* accessions representing the available chemical diversity. Fecundity, longevity and intrinsic growth rate of aphids was found to be significantly lower on some wild accessions. Moreover, we analyzed aphid feeding on artificial media containing leaf extracts with trichome derived terpenes from cultivated and wild tomato accessions. Our analysis of aphid developmental parameters, accumulation of salivary sheath and honeydew production of aphids suggest that trichome derived terpenes of some wild accessions influence aphid performance by potentially affecting their feeding behavior. We are now performing olfactometer choice assays to study the effect of terpenes emitted from tomato accessions on the pre-alighting search behavior of alatae aphids. Some wild accessions were repellent and their extracts significantly reduced the attractiveness of cultivated tomato plants to aphids.

#### **T18. Not Another *Miscanthus* × *giganteus* Nitrogen Trial. It is a REPLAY**

**Mauricio Tejera**, *Iowa State University, Ames, IA, USA*

Perennial crops have been widely studied in the last few decades given their potential for use as energy and fiber sources while providing greater environmental services (e.g. lower fertilizer and pesticide



requirements, soil carbon storage, and biodiversity). However, field research and data analysis on perennials have remained unchanged and ignored important sources of variability. Perennial field research is based on a single cohort established under certain, usually uncontrolled, conditions and studied over time. While convenient and widely used, this design lacks statistical power to separate the environmental effects on plants during the growing season from the stand age effect. This could result in misleading temporal dynamics of the crop and inconclusive long-term assessments of its productivity. In addition, since results are based on a single cohort, this design is not able to estimate the variability generated by planting year conditions (e.g. environment, management) therefore, conclusions should be restricted to similar environmental conditions. Using the bioenergy crop *Miscanthus × giganteus* as a model species, in 2015 we established a REplicated PLAnting Year (REPLAY) experiment. It was based on a split plot design with 3 planting years (2015, 2016, 2017) as whole-plot and 5 nitrogen (N) rates as split plot. Winter harvested biomass showed a strong interaction between N fertilization and establishment conditions, where one-year-old stands responded only in one of the three planting years. During 2017, N fertilization extended the growth cycle in two- and three-year-old stands but had a marginal effect on one-year-old stands. These results show how REPLAY experiments are able to characterize the variability generated by planting year conditions and separate stand age from environmental effects. Further research is needed to study how a longer growing season could drive a yield response and how this would affect internal nutrient recycling.

#### **T19. DROUGHT STRESS ACTIVATES NAC TRANSCRIPTION FACTOR RD26 through GSK3-LIKE KINASE BIN2 and PROTEIN PHOSPHATASE 2C ABI1 in ARABIDOPSIS**

**Hao Jiang**, *Iowa State University, Ames, IA, USA*

Plant steroid hormones Brassinosteroids (BRs) regulate plant growth and development in many different levels. Recent research reveals that stress-responsive NAC transcription factor RD26 is regulated by BR signaling and antagonizes with BES1 in the crosstalk between growth and drought stress signaling. In order to investigate the upstream signaling transduction components to activate RD26 during drought, we examine the function of GSK3-like kinase BIN2 and protein phosphatase 2C ABI1. Here, we show that ABI1, as the ABA signaling negative regulator, dephosphorylates and destabilizes BIN2, to inhibit BIN2 kinase activity. Moreover, RD26 protein is destabilized by BR, and stabilized by ABA and dehydration in a BIN2-dependent manner. BIN2 directly phosphorylates and interacts with RD26 in vitro and in vivo. The BIN2 phosphorylation is required for the RD26 transcriptional activation on drought-responsive genes. Accordingly, RD26 overexpression suppresses the growth of BIN2 triple mutant *bin2 bil1 bil2*, and enhances its drought resistance. As a novel drought signaling transduction mechanism, our data suggest that drought stress activates BIN2 activity through releasing the inhibition by ABI1, and then BIN2 positively regulates drought stress response by a phosphorylation-dependent regulation of RD26.

#### **T20. Antimicrobial Lipid Transfer Proteins are a Common Feature of Floral Nectar**

**Anthony Schmitt**, *University of Minnesota, Minneapolis, MN, USA*

The primary solutes in nectars are sugars, but proteins also often accumulate to high concentrations. Here we report that non-specific lipid-transfer proteins (nsLTPs) are a common feature of floral nectars across species and that they likely prevent microbial growth in vivo. For example, SDS PAGE analysis of raw nectar from two species, *Brassica rapa* and *Cucurbita pepo*, revealed an array of proteins in each nectar. Major bands at ~10 kDa for both *B. rapa* and *C. pepo* nectar were found to contain non-specific lipid transfer protein (termed BrLTP2.1 and CpLTP2.1, respectively). The genes encoding both nsLTPs were predicted to have signal peptides required for secretion from the cell and eight cysteines, which are characteristic of all nsLTPs. Heterologously expressed and purified BrLTP2.1 and CpLTP2.1 both bound strongly to saturated free fatty acids and had strong direct antimicrobial activity, particularly against

necrotrophic fungi. Interestingly, BrLTP2.1 displayed nectary-specific expression patterns, whereas CpLTP2.1 was expressed throughout the plant. Furthermore, we identified an LTP-like protein, AZELEIC ACID INDUCED 7 (AZI7), as being strongly expressed in the Arabidopsis nectary by microarray and AtAZI7pro::GUS analysis. Similar to BrLTP2.1 and CpLTP2.1, AZI7-GFP fusions were secreted into Arabidopsis nectar and heterologous AZI7 bound to lipids. However, a null mutant, azi7-1, showed a 40% reduction in floral nectar, suggesting a role for AZI7 in regulating nectar production. Cumulatively, our findings suggest that nsLTPs are a widespread feature of floral nectars that may help protect reproductive tissues from infection and may also play a role in regulating nectar synthesis and secretion.

## **T21. Enhanced MALDI-Mass Spectrometry Imaging of Amino Acids in Maize Root Tissue through Chemical Derivatization**

**Kelly O'Neill**, *Iowa State University, Ames, IA, USA*

Matrix assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) has become a widely used technique for the analysis of plant and animal tissues because of its availability, versatility, and ability to simultaneously obtain chemical and spatial information. However, some metabolites, such as amino acids, are difficult to detect in mass spectrometry due to their poor ionization. Derivatization of such compounds can enhance their signals and make detection simple and more reliable. Here, an on-tissue chemical derivatization method is utilized to visualize amino acids in various genotypes of maize roots and to determine any changes in localization or abundance among inbreds and hybrids. Three biological replicates from each of the inbreds, B73 and Mo17, and the hybrids, B73 x Mo17 (BxM) and Mo17 x B73 (MxB), were imaged. Six derivatized amino acids (glycine, alanine, valine, leucine/isoleucine, asparagine, and glutamine) were detected in positive mode that were not present without derivatization. Amino acids are primarily localized in the pith and xylem in the center of the root. In some instances, there is also localization in the outer cortex, although to a lesser extent. All of the signal intensities for the derivatized amino acids were normalized to that of the derivatized internal standard (deuterated alanine). Amino acids have about 2x greater signal intensity in Mo17 than in B73. However, both of the hybrids have higher signal intensities than either one of the parents (about 3x greater signal intensity than B73 for most amino acids), possibly due to hybrid vigor. Compared to the other amino acids, alanine had the most drastic increase in intensity from the inbreds to the hybrids, approximately a 20 fold increase. Due to the inherent issues of quantification using MALDI-MSI, we also plan to compare and confirm our results using the more quantitative method of gas chromatography mass spectrometry.

## **T22. Functional Characterization of Compromised Hydrolysis of Triacylglycerol 7 (CHT7) Protein and its CXC Domain in *Chlamydomonas reinhardtii***

**Nick Fekaris**, *Michigan State University, East Lansing, MI, USA*

In search for renewable energy sources, microalgae have widely been studied as a potential feedstock for biofuels. When deprived of nutrients, some algae go into a hibernation like state called quiescence where cells reversibly cease division and accumulate oil as triacylglycerol (TAG). Upon nutrient refeeding, TAG storage is degraded, and cells resume division. In a previous mutant screen in the green alga *Chlamydomonas reinhardtii*, a mutant with a delay in the remobilization of TAG and growth following N resupply was identified. The gene responsible for this phenotype was subsequently named Compromised Hydrolysis of TAG (CHT7). The CHT7 protein contains a CXC domain, which is thought to bind DNA. To determine which portions of CHT7 are integral to its function, fourteen CHT7 constructs expressing N and C-terminal truncation deletions of increasing size and a CXC domain deletion were generated by site-directed mutagenesis PCR in *E. coli*. The constructs were confirmed by restriction digest and DNA sequencing, and introduced into the cht7 mutant through electroporation. CHT7 proteins with the CXC domain deleted ( $\Delta$ CXC) as well as two CHT7 proteins truncated from the N-terminal end (N1, N2) were

detected in the transformants by immunoblot; however, no truncated CHT7 protein was detected in other mutant lines. During N deprivation and N resupply N1, N2, and  $\Delta$ CXC showed similar growth as the wild type. Additionally, N1, N2, and  $\Delta$ CXC degraded TAG normally following N refeeding, suggesting that these portions of CHT7 are dispensable for the phenotypes tested.

### **T23. Characterization and mapping of the *Suppressor of sessile spikelet 3 (Sos3)* mutant which functions in paired spikelet development in maize**

**Amanda Blythe**, *University of Missouri, Columbia, MO, USA*

*Zea mays* (maize) and rice are two of the most important grasses in the world due to their central role in agriculture. The spikelet, a short branch which produces florets, is the fundamental unit of grass inflorescences. However, the key difference between grasses is the number of spikelets produced. In particular, maize produces paired spikelets while rice and wheat produce single spikelets. These spikelets are produced from niches of undifferentiated stem cells, called meristems, which must be maintained for proper plant growth. In order to study paired spikelet development and meristem maintenance, the Suppressor of sessile spikelet 3 (*Sos3*) mutant of maize is being analyzed. *Sos3* mutants produce single instead of paired spikelets, causing defects in the development of the male (tassel) and female (ear) inflorescences. The resulting phenotype is characterized by fewer tassel branches and gaps between kernels on the ears. Therefore, the *sos3* gene may play a role in paired spikelet development. Histology and scanning electron microscopy (SEM) analyses show that *Sos3* mutants produce single spikelet meristems in place of spikelet pair meristems. Moreover, preliminary results indicate *Sos3* shows a decrease in meristem size, suggesting that the *sos3* gene functions in meristem maintenance. To determine the location and identity of the mutated gene, the *Sos3* mutant is being mapped. Linkage analysis with microsatellite markers shows the *sos3* gene maps to chromosome 1 (bin 6) between markers *umc1988* and *umc2025*, and fine mapping is continuing. Identifying the *sos3* gene will provide valuable insight into paired spikelet development, which could lead to increased yields in important single spikelet cereal crops.

### **T24. Understanding the Metabolic Flux of Rhamnose in Plant Cells**

**Kyler Weingartner**, *Kansas State University, Manhattan, KS, USA*

Flavonoids are polyphenolic secondary metabolites that occur naturally in plants. One characteristic feature of flavonoids, which comprise a large family of thousands of compounds, is that they can be decorated with hexose sugars such as glucose or rhamnose. In the model system *Arabidopsis*, four rhamnose synthase genes (*RHAMNOSE SYNTHASE1 (RHM1)*, *MUM4/RHM2*, *RHM3* and *UER1*) code for enzymes that catalyze the conversion of UDP-glucose to UDP-rhamnose. Mutations in *RHM1* and *MUM4* result in visible phenotypes that are related to cell wall defects. Our hypothesis is that the rhamnose synthase enzymes generate a finite amount of rhamnose based on their expression level that is partitioned between flavonoids and the cell wall. In the rhamnose synthase mutants, the phenotypes observed are due to lower levels of rhamnose that result in compromised cell wall integrity. To test this hypothesis, a variety of different genetic, biochemical, and biomolecular approaches are being conducted. The first being that double mutants have been constructed and genotyped by PCR to probe for novel functions affecting various aspects of growth and development. All the expected double mutants have been observed except for the *rhm3 mum4* combination. One possible explanation is that pollen containing both mutations is not viable. Second, transparent testa mutations in the flavonoid synthesis pathway have also been crossed with *rhm1* and *mum4* in order to lower the level of flavonoids and potentially repress the phenotypes. Third, a feeding experiment is being conducted by growing the plants on sugar supplemented media at varying concentrations in attempt to repress the developmental defects. The repression of the developmental defects is being quantified by two independent methods. Microscopy is being used for quantification of defective

trichomes on first leaves. Cell wall analysis will be used to determine the overall monosaccharide composition as well as their linkage to form polymers.

**T25. An acyl carrier protein mutant links auxin signaling and fatty acid biosynthesis**

**Clarissa Lewis**, *Iowa State University, Ames, IA, USA*

Auxin regulates several aspects of plant development, including hypocotyl elongation and root growth. Our current knowledge of auxin regulated proteins that drive such phenotypic changes is limited. Recently, early auxin regulated protein signaling events were characterized by profiling picloram (a synthetic auxin) treated hypocotyls at 30 min and 120 min using an iTRAQ (isobaric tags for relative and absolute quantification) based quantitative proteomics. Hundreds of differentially expressed proteins were identified and candidate proteins were selected for functional characterization. One such protein is ACYL CARRIER PROTEIN3 (ACP3), which exhibits reduced protein expression rapidly after picloram treatment in hypocotyls. Loss of ACP3 function results in short hypocotyls and roots. Functional characterization of *acp3* mutant alleles will increase our understanding of how auxin pathways are linked to fatty acid biosynthesis during *Arabidopsis* seedling development.

**T26. A Homeodomain Transcription Factor, its START Domain and Epidermal Development in Plants**

**Thiya Mukherjee**, *Kansas State University, Manhattan, KS, USA*

During development of multicellular organisms, the metabolic states of cells and their corresponding gene expression are connected at the molecular level to orchestrate the events underlying cell-type differentiation. This involves protein-metabolite interactions that are outputs of various metabolic pathways. Key regulators of epidermal development in plants are the highly conserved Class IV homeodomain leucine zipper (HD-Zip IV) transcription factors that contain Steroidogenic Acute Regulatory protein (StAR)-related lipid transfer (START) domains. START domains were first identified in mammalian StAR proteins and are implicated in binding to lipid/sterol ligands. However, the lack of information on how START regulates HD-Zip IV proteins serves as a major stumbling block in deciphering their function in plant epidermal development. The goal of this project is to gain a mechanistic understanding of the role of START in HD-Zip IV transcription factors from *Arabidopsis*. Our working hypothesis is that lipid binding to the START domain regulates the activity of the transcription factor, at least in part by modulating the affinity of the homeodomain (HD) for the DNA. Using enhanced yellow fluorescent protein (EYFP) fusions to GLABRA2 (GL2), a representative HD-Zip IV family member that is critical for epidermal differentiation, we showed that deletion of the START domain results in complete loss-of-function despite nuclear localization of the protein. Similarly, missense mutations in the START domain affect transcription factor activity but not nuclear localization. Deletion of the HD or missense mutations of conserved residues therein also result in a loss-of-function phenotype, while nuclear localization is intact. Additional deletion analysis revealed a monopartite nuclear localization sequence (NLS) of 13 amino acids that occurs upstream of the HD. Future work will provide a more detailed understanding of the structure-function relationship of HD-Zip IV transcription factors including identification of novel protein-metabolite interactions underlying epidermal development in plants.

**T27. NEMATE, a Nectary-enriched Multidrug and Toxic Extrusion Protein Involved in Nectar Production in *Arabidopsis***

**Rahul Roy**, *University of Minnesota, Minneapolis, MN, USA*

Nectar is a sugary solution produced by the floral nectary as a reward for pollinators. A better understanding of nectar production mechanisms has implications for improving pollinator health and promoting their crop visitation. Transcriptomic analysis of the *Arabidopsis* nectary has revealed numerous genes that are highly expressed during nectar production. Some, such as SWEET9 (sucrose transporter),

CWINV4 (invertase) and PIN6 (auxin transport) have been previously characterized. We report the role of a Nectary-enriched Multidrug and Toxic Extrusion protein (NEMATE) transporter in nectar production. NEMATE belongs to a family of secondary active transporters that have been studied in bacteria, plants and humans. MATE proteins are antiporters, coupling the transport of substrates to H<sup>+</sup>/Na<sup>+</sup> exchange. We confirmed the nectary-enriched expression of NEMATE with transgenics expressing GUS and GFP reporter genes under the control of the native promoter. Analysis of both N- and C-terminal translational GFP fusions reveal localization at the plasma membrane. Two T-DNA insertional mutants with reduced expression, *nemate1-1* and *nemate1-2*, produce significantly less nectar than wild-type but display no differences in the expression of genes essential for nectary function - CWINV4, SWEET9 and PIN6. Surprisingly, nectary-specific NEMATE overexpressors produce extremely low amounts of nectar and display a reduced expression of CWINV4 and SWEET9 but not PIN6. Transcriptomic analysis of PIN6 loss-of-function (*pin6-2*) nectaries reveals almost a 23-fold upregulation of NEMATE, suggesting a link between auxin homeostasis, NEMATE expression and nectar production. NEMATE expression is also upregulated in transgenic flowers with high levels of endogenous auxin in the nectaries due to the expression of the *iaaM* auxin synthesizing gene (from *Pseudomonas*). Studies with an auxin reporter (DR5-GUS) in the mutant flowers also reveals lower level of auxin response in nectaries when compared to wildtype DR5-GUS flowers. Based on these results we predict that NEMATE functions downstream of PIN6 and is required for auxin responses and nectar production in *Arabidopsis* nectaries.

#### **T28. Type-I MADS-box Transcription Factors Play Critical During Early Seed Development in Rice**

**Puneet Paul**, *University of Nebraska-Lincoln, Lincoln, NE, USA*

MADS box transcription factors are well-known to be involved in all major aspects of a plant's life. In the present study, we report two type-I MADS-box transcription factors critical for regulating early seed development in rice. The two transcription factors are specifically expressed during a crucial developmental window where endosperm transits from syncytium to cellularization stage. Over-expression of these genes result in delayed cellularization, and high levels of spikelet sterility. Double knock-out (CRISPR-Cas9) of these genes is lethal, depicting their indispensable role during early seed development. Moreover, the expression of these genes is negatively correlated to the expression of FIE1, master regulator of Polycomb Repressive Complex2. To summarize, we have characterized the role two Type-I MADS-box from physiology, molecular and epigenetic point of view, thereby providing possible clues to the regulatory mechanism controlling early seed development in rice.

#### **T29. An Engineered Novel Plasma Membrane Protein Provides Broad-Spectrum Pathogen and Pest Immunity in Soybean.**

**Micheline Ngaki**, *Iowa State University, Ames, IA, USA*

Sudden death syndrome (SDS) is an emerging soybean [*Glycine max* (L.) Merr.] disease caused by the soil-borne fungal pathogen *Fusarium virguliforme*. The pathogen causes root rot symptoms including brown discoloration and rotting, and produces toxins that cause leaf scorch or foliar SDS. There is a dire need to develop SDS resistant soybean varieties to manage this disease. Over 40 quantitative trait loci conditioning partial host resistance to *F. virguliforme* have been reported and several SDS resistant cultivars have been developed for commercial cultivation. However, major genes conditioning SDS resistance are most unlikely available in nature. Glycine max disease susceptibility 1 (GmDS1) gene encoding a novel protein with unknown function was identified through a transcriptomic study of the soybean - *F. virguliforme* interaction. The gene is repressed in soybean roots following *F. virguliforme* infection. We hypothesized that the pathogen suppressed the expression of this gene to overcome its possible defense function. We expressed GmDS1 during *F. virguliforme* infection by replacing its promoter with three infection-inducible/root specific promoters. Under growth chamber and field conditions most of the transgenic lines

carrying the GmDS1 fusion genes showed enhanced SDS resistance. Interestingly, the altered expression of GmDS1 also resulted in resistance to soybean pests such as spider mites, soybean aphids, and soybean cyst nematodes in transgenic soybean plants. We localized the GmDS1 protein to plasma membrane by transiently expressing the GFP-tagged protein in *Nicotiana benthamiana*. Intriguingly, GmDS1 signal remains on cell wall following bacterial inoculation. GmDS1 is a 7.9 kDa protein with two membrane-spanning domains. The discovery of GmDS1 opens the door to the characterization of a novel putative receptor-like protein that either directly or indirectly binds to pathogen and pest-associated molecular patterns (PAMP) to signal the activation of host defenses.

### **T30. Traveling Two Diverging Roads, Cytochrome-P450 Catalyzed Demethylation and Lactone Formation in Bacterial Gibberellin Biosynthesis**

**Raimund Nagel**, *Iowa State University, Ames, IA, USA*

Biosynthesis of the gibberellin A (GA) plant hormones evolved independently in certain plant-associated fungi and bacteria. Although the relevant enzymes are phylogenetically distinct, the pathways proceed via essentially identical transformations. One particularly complex step involves combined demethylation and g-lactone ring formation, but the mechanism by which this is accomplished has remained opaque. Here the recently identified cytochrome P450 (CYP) from bacteria that catalyzes this transformation, CYP112, was probed via extensive activity assays, UV-Vis spectral binding studies, and <sup>18</sup>O labeling experiments. CYP112 tightly binds and reacts not only with its native substrate GA12, but also the stable 20-hydroxylated reaction intermediate GA15 and subsequently formed aldehyde equivalent GA24, as well as the methylated derivatives of GA12 and GA15, along with the preceding pathway metabolite GA12-aldehyde, and will bind (although not react with) even earlier ent-kaurene derived metabolites. Notably, CYP112 can utilize H<sub>2</sub>O<sub>2</sub> as an alternative oxygen and electron donor, ruling out use of the ferric-superoxo intermediate from the CYP catalytic cycle. Together with the loss of carbon-20 as CO<sub>2</sub>, demonstrated by the labeling studies, this necessitates separate carbon-carbon bond scission and g-lactone forming reactions, although CYP112 does not react with, or even bind to the potential intervening C-20 carboxylate intermediate GA25. The ability of CYP112 to hydroxylate the d-lactone form of GA15 also shown by the labeling studies is consistent with the implied use of such a further oxygenated heterocycle in the final conversion of GA24 to GA9, and indicates that catalysis by CYP112 partitions its reactants between two diverging, parallel mechanisms.

## **Posters abstracts**

### **P1. Identification of Dynamic Transcriptional Regulators of Plant Disease Resistance, using the Barley-Powdery Mildew Pathosystem**

**Valeria Velásquez-Zapata**, *Iowa State University, Ames, IA, USA*

Obligate fungal pathogens, notably mildews and rusts, are a major threat to cereal grain production worldwide. Because they are unable to survive autonomously, they represent ideal tools for exploring interdependent signaling between disease agents and their hosts. Individual factors in pattern- and effector-triggered immunity have been identified over the years, yet the dynamics of these immune processes are not well understood. To tackle this challenge, we performed an expression Quantitative Trait Locus (eQTL) analysis to interrogate the temporal control of immunity-associated gene expression in barley (*Hordeum vulgare* L.) challenged with the powdery mildew fungus, *Blumeria graminis* f. sp. hordei (Bgh). The key result was the genetic identification of two highly significant clusters of trans eQTL near the telomeric ends of chromosomes 2HL and 1HS. Using these data, we outlined computational steps to discover transcriptional regulators that govern the temporal dynamics of plant immunity. We paired the

barley genome assembly with extensive barley-Bgh expression data using two complementary approaches to predict defense gene modules, immune-active cis-regulatory elements (CRE) and their cognate transcription factors (TFs): First, we compared experimentally validated Arabidopsis and rice TF-CRE pairs with barley promoter sequence sets and calculated an enrichment score and FDR-adjusted p-value using Fisher's exact test. Consistent with our hypothesis, we identified overrepresented CREs in promoters of the previously identified trans eQTL-associated gene sets. Second, we performed de novo CRE discovery. Over 70% of the recovered motifs were consistent to known motifs identified as significant in our targeted analysis. Many of the remaining motifs exhibited low information content (poly-A/T) but several appear to be novel based on extensive database searching. Finally, these results were represented with unrooted phylogenetic trees of each barley TF family, and used as a selection tool for selection experimental validation of regulators of defense gene expression at key stages of pathogen infection.

## **P2. Computational Classification of Phenologs Across Biological Diversity**

**Ian Braun**, *Iowa State University, Ames, IA, USA*

Phenotypic diversity analyses are the basis for research discoveries that span the spectrum from basic biology (e.g., gene function and pathway membership) to applied research (e.g., plant breeding). Phenotypic analyses often benefit from the availability of large quantities of high-quality data in a standardized format. Image and spectral analyses have been shown to enable high-throughput, computational classification of a variety of traits across a wide range of phenotypes. However, equivalent phenotypes expressed across individuals or groups that are not anatomically similar can pose a problem for such classification methods. In these cases, high-throughput, computational classification is still possible if the traits and phenotypes are documented using standardized, language-based descriptions. In the case of text phenotype data, conversion to computer-readable “EQ” statements enables such large-scale analyses. EQ statements are composed of entities (e.g., leaf) and qualities (e.g., length) drawn from terms in ontologies. In this work, we present a method for automatically converting free-text descriptions of plant phenotypes to EQ statements using a machine learning approach. A random forest classifier identifies potential matches between phenotype descriptions and terms from a set of ontologies including GO (gene ontology), PO (plant ontology), and PATO (phenotype and trait ontology), among others. The features used by this classifier include semantic, syntactic, and context similarity metrics between words and ontology terms. This classifier is trained and tested using a dataset of manually converted plant descriptions and EQ statements from the Plant PhenomeNET project (Oellrich, Walls et al., 2015). The most likely matching terms identified by the classifier are used to compose final EQ statements with confidence scores. Results of evaluating the accuracy of this approach are presented, and potential use across datasets to enable automated phenolog discovery are discussed.

## **P3. Maize GO Annotation - Methods, Evaluation, and Review (maize-GAMER)**

**Kokulapalan Wimalanathan**, *Iowa State University, Ames, IA, USA*

We created a new high-coverage, robust, and reproducible functional annotation of maize protein coding genes based on Gene Ontology (GO) term assignments. Whereas the existing Phytozome and Gramene maize GO annotation sets only cover 41% and 56% of maize protein coding genes, respectively, this study provides annotations for 100% of the genes. We also compared the quality of our newly-derived annotations with the existing Gramene and Phytozome functional annotation sets by comparing all three to a manually annotated gold standard set of 1,619 genes where annotations were primarily inferred from direct assay or mutant phenotype. Evaluations based on the gold standard indicate that our new annotation set is measurably more accurate than those from Phytozome and Gramene. To derive this new high-coverage, high-confidence annotation set we used sequence-similarity and protein-domain-presence methods as well as mixed-method pipelines that developed for the Critical Assessment of Function

Annotation (CAFA) challenge. Our project to improve maize annotations is called maize-GAMER (GO Annotation Method, Evaluation, and Review) and the newly-derived annotations are accessible via MaizeGDB (<http://download.maizegdb.org/maize-GAMER>) and CyVerse (B73 RefGen\_v3 5b+ at [doi.org/10.7946/P2S62P](https://doi.org/10.7946/P2S62P) and B73 RefGen\_v4 Zm00001d.2 at [doi.org/10.7946/P2M925](https://doi.org/10.7946/P2M925)).

#### **P4. Effector Gene Expression Regulation by Small RNAs in Barley Powdery Mildew**

**Matthew Hunt**, *Iowa State University, Ames, IA, USA*

Powdery mildews inflict heavy yield losses on diverse crops worldwide, but the host range for most species of powdery mildew is relatively narrow. Each species produces a large variety of effector proteins that reduce defense responses and modifies host metabolism to enhance fungal growth and reproduction. The regulation of effector genes, especially at the post transcriptional level, is mostly unknown. In this study we sought to understand how small RNA (sRNA) expression in barley powdery mildew (*Blumeria graminis* f. sp. hordei) regulates effector and other gene expression during an infection time course from 0 to 48 hours after infection (HAI). Using small RNA-Seq Illumina Sequencing and a custom bioinformatics pipeline, we identified over 1700 micro RNA-like (milRNA) sRNAs produced in *Blumeria*. About 37% percent of the precursors of the predicted milRNAs have homology to known *Blumeria* transposable elements (TEs), implying a direct evolution of TEs into sRNA producing hairpins, as has been described previously in plants and animals. Differential expression (DE) analysis was carried out on the milRNAs, and 268 were identified that are differentially expressed in susceptible barley lines when compared with wild type. All of the DE milRNAs were only DE at the 48 HAI infection time point. To identify direct targets of the milRNAs we carried out parallel analysis of RNA Ends (PARE) analysis. This technique identifies in vivo transcript cut sites in a high throughput manner. Using the PARE data we have identified milRNAs that are predicted to cleave targets in both the Candidate Secreted Effector Proteins (CSEP) and AVRk1 and AVRa10 (EKA) effector families. These results indicate that *Blumeria* is regulating effector expression at the post transcriptional level in a developmentally timed manner. This shift both in regulation of effector and metabolic gene expression may be related to a shift from defense to nutrient acquisition.

#### **P5. Control of Quiescence and Cell Division by Compromised Hydrolysis of TAG 7 (CHT7) in**

*Chlamydomonas reinhardtii*

**Tomomi Takeuchi**, *Michigan State University, East Lansing, MI, USA*

Faced with nutrient scarcity, many microorganisms exit the normal cell division cycle and enter a temporary resting state called quiescence, where cells arrest replication but stay viable. In the microalga *Chlamydomonas reinhardtii*, quiescence is induced upon deprivation of nutrients such as nitrogen (N) and is accompanied by the accumulation of storage molecules such as triacylglycerol (TAG). When nutrients are resupplied, cells degrade TAG and re-enter the cell division cycle. A *Chlamydomonas* mutant, compromised hydrolysis of TAG 7 (*cht7*), was previously isolated in a screen for mutants that show a delay in remobilization of TAG during N refeeding. CHT7 was found to be a member of highly conserved CXC domain proteins, some of which are known to exist in a transcriptional regulatory complex termed DREAM along with the retinoblastoma tumor suppressor (RB) protein. During phylogenetic analysis the CXC domain of CHT7 was found to cluster closely with the CXC domains of proteins found in the DREAM complex. Co-immunoprecipitation assays showed interaction between CHT7 and *Chlamydomonas* RB protein during N deprivation and N replete growth. Detailed phenotypic characterizations of the *cht7* mutant revealed that CHT7 was necessary for maximal survival under N deprivation and orderly resumption of cell growth and division upon N refeeding. Under these conditions, the *cht7* mutant showed cytological defects and a number of cell cycle genes were found to be highly



misregulated. These results point to the role of CHT7 as a regulator of cell cycle gene expression during nutrient deprivation-induced quiescence and cell division.

#### **P6. Putative Plastid Rhomboid Protease Plays a Role in Phosphatidate Metabolism in *Arabidopsis thaliana***

**Anastasiya Lavell**, *Michigan State University, East Lansing, MI, USA*

The thylakoid membranes of the chloroplast house the photosynthetic machinery that converts light into chemical energy. Chloroplast membranes are unique from other plant organelles in their lipid makeup, which is dominated by mono and digalactosyldiacylglycerol (MGDG and DGDG). The predominant galactolipid, MGDG, can be made through both plastidic (prokaryotic) and ER (eukaryotic) pathways in *Arabidopsis*, resulting in two distinct species of lipid. Phosphatidate has been shown to be the first acylated lipid species in the plastid galactolipid biosynthetic pathway, providing a pool of diacylglycerol for MGDG Synthase. The enzymatic reactions yielding these galactolipids have been well-described, however, regulation of these steps is unknown at this time. Intramembrane proteolysis, as demonstrated by members of the rhomboid-like family of proteins, is one example of regulation through proteolysis. One such rhomboid-like protein 10 (RBL10), found in the chloroplasts of *Arabidopsis thaliana*, may be involved in maintaining biosynthesis of MGDG through the plastidic pathway. Plants disrupted in the gene encoding RBL10 have greatly decreased 16:3 and increased 18:3 acyl chain abundance in MGDG in leaves. Additionally, *rb10* mutants show reduced <sup>14</sup>C – acetate incorporation into MGDG during the first hour of pulse-chase labeling, indicating a reduced flux through the prokaryotic galactolipid biosynthesis pathway. While plastid MGDG biosynthesis is reduced in *rb10* mutants, they are capable of synthesizing PA, as well as making normal amounts of MGDG by compensating with ER lipid precursors. Though the molecular mode of action remains to be described, these preliminary findings link this protease to utilization of PA for galactolipid biosynthesis and give an opportunity to characterize a novel lipid regulatory mechanism.

#### **P7. Exploring the Intracellular and Extracellular Lipid Metabolic Networks at the Level of Individual Cells**

**Liza Alexander**, *Iowa State University, Ames, IA, USA*

Plant epidermal cells express unique molecular machines that juxtapose the assembly of intracellular lipid components and the unique extracellular lipids that are unidirectionally secreted to the surface of the plant. Physiologically this lipid-trafficking process is genetically programmed, but can change in response to environmental stimuli (e.g., drought, temperature, pathogens), making them important to agricultural crop productivity. Additionally, these lipids are chemically most akin to petroleum hydrocarbons making this research insightful towards the development of biorenewable fuels and chemicals. This study uses genetic stocks of maize and *Arabidopsis* designed to reveal the biochemical function of specific genes that affect the deposition of extracellular cuticular lipids. One such gene, maize *Glossy2* is the sequence homolog of the *Arabidopsis* *Cer2* gene, and both encode proteins that have proven to be archetypal of BAHD class of enzymes that catalyze acyl transferase reactions using acyl-CoA substrates, to produce either ester-linked or amide-linked specialized metabolites. Recent studies suggest that *Cer2* is involved in fatty acid elongation, but this functionality may not require the BAHD acyl transferase catalytic function. We have recently shown that not only are *Glossy2* and *Cer2* sequence homologs, but they are also functional homologs. A comprehensive analysis of the intracellular lipid profiles via liquid chromatography/quadrupole time-of-flight mass spectrometry (LC/Q-TOF/MS) and extracellular lipid data via gas chromatography mass spectrometry (GC-MS) are used to provide invaluable insights on the interrelationships between different molecular machines as disrupted by genetic permutations, and in turn provide models on the physiological functions of lipid metabolism genes.

**P8. Role of the *Agrobacterium* virulence effector protein VirE2 in modulating plant gene expression**

**Rachelle Lapham**, *Purdue University, West Lafayette, IN, USA*

VirE2 is an *Agrobacterium* effector protein that is important for plant transformation. VirE2 likely coats T-strands after they enter the plant cell to protect them from degradation. VIP2 is a host transcription factor that interacts with VirE2 and is involved in microRNA biogenesis and defense responses. We hypothesized that in addition to VirE2's proposed structural role in T-DNA trafficking, VirE2 may interact with VIP2 and alter the expression of VIP2-regulated genes, especially those important for suppression of defense responses. This, in turn, may facilitate transformation. Additionally, VirE2 may influence the plant transcriptome independent of VIP2. We have investigated these possibilities by individually placing VirE2 and VIP2 under the control of an inducible promoter in *Arabidopsis* and performing RNAseq under non-induced and induced conditions, and in the presence of *Agrobacterium*, to determine the effects of VirE2 or VIP2 on plant gene expression during infection. We have thus defined the VIP2 regulon and also identified a subset of genes that are differentially expressed in the presence of VirE2. Some of these plant genes are involved in gene silencing. Other genes differentially expressed after VirE2 induction are known to be important for transformation. Genes encoding arabinogalactan protein (AGP) family members were enriched in the VirE2 expression data. We are analyzing plant lines mutant for various AGP genes or genes involved in gene silencing for altered transformation susceptibility.

**P9. Small RNA, DNA Methylation and Gene Expression in Soybeans with Copy Number Variation at Soybean Cyst Nematode Resistance Locus (*Rhg1*)**

**Usawadee Chaiprom**, *University of Illinois at Urbana-Champaign, Champaign, IL, USA*

Small RNAs (sRNA) are involved in RNA-directed DNA Methylation and control gene expression. The sRNA regulation contributes to plant disease resistance. Soybean cyst nematodes (SCN) damage soybean roots and lead to severe yield reduction. Previous studies found that copy number variation (CNV) of a 31.2-kb segment at the *Rhg1* locus confers SCN resistance. However, the regulation of *Rhg1* genes has not been investigated. In this study, we aimed to examine sRNA populations in soybeans with CNV at the *Rhg1* locus, including Williams 82 (one copy), Peking (three copies) and PI 88788 (9 copies). Known microRNA families were differentially expressed between SCN-susceptible and resistant soybeans, for instance, miR390, miR395 and miR1510. An accumulation of 24-nt sRNAs at the *Rhg1* locus was found in Peking and PI 88788, suggesting an involvement of sRNAs in transcriptional regulation of *Rhg1* genes. We further investigated sRNA, mRNA and DNA methylation profiles in uninfected roots of isogenic Fayette plants with CNV at the *Rhg1* locus ranging from 9 to 11 copies. The sRNAs were mapped to the *Rhg1* locus; however, the abundances of sRNA and mRNA at this locus were not statistically different among Fayette plants. Interestingly, differentially methylated regions were identified at promoters of *Rhg1* genes within Fayette plants. The co-occurrence of sRNAs and DNA methylation indicates a role of sRNAs in regulating DNA methylation. For genome-wide analysis, a co-expression network constructed from differentially expressed genes within Fayette uninfected roots revealed candidate genes related to the ethylene-dependent defense response and oxidation-reduction process. Fluidigm-based qRT-PCR is used to profile the temporal expression of 24 selected candidate genes in SCN-infected roots of soybeans with CNV at the *Rhg1* locus. The results showed similar expression patterns between SCN-infected and uninfected roots, but there were some differences in expression levels within genotypes and time points.

**P10. The AP2/ERF Transcription Factor TINY Modulates Brassinosteroid-Regulated Plant Growth and Drought Response in *Arabidopsis***

**Zhouli Xie**, *Iowa State University, Ames, IA, USA*

In order to survive environmental stresses while optimizing their growth plants must precisely modulate their growth and stress response programs. Plant steroid hormone Brassinosteroids (BRs) promote plant

growth and inhibit drought responses through central transcription factors BES1 and BZR1 in the BR signaling pathway. Here, we found that drought inducible AP2/ERF A-4 family transcription factor TINY inhibits BR-regulated growth while promoting drought stress response. TINY interferes with BR-induced plant growth by reducing BES1 levels and BR signaling. Accordingly, TINY overexpression plants displayed a dwarf growth phenotype, were more sensitive to inhibition of BR biosynthesis and suppressed the constitutive growth phenotype of *bes1-D*. In contrast, knockout of TINY and its homologs led to increased plant growth and were resistant to inhibition of BR biosynthesis. TINY promotes plant survival under drought conditions at least partially by interacting with and antagonizing BES1 on drought responsive genes. Overexpression of TINY led to increased survival during drought and global gene expression studies revealed that TINY functions to reduce the expression of BR-induced genes and increase the expression of BR-repressed stress responsive genes to inhibit plant growth and promote drought response. On the other hand, BIN2, a negative regulator in the BR pathway, phosphorylates and stabilizes TINY, providing a mechanism by which BRs function to inhibit TINY function to prevent unnecessary activation of drought responses. Our results thus reveal that TINY not only functions to promote drought response and inhibit plant growth by modulating BES1 function under drought condition, but also acts as a target for BR signaling during plant growth.

#### **P11. Use of Ribosome Profiling to Decipher Translational Control of Gene Expression during the Unfolded Protein Response in Maize Roots**

**Pulkit Kanodia**, *Iowa State University, Ames, IA, USA*

Detection of steady state RNA levels by RNAseq has been used widely to assess gene expression, but these measures do not reveal how well the RNAs are translated. Instead, ribosome profiling or Riboseq provides information about how efficiently the mRNAs are translated. Riboseq involves deep sequencing ribosome protected footprints (RPFs) obtained by RNase digestion of translation-arrested polyribosomes. By counting the RPFs that map to each position in an mRNA, we can identify how efficiently each mRNA is translated with single nucleotide resolution. We have honed the technique in our lab to obtain good quality RPFs. A majority of reads map to coding sequences and the RPFs show triplet periodicity owing to the brief pause of the ribosome at each codon during elongation. We used Riboseq to decipher the global change in protein synthesis under persistent endoplasmic reticulum (ER) stress in maize roots. The unfolded protein response (UPR) is elicited by ER stress, defined as the accumulation of misfolded protein in the ER. Global translation down-regulation occurs in mammalian cells during UPR by inactivation of eukaryotic translation initiation factor 2 $\alpha$  (eIF2 $\alpha$ ) via phosphorylation by a UPR activated protein kinase, PERK. Unlike mammalian cells, plant cells lack PERK, but phosphorylate eIF2 $\alpha$  depending on another protein kinase, GCN2. Our analysis reports a global down-regulation of protein translation in maize roots treated with ER-stress agent, tunicamycin. Riboseq revealed that thousands of mRNAs were reduced in translation, including mRNAs encoding ribosomal proteins. This illustrates the importance of reducing the input load on ER so the transcriptionally upregulated or ER-stress-activated factors can alleviate the ER-stress. Validation of several down-regulated genes and few up-regulated genes using qPCR and western blotting is in progress. In summary, ribosome profiling is a powerful tool to investigate global changes in the translome in response to any biotic or abiotic stress.

#### **P12. Mechanism of cap-independent translation by maize chlorotic mottle virus: a step toward genome-engineered resistance.**

**Elizabeth Carino**, *Iowa State University, Ames, IA, USA*

Maize chlorotic mottle virus (MCMV) is the key player in the synergistic interaction with potyviruses that causes maize lethal necrosis disease (MLND). An expanding MLND epidemic is causing serious yield losses across East Africa. As a member of the Tombusviridae family, MCMV lacks the 5' cap required for

conventional translation of host mRNAs. We identified a cap-independent translation element (CITE) in the 3' untranslated region (UTR) of the MCMV genome. The presence of this MCMV CITE (MTE) in the 3' UTR helps the virus boost its translation efficiency, minimize genome length, and may permit the viral replicase to shut off translation of the genomic RNA from the 3' UTR. We determined the secondary structure of the MTE and how it base pairs to the 5' UTR to facilitate translation initiation. There are different interactions used by 3'-CITEs to recruit the translation machinery: some recruit eukaryotic initiation factor (eIF) 4E, others recruit eIF4G, while some interact only with the eIF4F heterodimer (eIF4E+eIF4G). Despite lacking a cap, the MTE binds the cap-binding translation initiation factor eIF4E with high affinity. We now seek to identify mutations in eIF4E that disrupt binding to the MTE but not to capped RNA. This should allow engineering (or screening for) resistance to MCMV by using a mutant eIF4E that functions normally with capped mRNA, but does not bind MCMV RNA, thus preventing viral RNA translation and ensuing infection. This approach is promising, as natural mutations in eIF4E confer resistance to many other plant viruses.

**P13. Dissecting the Subcellular Compartmentation of Acetyl-CoA Metabolism in *Arabidopsis thaliana* Using Integrated Genetic and Metabolomics Approaches**

**Xinyu Fu**, *Iowa State University, Ames, IA, USA*

Acetyl-CoA is a key metabolite at the crossroads of many metabolic pathways that are responsible for the biosynthesis of diverse phytochemicals (e.g., lipids, flavonoids, and terpenes). These end products of metabolism are ideal targets for biofuels and bioactive applications. Therefore, understanding the dynamic changes and spatial organization of acetyl-CoA metabolism is fundamental to precise control and regulation of carbon fluxes to bioengineer plants for these applications. Due to the impermeability of acetyl-CoA across membranes, its metabolism is segregated into several subcellular compartments. Two distinctly localized enzymes, acetyl-CoA synthetase (ACS) in the plastids and acetate non-utilizing (ACN) in the peroxisomes, have been identified to generate acetyl-CoA via the activation of acetate. However, the physiological functions and biological relevance of these two enzymes remain unclear. To address this question, we characterized several mutant lines of *Arabidopsis thaliana*, that lack the ability to express each enzyme or both in combination. Although the *acs* and *acn* single knockout mutants grow normally under standard conditions, they accumulate higher levels of endogenous acetate, and they are more sensitive to externally supplied acetate than wild-type plants. Strikingly, the *acs acn* double mutant that simultaneously lacks both enzymes display a drastic growth defect phenotype (e.g., slow and prolonged vegetative growth and infertility) and accumulate even high levels of acetate than either single mutant. This indicates that the plastidial ACS and peroxisomal ACN provide redundant functions that in combination are required for normal plant growth and development. We further applied stable isotope-assisted metabolomics to fingerprint the in vivo carbon flux from labeled precursors to metabolites synthesized by distinct acetyl-CoA pools that are dependent on a functional ACS or ACN gene. Namely, feeding *acs* and *acn* mutant plants with <sup>13</sup>C-acetate, unique isotopic patterns of fatty acids, organic acids, and amino acids were observed. The kinetics of isotope incorporation into the metabolome is being measured to identify the metabolic flux changes that lead to the altered metabolic phenotypes. This study will advance our understanding of compartmentalized metabolic networks by providing more spatial and temporal information of enzymes, intermediates, and end products of metabolism.

**P14. Fine Mapping of Metabolite-QTLs for Extracellular Surface Lipid Accumulation on Maize Silks**  
**Tes Posekany**, *Iowa State University, Ames, IA, USA*

Upon emergence from the husk leaves, maize silks are exposed to numerous environmental stresses (e.g., UV radiation, insect damage, and desiccation). Like most other aerial plant surfaces, the maize silk has a cuticle infused with and coated by extracellular surface lipids (SLs) that act as an environmental barrier.

The silk SL metabolome includes at least 50 metabolites that are primarily linear hydrocarbons, fatty acids, and aldehydes ranging in chain lengths from 16 to 35 carbon atoms. To identify the genomic loci controlling the biosynthesis of these metabolites, we performed metabolite-quantitative trait locus (mQTL) mapping using the intermated B73xMo17 recombinant inbred line (IBMRIL) population, which harbors considerable variation in the silk SL metabolome. Surface lipids were extracted from emerged silks at three days post-silk emergence and subsequently identified and quantified using gas chromatography-mass spectrometry (GC-MS) or GC-flame ionization detection (GC-FID). mQTL analysis of constituent traits, metabolite-class traits, and relative composition traits identified >500 mQTLs that modulate the abundance and composition of the silk SL metabolome, with some mQTLs detected in more than one environment. A more complete characterization of the genetic network has been pursued through inclusion of traits that are precursor (fatty acids), proposed intermediate (aldehydes), and end-product (hydrocarbons) lipids. To connect this genetic network to the predicted biochemical network for hydrocarbon biosynthesis, identification of causal genetic polymorphisms or the ability to discriminate among competing candidate gene hypotheses is required. Here we report our progress in dissecting two genomic loci that are particularly influential in shaping the silk SL metabolome. Fine mapping results are reported from three complementary breeding and analysis approaches: isogenic dual testcross, heterozygous inbred family and bi-parental introgression, each of which are used to interrogate potentially informative recombination events.

**P15. Reducing Seed Coat Fiber Content to Improve Seed Meal Nutritional Value of the Oilseed Crop Pennycress (*Thlaspi arvense*)**

**Taylor Suo, Illinois State University, Normal, IL, USA**

Pennycress (*Thlaspi arvense*; Field pennycress) is an oilseed plant of the Brassicaceae family that is closely related to Arabidopsis, camelina, and rapeseed canola. Pennycress is native to Eurasia and naturalized to North America. Wild strains grow widespread throughout temperate regions of the world. Efforts are underway to rapidly domesticate pennycress to be grown as a winter annual oilseed-producing cover crop. For example, pennycress can be planted in the fall in standing corn and harvested in the spring in time to plant full-season soybeans throughout the 80 million-acre U.S. Midwest Corn Belt. Once commercialized, elite pennycress varieties will provide additional income to farmers and agribusinesses thereby strengthening rural communities. Pennycress will also provide ecosystem services as a cover crop, reducing soil and nutrients runoff from otherwise vacant farmland. About two-thirds of the value of the pennycress seed is in the oil, which can be crushed out of the seed and used as a biodiesel or jet fuel feedstock. The remaining one-third value is the left-over seed meal, which can be used as a protein supplement in animal feed. However, the meal from wild pennycress strains is of relatively low quality due to the seed coat having high fiber content. This presentation will detail our efforts to identify and characterize EMS-generated pennycress mutants having lighter-colored seed coats (so-called transparent testa mutants), which is a trait that commonly accompanies reduced seed coat fiber in Brassica species.

**P16. Bulked Segregant - genotyping-by-sequencing: Cost-effective and background independent genetic mapping of mutants and QTL**

**Kokulapalan Wimalanathan, Iowa State University, Ames, IA, USA**

Genetic mapping of new mutants, which allows us to map a mutant phenotype to a causal locus or loci in the genome, is a crucial step in forward genetics. Construction of a mapping population that consists of mutant and normal individuals is essential for genetic mapping. The mapping population can be used by different high-throughput methods for genetic mapping. Single Nucleotide Polymorphism (SNP) arrays and Sequenome-based methods detect presence and absence of pre-discovered SNPs, and therefore are not background independent. In contrast, high-throughput sequencing (HTS) based methods used for genetic

mapping are generally background independent. Some HTS methods such as Genotyping-by-sequencing (GBS) and RAD-seq use DNA for mapping, while other methods such as BSR-seq and MMAPP use RNA. Current DNA-based methods barcode DNA extracted from each individual in the mapping population to construct the sequencing library, and RNA-based methods construct a separate library from each of two pools, namely mutant and normal. Both approaches provide high resolution maps to identify causal loci, but are not cost-effective for screening a large number of mutant families such as may be recovered from an enhancer/suppressor screen. Here we present a low-resolution, but cost-effective, HTS-based method for genetic mapping. For each new mutant we pooled tissue from phenotyped individuals to create a mutant pool and a normal pool. We adapted the original GBS method to construct sequencing libraries, prepared libraries for several pairs of pools and determined rough map positions. Our method is cheaper than the current GBS protocol, easier than using RNA for library construction, and without sampling biases inherent in using RNA expressed in a certain tissue type(s). We are currently fine mapping the intervals identified by BS-GBS, and extending the method to map natural modifiers. Here we present the pipeline and results from these genetic mapping efforts in maize.

#### **P17. Deacclimation of cold-acclimated canola: GWAS and physiology**

**Jiaping Zhang**, *USDA-ARS-RRVARC, Fargo, ND, USA*

Winter canola produces greater yields and can flower earlier than spring canola. However, its range is limited by the inability of this crop to withstand the harsher winters that occur in more northern regions of the US. Two basic factors result in reduced survival of winter canola in the Northern Great Plains. One is over wintering survival, and the other is freezing damage that can occur in the winter or spring when plants deacclimate following short-term warm spells followed by return of freezing temperatures. To investigate genes that might mitigate the losses caused by deacclimation followed by subsequent freezing, we have investigated the temporal and temperature thresholds that result in deacclimation. Towards this end, we have determined that plants that had been fully acclimated at 5 C for 4 weeks and can normally withstand freezing to -10 C show signs of deacclimation following as little as 3 days at 10 C. Similar levels of deacclimation occur at 3 days with 10 C nights and 20 C day light. Complete deacclimation (to non-acclimated levels) can occur in as little as 3 days at 15 C. Interestingly, plants can also partially acclimate after 4 weeks at 10 C suggesting that there is the potential for an equilibrium of acclimation and deacclimation processes that occur at this temperature. To identify genes or loci that might be involved in these deacclimation processes, we initiated a genome wide association study using a previously genotyped diversity panel containing 429 primarily winter canola varieties. To date, over 300 lines (3 replicates/line) from this population have been subjected to two rounds of analysis where plants were acclimated for 4 weeks at 5 C and then deacclimated for 3 days, frozen to -10 C and then allowed to recover. Plants were scored visually for damage 1 week following freezing using a 0 to 3 scale. The resulting data identified several regions of the genome, one of which identified a gene encoding subunit 5 of the DNA replication complex.

#### **P18. Gene Regulatory Network Analysis of NKD1, NKD2 and OPAQUE2 in Maize Endosperm Development**

**Hao Wu**, *Iowa State University, Ames, IA, USA*

Maize endosperm provides essential resources for food, feed and industrial raw materials. Prior studies revealed that transcription factors (TFs) NKD1, NKD2 and OPAQUE2 (O2) are three key players in endosperm development. The o2 mutant and nkd1, nkd2 double mutant each altered expression of many genes, including genes associated with hormone response, cell cycle, storage material biosynthesis, resulting in dramatic mutant kernel phenotypes. However, the underlying regulatory networks of these factors remain to be characterized. In this study, a family of nkd1, nkd2 and o2 homozygous mutants,

including wild-type, 3 single mutants, 3 double mutants and the triple mutant, was developed. They manifested diverse phenotypes in kernel surface characteristics, endosperm vitreousness and aleurone cell development, indicating interactions among *nkd1*, *nkd2* and *o2* regulatory networks. Endosperm RNA samples from 8, 12 and 16 DAP of the 8 genotypes were collected and 3'-RNA-sequencing was performed. Based on the normalized data, gene correlation analysis and weighted gene co-expression network analysis (WGCNA) were performed to identify gene expression modules (expression correlation  $p < 0.01$ ) and corresponding hub genes (intramodular connectivity by Module Membership,  $kME > 0.9$ ). In correlation analysis of the entire sample set, 424, 232 and 1064 genes were significantly correlated with *nkd1*, *nkd2* and *o2*, respectively. Putative direct targets of TFs tend to be enriched in genes with very low *p* values, based on the analysis combining O2 Chip-seq data with *o2* correlation gene lists. Each of the 3 TFs showed different correlated gene sets and enriched Gene Ontology (GO) terms in each development stage, suggesting that they regulate different sets of downstream genes with varied roles throughout development. WGCNA identified several hub genes significantly correlated with each of the 3 TFs, indicating that these genes, directly regulated by or closely related with the 3 TFs, could be key regulators of their co-expression modules, responsible for particular developmental events. As a work-in-progress, epitope-tagged NKD1 and NKD2 transgenic lines will be developed for Chip-seq to confirm direct target genes. Additionally, hub genes and their associated metabolic pathways or downstream targets will be further investigated, in order to gain deeper insight into the regulatory networks controlling maize endosperm development.

#### **P19. CRISPR-Cas9 Ribonucleoprotein Complex Delivery in Plants for DNA Free Genome Editing**

**Raviraj Banakar**, *Iowa State University, Ames, IA, USA*

CRISPR-Cas9 Ribonucleoprotein Complex Delivery in Plants for DNA Free Genome Editing The clustered regularly interspaced short palindromic repeat (CRISPR)-CRISPR-associated-protein (Cas) system has been a method of choice for precise genome editing in many organisms, including plants. However, thus far the CRISPR studies in plants have focused on stable integration of CRISPR-Cas9 reagents delivered as plasmids. Because Cas9 is mutagen, there is a considerable concern over environmental safety and gene drive when it is stably and constitutively expressed in plants. In this study, we describe a protocol for the co-delivery of CRISPR reagents as a ribonucleoprotein (RNP) complex and a DNA selectable marker into rice (*Oryza sativa*) and *Nicotiana benthamiana*. Single-copy endogenous gene encoding phytoene desaturase (PDS1) and a transgene encoding green fluorescent protein (mGFP5) were targeted in rice and the 16C line of *N. benthamiana*, respectively. Hygromycin-resistant transgenic T0 events were regenerated in the case of rice, of which 80% carried a mutation in the PDS1 gene. Similarly, bialaphos-resistant transgenic T0 events were regenerated in the case of *N. benthamiana*, of which 85% of the events carried mutations in the mGFP5 gene. Deletion, insertion and substitution mutations were observed in both rice and *N. benthamiana*. Methodology demonstrated here can be used to generate transgene-free mutant crops that can help advance basic and applied research.

#### **P20. Soybean Aphids Exploit Soybean Abscisic Acid Signaling to Promote Susceptibility**

**Jessica Hohenstein**, *Iowa State University, Ames, IA, USA*

The soybean aphid (*Aphis glycines*) is an economically important insect pest of soybean (*Glycine max*) in the Midwest. Unmanaged aphid populations can reduce yields by up to 40%. Plant defenses mediated by the phytohormone jasmonic acid (JA) are effective against soybean aphids yet previous transcriptome and metabolome data suggests that aphids may alter JA biosynthesis and/or block the JA response in susceptible plants. The mechanism of defense suppression is unknown but it has been hypothesized that aphids induce an antagonistic decoy pathway to suppress JA responses. Transcriptome data revealed marked induction of the abscisic acid (ABA) pathway during aphid infestation; we tested the hypothesis that soybean aphids block JA-mediated responses by induction of the ABA pathway. Consistent with

previous data, we showed that aphid feeding attenuated JA- and wound-induced JA responses as measured by the JA-inducible cysteine proteinase inhibitor N2 (PinN2). Following this, growth of the chewing caterpillar *Helicoverpa zea* was facilitated by the suppression of JA responses. In plants infested with aphids, we found increased levels of cis-JA but not biologically active JA-isoleucine suggesting JA is being synthesized but not perceived. We also found significantly elevated levels of ABA. Using chemical elicitor treatment and knockdown mutants impaired in ABA biosynthesis (*aba2-RNAi*) or signaling (*scof-1-RNAi*), we showed that both endogenous and exogenous ABA suppressed wound-induced JA responses. Aphid populations were significantly reduced in the absence of a functional ABA biosynthetic or signaling pathway. Moreover, attenuation of wound-induced PinN2 transcripts was abolished in these mutants. Our results suggest the aphid-regulated repression of JA responses was mediated by soybean's endogenous ABA pathway, consistent with the hypothesis that soybean aphids exploit the stress hormone abscisic acid (ABA) to suppress effective JA-mediated plant defenses.

**P21. Increased Transpiration Is Correlated with Reduced Boron Deficiency Symptoms in the Maize *Tassel-less1* Mutant**

**Michaela Matthes**, *University of Missouri, Columbia, MO, USA*

Boron (B) is an essential micronutrient whose deficiency is widespread in the US and worldwide causing yield decline in many crops. While plants can take up B passively via diffusion, it was shown that active transport is particularly important under B deficient conditions. We have identified the *tassel-less1* (*tls1*) mutant in maize, which is the co-orthologue of the Arabidopsis B importer gene *NIP5;1*. *tls1* is inherently B deficient, due to its impaired uptake of B out of the soil. The mutation particularly affects meristematic tissues, leading to vegetative and/or reproductive defects depending upon the B concentration in the soil. All B deficiency symptoms can be rescued by B supplementation. In the field the B mediated rescue is variable, suggesting that there are additional factors contributing to the severity of B deficiency under field conditions. We found that B deficiency symptoms of *tls1* are alleviated, when it is grown in a greenhouse with a higher light intensity. Our results suggest that this is due to an increase in leaf B content correlated with an increased transpiration and passive B transport. This implies that passive B transport is a major B source in maize. Our studies will aid in improving crop tolerance to this environmental stress and will eventually lead to the development of high-yielding plants with optimized growth in marginal soils.

**P22. *barren stalk3* is Required for Axillary Branch Development and Maps to the Same Location as *barren stalk2***

**Norman Best**, *University of Missouri, Columbia, MO, USA*

*Zea mays* (maize) bears female reproductive inflorescences, ears, on axillary branches. Both initiation and maintenance of the female axillary meristems are necessary for their proper development. Previously characterized barren stalk (*ba*) mutants have determined that auxin is required for initiation of these axillary branches. The *ba* mutants, *ba1* and *ba2*, encode a transcription factor and interacting protein, respectively, that function downstream of auxin to control development of axillary meristems. A new mutant, barren stalk3 (*ba3*), was identified in 1990 in an Ubiquitous transposon active population by Pan and Peterson as a novel locus controlling this process. The *ba3* mutant failed to initiate an axillary ear branch and the grooves on the stem that normally bear ears do not develop. In the B73 genetic background, *ba3* mutants have shorter tassel branches and fewer secondary branches but there was no effect on plant height or tassel length. An enhancer of the *ba3* mutant phenotype was discovered when introgressed into the Mo17 background. The *ba3* mutants were significantly shorter for plant height and tassel length and there were fewer primary and secondary tassel branches on *ba3* mutants as compared to normal siblings. Therefore, the *ba3* locus was necessary for ear initiation without affecting plant height and the mutant phenotype was enhanced by maize standing variation and we infer that the *ba3* gene is necessary for



axillary meristem initiation or maintenance. We used a next generation sequencing and bulk-segregant analysis approach to map the ba3 locus to the short arm of chromosome 2, indicating that it could, in fact, be a new allele of ba2. Current endeavors are underway to identify the causative mutation of the ba3 phenotype and confirm if it is an allele of ba2. These results indicate that there may only be two identified barren stalk loci in maize.

**P23. Utilization of a split-root system for controlled, reproducible imposition of water deficit on maize seedlings**

**Rachel Mertz**, *University of Missouri, Columbia, MO, USA*

Drought is the major limiting factor for agricultural production worldwide, and improved crop varieties that maintain yields under water deficit are imperative to feed the estimated 9 billion global population by midcentury. In maize and other cereals, most water is acquired by whorls of nodal roots that develop sequentially from subterranean stem nodes rather than by seedling (primary and seminal) roots. As 85% of domestic maize experiences drought stress within a growing season, nodal roots frequently must emerge and elongate through very dry topsoil to access water at depth. The molecular genetic mechanisms of nodal root elongation maintenance at tissue water potentials that inhibit leaf and stem growth remain largely uncharacterized. We utilized a split-root system to impose precise, constant water deficits on seedling and nodal roots of the maize inbred lines FR697 and B73. Two water deficit regimes were imposed: nodal root growth at low soil water potential with well-watered seedling roots (moderate stress), and both nodal and seedling root growth at low soil water potential (severe stress). Under both stress regimes, FR697, but not B73, completely maintained node 2 root elongation. Intriguingly, although leaf expansion was reduced and tissue water potential declined under severe stress, leaves of FR697 seedlings did not wilt. In contrast, leaves of B73 showed greater wilting but less growth inhibition. Thus, FR697 exhibited greater enhancement of root-to-shoot biomass ratio under stress, and in addition, nodal root length per unit mass was attenuated, suggesting enhanced carbon partitioning to roots and altered root anatomy. To investigate the role of carbon partitioning in nodal root elongation and osmotic adjustment, we identified candidate genes from several sugar transporter families. These candidates will be mutated by targeted reverse genetics and evaluated for nodal root phenotypes using the split root system.

**P24. Directed Evolutionary Study of Class I Diterpene Synthases**

**Meirong Jia**, *Iowa State University, Ames, IA, USA*

Class I diterpene synthases act as pyrophosphate lyases, and contribute a large portion of the structural diversity of the labdane-related diterpenoids, a large super-family of 12,000 natural products predominantly found in plant kingdom. The ent-kaurene synthases (KSs) are required for biosynthesis of gibberellins or other signaling molecules across the embryophyta, providing a genetic reservoir that has given rise to other diterpenoids. Specifically, via gene duplication and neo-functionalization, leading to the so-called KS-like diterpene synthases (KSLs). Herein, directed evolutionary study has been intensively conducted in this enzymatic (sub-)family. A highly conserved Ile residue located in a helical kink region within the KS active site was characterized, whose replacement by Thr overwhelmingly switched enzyme from KSs to pimaradiene synthase(s). This remarkable single residue mutation effect demonstrates the intrinsic plasticity of KSs, which enables their ready conversion into alternative catalytic activity. Further phylogenetically directed evolutionary study was carried out with several KSLs, where we were able to not only alter enzymatic activity, but also obtain fundamentally new catalytic mechanisms – i.e., addition of water to yield alcohols rather than olefins. Notably, the product-determining residue(s) in these KSLs also fall within the kink region. This functional conservation pattern further supports the hypothesis that KSLs share the same ancestor. More importantly, these profound changes in product outcome by simply introducing single residue substitution offer us a powerful tool to manipulate enzymatic activities to yield

additional (un)natural products. Here the most current results from our directed evolutionary investigations of KSLs will be presented.

**P25. Biodiversity, Geographical Distribution and Phylogenetic Analysis of Geminivirus Associated Alphasatellites from Cotton Crop in Pakistan**

**Muhammad Shafiq**, *University of the Punjab, Lahore, Punjab, Pakistan*

The complete nucleotide sequence of alpha satellite associated with monopartite begomoviruses complex isolated from cotton from Pakistan was determined and evaluated. The present study represent new complexes of these satellite molecules present in nature. Isolation of 8 alpha satellite molecules including 6 from cotton were identified as strains of PaLCuA (Papaya leaf curl alpha satellite) with 90 % homology, one with 98 % similarity score being an isolate of CLCuMA (Cotton leaf curl Multan alpha satellite) and a new strain of GDSA (*Gossypium dawanii* symptomless alpha satellite) with 93 % similarity. These sequences could be a new strains of PaLCuA first time reported from cotton plants in Pakistan. As they were only recently discovered so knowledge about these satellite molecules, their structure and function is limited. Further study could reveal their impact on the host and their role in evolution, survival and diversification of begomoviruses.

**P26. Antifungal Plant Defensins: Mechanisms of Action and Engineering Disease Resistance.**

**Dilip Shah**, *Donald Danforth Plant Science Center, Saint Louis, MO, USA*

Fungal pathogens impose major constraints on crop yields globally. Host defense peptides have evolved to protect plants from pathogen attack. Defensins are cysteine-rich antifungal peptides expressed in all plants. They exhibit potent antifungal activity and therefore have potential for use in transgenic crops for enhanced resistance to fungal pathogens. MtDef4 and MtDef5 are two sequence-divergent apoplast-localized defensins expressed in *Medicago truncatula*. MtDef4 is a monomeric defensin of 47 amino acids, whereas MtDef5 is a novel bi-domain defensin containing two monomeric domains linked by a 7-amino acid peptide. They differ from each other in sequence, net charge and hydrophobicity. MtDef4 inhibits the growth of several filamentous fungi including *Fusarium graminearum* at micromolar concentrations. In contrast, the bi-domain MtDef5 inhibits the growth of these fungi at submicromolar concentrations. Sequence motifs governing the antifungal activity of these defensins have been identified. MtDef4 and MtDef5 permeabilize the plasma membrane of fungal pathogens. They translocate into fungal cells, but use spatially distinct modes of entry and localize to different subcellular compartments. MtDef4 and MtDef5 bind to different plasma membrane resident phospholipids in fungal cells and disrupt the plasma membrane. MtDef5, but not MtDef4, forms oligomers in presence of phosphatidylinositol monophosphates. MtDef4 and MtDef5 exhibit different modes of antifungal action and show promise as novel antifungal agents in crops. Aflatoxins, secondary metabolites produced by *Aspergillus flavus*, are extremely toxic carcinogenic compounds. Aflatoxin contamination of peanuts poses a major threat to public health in sub-Saharan Africa and Asia. Transgenic peanut lines expressing MtDef4 have been generated. Peanut seeds expressing this defensin exhibit strong resistance to *A. flavus* and accumulate extremely low levels of aflatoxins. This is the first study to demonstrate highly effective biotechnological strategy for generating peanuts that are near-immune to aflatoxin contamination, offering a panacea for food safety for people in developing countries.

**P27. Targeted subfield switchgrass integration could improve the farm economy, water quality, and bioenergy feedstock production**

**Emily Heaton**, *Iowa State University, Ames, IA, USA*

Progress on reducing nutrient loss from annual croplands has been hampered by perceived conflicts between short-term profitability and long-term stewardship, but these may be overcome through strategic

integration of perennial crops. Perennial biomass crops like switchgrass can mitigate nitrate-nitrogen (NO<sub>3</sub>-N) leaching, address bioenergy feedstock targets, and - as a lower-cost management alternative to annual crops (i.e., corn, soybeans) - may also improve farm profitability. We analyzed publicly available environmental, agronomic, and economic data with two integrated models: a subfield agroecosystem management model, Landscape Environmental Assessment Framework (LEAF), and a process-based biogeochemical model, DeNitrification-DeComposition (DNDC). We constructed a factorial combination of profitability and NO<sub>3</sub>-N leaching thresholds and simulated targeted switchgrass integration into corn/soybean cropland in the agricultural state of Iowa, USA. For each combination, we modeled (i) area converted to switchgrass, (ii) switchgrass biomass production, and (iii) NO<sub>3</sub>-N leaching reduction. We spatially analyzed two scenarios: converting to switchgrass corn/soybean cropland losing > US\$ 100 ha<sup>-1</sup> and leaching > 50 kg ha<sup>-1</sup> ('conservative' scenario) or losing > US\$ 0 ha<sup>-1</sup> and leaching > 20 kg ha<sup>-1</sup> ('nutrient reduction' scenario). Compared to baseline, the 'conservative' scenario resulted in 12 % of cropland converted to switchgrass, which produced 11 million Mg of biomass and reduced leached NO<sub>3</sub>-N 18 % statewide. The 'nutrient reduction' scenario converted 37 % of cropland to switchgrass, producing 34 million Mg biomass and reducing leached NO<sub>3</sub>-N 38 % statewide. The opportunity to meet joint goals was greatest within watersheds with undulating topography and lower corn/soybean productivity. Our approach bridges the scales at which NO<sub>3</sub>-N loss and profitability are usually considered, and is informed by both mechanistic and empirical understanding. Though approximated, our analysis supports development of farm-level tools that can identify locations where both farm profitability and water quality improvement can be achieved through the strategic integration of perennial vegetation.

#### **P28. Pod Indehiscence – a Key Factor of Soybean Geo-climate Adaptation during Domestication**

**Jiaoping Zhang**, *Iowa State University, Ames, IA, USA*

Loss of pod dehiscence is a key step during soybean [*Glycine max* (L.) Merr.] domestication. Here we report a novel locus containing NST1A, a paralog of SHAT1-5, is associated with pod indehiscence in soybean. Further analysis identified that a single nucleotide substitution leading to a truncated NST1A is associated with the trait. However, the pod indehiscence conferred by NST1A can be overcome by the dehiscence at Pdh1 that is known to control the torsion force of the pod walls. Alleles distribution analysis indicated that the resistance to pod dehiscence among the soybean landraces in China increases from South to North. In Southern China, the indehiscence at NST1A is prevalent, whereas the indehiscent Pdh1 predominates the resistance to shattering at the Huang-Huai-Hai (HHH) valleys. In Northeast China (NEC), however, the indehiscence at both loci is required. Further investigation of the geo-climate adaptation of the indehiscent alleles suggests that the relative humidity is the driving force of above geographic distribution pattern. Additionally, the origin and the distribution of the indehiscent Pdh1 support that HHH valleys, but not NEC, is the center of origin of cultivated soybean. This study demonstrated that soybean domestications have strong topographic signatures as part of the genome adaptation to the environmental conditions. It also provides insights into the soybean expansion and rapid domestication of wild legume species.

#### **P29. CRISPR-based genome editing of grain size regulators in wheat**

**Wanlong Li**, *South Dakota State University, Brookings, SD, USA*

The overarching goal of the IWYP (International Wheat Yield Partnership) is to increase wheat yield by 50% in 20 years, which demands an increase of annual genetic yield gain from the current level of below 1% to at least 1.7%. This quantum gain in genetic yield will require the development of breakthrough approaches and novel genetic resources for wheat improvement. This NIFA-IWYP project aims to develop an improved CRISPR/Cas9 system, create edit mutations for grain size (GS) candidate genes, characterize phenotypic effect of the mutations and transfer them into durum wheat. We have identified and in silico

mapped 45 candidate genes regulating grain size from the wheat and developed two CRISPR platforms for targeting single genes with two guide RNA genes and targeting multiple genes with up to eight guide RNA genes. Using these systems, we generated 13 CRISPR constructs targeting the negative GS regulatory genes for wheat transformation and obtained 44 T0 transgenic plants. From the pgWGS1 transgenic plants targeting CKX2-1, we identified five mutations on the 3AS and 3BS homoeologs among 45 T1 generation plants. This result indicated that our CRISPR system is effective and encourages us to improve its gene editing efficiency. We also established a wheat transformation facility to increase the transgenic plant number. Grain size is the major driver for further growth of wheat yield. The success of this project will not only deepen our understanding of genetic control of wheat grain size but also deliver an improved genome editing system, a package of novel germplasm with enhanced grain yield potential and functional markers, thus significantly contributing to the IWYP's goal. This work is funded by the IWYP grant 2017-67008-25934 from the USDA National Institute of Food and Agriculture.

**P30. Sequencing the sea wheatgrass genome and developing genome-specific markers to transfer biotic stress resistance and abiotic stress tolerance into wheat**

**Wanlong Li**, *South Dakota State University, Brookings, SD, USA*

Wheat production is facing numerous challenges from biotic and abiotic stresses. Alien gene transfer has been an effective approach for wheat germplasm enhancement. Sea wheatgrass (SWG, *Thinopyrum junceiforme*) is a distant relative of wheat and a relatively untapped source for wheat genetic improvement. We identified high tolerance to waterlogging, manganese toxicity, salinity, heat and low nitrogen and resistance to wheat streak mosaic virus (temperature-insensitive), and sawflies (due to solid stem) in SWG and developed large number of SWG-derived populations. This project has two objectives: (1) develop a draft SWG genome assembly for genome-specific markers, and (2) construct a SWG chromosome library in wheat consisting of 14 wheat-SWG addition lines. We have generated 465Gb sequences of the SWG genome and developed a draft assembly of ~24 million contigs with a total assembly size of ~10 Gb. Using the assembled sequences, we have developed 12 SWG-specific PCR markers and localized on seven homoeologous groups. We are screening populations of ~600 plants derived from wheat-SWG backcrosses using the SWG-specific markers and found that >50% of the plants carrying 1 to 2 SWG chromosomes. The SWG-derived populations segregate for numerous traits including spike morphology, male fertility, grain color, grain size, and waterlogging tolerance. In addition to the development of SWG-specific markers, the SWG genome assembly represents an important genomic resource for molecular study of abiotic stress tolerance in the Triticeae crops. This work is funded by the Plant Breeding grant 2017-67014-26210 from the USDA National Institute of Food and Agriculture.

**P31. Uncovering the Genetic Regulation of Seed Amino acid Levels and Composition Using High-throughput Detection Method Combined with GWAS**

**Ruthie Angelovici**, *University of Missouri, Columbia, MO, USA*

Seeds are a major source of protein in human and livestock diets. However, the seeds of major staple crops such as maize, soybeans and rice are deficient in several essential amino acids (EAA). Failure to consume sufficient levels of EAA per day leads to severe malnutrition, even if one's calories requirements are met. In order to improve the amino acid composition in staple crop seeds, we need a more fundamental understanding of the metabolic and genetic basis that underlie their regulation. There are two functional pools of amino acid: the free amino acid pool, which comprises ~5% of the total amino acid in seeds and the protein bound amino acid pool, which comprises ~95% of the total amino acid in seeds. Both pools can be manipulated and contribute to biofortification, nevertheless, the relationship between these two functional pools nor their regulation are understood. To shed light on this issue, we have performed GWAS on both free and total amino acids measured from dry maize seeds taken from a subset of the Goodman

association panel. To this end, we have developed a targeted high throughput method to measure the absolute levels of free and bound amino acid from seed sample using the Multiple reaction monitoring LC-MS/MS approach. Our results show that not only can we uncover part of the genetic architecture of the absolute levels of both free amino acids and protein bound amino acids but we can also detect genetic loci that are significantly associated with the relative ratio between the two functional pools. These loci have the potential to serve as new targets for future biofortification efforts.

**P32. Image-Based Analysis to Dissect Vertical Distribution and Horizontal Asymmetry of Conspecific Root System Interactions in Response to Planting Densities, Nutrients and Root Exudates in *Arabidopsis thaliana***

**Jane Geisler-Lee**, *Southern Illinois University Carbondale, Carbondale, MO, USA*

Intraspecific competition is an important plant interaction that has been studied extensively aboveground, but less so belowground, due to the difficulties in accessing the root system experimentally. Recent in vivo and in situ automatic imaging advances help understand root system architecture. In this study, a portable imaging platform and a scalable transplant technique were applied to test intraspecific competition in *Arabidopsis thaliana*. A single green fluorescent protein labeled plant was placed in the center of a grid of different planting densities of neighboring unlabeled plants or empty spaces, into which different treatments were made to the media. The root system of the central plant showed changes in the vertical distribution with increasing neighbor density, becoming more positively kurtotic, and developing an increasing negative skew with time. Horizontal root distribution was initially asymmetric, but became more evenly circular with time, and mean direction was not affected by the presence of adjacent empty spaces as initially hypothesized. To date, this is the first study to analyze the patterns of both vertical and horizontal growth in conspecific root systems. We present a portable imaging platform with simplicity, accessibility, and scalability, to capture the dynamic interactions of plant root systems.

**P33. Bidirectional Interaction between Plant Root Circadian Clock and Cyst Nematodes**

**Wei Wang**, *Iowa State University, Ames, IA, USA*

The plant circadian clock has emerged as a novel signaling hub integrating diverse pathogenic and immune signals to gate plant immunity and coordinate metabolic needs for growth. While all the discoveries so far were made through studies on leaf pathogens, virtually nothing is known about the interactions between the root circadian clock and root pathogens. However, the soil is where an extraordinary diversity of microbes actually resides, and roots are at the very battle front against soil pathogens. To fill this critical knowledge gap and identify novel molecular targets for breeding and bioengineering to enhance crop resistance to the root pathogens, we initiated a comprehensive study on the bidirectional interactions between root circadian clock and economically important root pathogens, cyst nematodes. Bioinformatics analysis using the “molecular timetable” method suggested that cyst nematodes may perturb the root circadian rhythm in both *Arabidopsis* and soybean. These computational predictions in soybean were further confirmed by targeted sequencing analysis and time-course live imaging using the transgenic soybean hairy root system. On the other hand, soybean resistance to soybean cyst nematode (SCN) showed robust diurnal and circadian rhythms. Using transgenic “clock-dead” soybean hairy root, we found that these diurnal and circadian rhythms depend on soybean root circadian clock rather than the nematode clock. More importantly, “clock-dead” mutant showed more resistance against SCN. Taken together, our results highlighted a critical role of the root circadian clock in the interactions between soybean and SCN.

**P34. Stress Granules-mediated Translational Control of the Plant Immunity**

**Mian Zhou**, *Iowa State University, Ames, IA, USA*

Salicylic acid (SA), a key plant immune signal, induces dramatic transcriptome changes as an important

immune responses. However, SA-induced translational control of immunity remains largely unknown. Here we report that the exogenous SA treatment inhibits translation and triggers the formation of stress granules (SGs), which are assemblies of translational-stalled mRNAs and specific proteins. Surprisingly, this SA-induced SGs formation is independent of both known SA signaling pathway and canonical SGs formation pathway. We further conducted a mass spectrometry experiment using seedlings containing GFP-tagged Rbp47b, a common SG marker protein in Arabidopsis. The result showed that SGs marker proteins, such as UBPI and poly-A binding proteins, as well as specific signaling proteins are recruited into SGs, indicating that SGs may function as a signaling hub to communicate a ‘state of emergency’ and modulate growth and defense accordingly. Consistent with this hypothesis, the mutant of a SG component Tudor Staphylococcal Nuclease (TSN) displays a hyper sensitivity to SA with a compromised growth phenotype. In summary, our study provides another layer of translational regulation of plant immunity, which involves translation repression triggered-SG formation and recruitment of signaling proteins into SGs to provide a cellular protection mechanism against a lethal outcome and ensure a rapid recovery after stress.

### **P35. Analysis of DNA Double Strand Breaks Induced by *Pseudomonas syringae* Virulence Factors**

**Andrew Russell**, *Northern State University, Aberdeen, SD, USA*

Organisms encounter DNA damage from a wide variety of sources including ultraviolet light, genotoxic chemicals, reactive oxygen species, and DNA replication errors. Perhaps the most devastating damage that can occur is a double strand break (DSB) in which both sides of the DNA double helix become severed. Recently, DSBs were shown to occur in plants after challenge with known pathogenic microorganisms including bacteria, fungi, and oomycetes. This phenomenon is also triggered by many animal and human pathogens and may be a common strategy used to inflict damage on host cells. Collectively, these studies give evidence of a conserved pathogenic mechanism; however, we know very little about how microorganisms inflict such damage to the host’s genetic material. To elucidate the molecular basis of how pathogens induce DSBs, I am studying the virulence factors from various pathovars of the plant pathogen *Pseudomonas syringae*. One pathovar, *P. s.* DC3000, has been shown to induce DSBs in the plant model system, *Arabidopsis thaliana*, yet the molecular weapons that cause this damage are unknown. My current approach is two pronged: First, I am inoculating several related pathovars onto *Arabidopsis* in an attempt to find at least one that does not induce DSBs. Second, I am using comparative genomics to catalog genetic differences between these pathovars that provide a potential genetic basis for this phenomenon. Future studies will focus on reconstruction of the molecular interactions necessary for DSBs. The resulting work should provide valuable information about how microorganisms exploit normal cellular processes in their host. Moreover, it will give us a clearer picture of how pathogens establish successful infections in plants, animals, and humans alike.

### **P36. Role of Bacterial Volatiles in Plant Defense Signaling and Disease Resistance**

**Muthu Venkateshwaran**, *University of Wisconsin-Platteville, Platteville, WI, USA*

Chemical control of plant diseases is the most popular management strategy in many crop production regimes. In sustainable agriculture, utilizing host plant resistance is considered as an important component of plant disease management. Volatile Organic Compounds (VOCs) isolated from plant growth promoting rhizobacteria (PGPR) have been shown to confer resistance in plants to a variety of pathogens, leading to reduced disease incidence and severity. However, the molecular mechanisms of VOC-mediated plant defense responses are rudimentary. We hypothesized that the bacterial VOCs act as elicitors of defense signaling by triggering the expression of an array of defense-related genes in plants that play a role in either salicylic acid (SA)- or jasmonic acid (JA)-mediated defense signaling pathways. The role of VOCs as elicitors of defense signaling was investigated through gene expression studies (reverse transcriptase-PCR

and promoter-GUS assays). We also performed pathogenicity assays to evaluate the role of bacterial VOCs in conferring resistance to soybean white mold caused by *Sclerotinia sclerotiarum*. To analyze VOC-mediated defense signaling, we used *Arabidopsis thaliana*, *Medicago truncatula* (dicots) and rice (a monocot) as model systems. Ten different bacterial VOCs were tested for their role as elicitors of defense signaling. The expression profile of genes that are known to be involved in the SA- or JA-mediated defense signaling pathway were monitored in each model plant system. In addition, we used *A. thaliana* seedlings stably transformed with the promoter of defense-related genes fused to the coding sequence of beta-glucuronidase (GUS) to monitor the expression of VOC-induced defense genes using colorimetric assays. Using gene expression studies, plant morphometric analyses and pathogenicity assays, we have identified a set of promising VOCs that can be used as elicitors of plant defense. We will discuss the scope of using biopolymers for the controlled delivery of VOCs to induce plant defense against pathogens.

### **P37. The Role of Hydroxyproline O-Arabinosylation in Flowering Plant Reproduction**

**Cora MacAlister**, *University of Michigan, Grand Rapids, MI, USA*

For flowering plants, sexual reproduction relies on a series of complex and intimate interactions between three distinct parties: the diploid floral tissue of the pistil and the haploid male and female generations (the pollen and embryo sac, respectively). The milieu of the interaction is largely extracellular. While the pollen tube relies on its unique cell wall for rapid elongation and the ability to reorient growth in response to guidance cues, the pistil supports and regulates the growth of compatible pollen through the specialized extracellular matrix of the transmitting tract. Both the pollen tube cell wall and transmitting tract extracellular matrix are composed of polysaccharides and various hydroxyproline-rich glycoproteins. These glycoproteins differ in their amino acid bias, characteristic repeat motifs and types and degree of glycosylation. We have found that hydroxyproline O-arabinosylation (the addition of short chains of arabinose sugars onto hydroxyproline residues) is required for full pollen fertility. Pollen from *Arabidopsis* mutants of the enzymes that initiate or extend the arabinose chain fail to fertilize ~90% of their available ovules leading to poor seed yield. The mutant pollen tubes show reduced elongation, disrupted pollen tube morphology and meandering in vivo growth. Despite this strong effect on male fertility, female fertility and vegetative growth are normal. This modification and the enzymes required for its production are plant specific, but deeply conserved across all plants and green algae. In tomato, two of the four hydroxyproline O-arabinosyltransferase homologues are expressed specifically in pollen and are non-redundantly required for efficient male transmission. We have also found that the functionally distinct regions of the tomato pistil, the stigma, apical and basal style and ovary, also express unique sets of glycoproteins.

### **P38. Integrated Multispectroscopic In Situ Imaging of Plant Metabolism at the Level of Subcellular Compartments**

**Geng Ding**, *Iowa State University, Ames, IA, USA*

Understanding the remodeling of membrane lipid topology in plant cells has major consequence in optimizing plant biomass productivity. The integrated molecular imaging technologies are being developed in the biological context of autophagy, which remodels membrane lipid topologies. These autophagy-induced changes in lipid topologies control spatially defined subcellular regions within plant cells and optimizes plant biomass productivity in response to environmental signals. Genetic stocks that will enable the dissection of membrane lipid dynamics have been identified and analyzed to identify specific target lipid molecules for molecular imaging. Analytic technologies for imaging these specific target lipid molecules via fluorescence, Raman and mass spectroscopy have been established. The imaging technology is being developed in the context of computational capabilities that will integrate multi-spectral images with genome scale modeling and thus contribute to the better understanding how biomass-based biofuel producing metabolic pathways are interconnected and controlled within topological constraints in spatially

defined membrane-bounded regions within plant cells. Thus, this multi-disciplinary project will develop new integrated multi-spectral imaging technologies that will assess and quantitatively model metabolic processes that are non-symmetrically distributed at the cellular and subcellular levels of plant organs.

**P39. Comparative functional genomics of nectaries and nectars in the dicots**

**Clay Carter**, *University of Minnesota, Minnesota, MN, USA*

Plants attract mutualistic animals by offering a reward of nectar. Specifically, floral nectar (FN) is produced to attract pollinators, whereas extrafloral nectar (EFN) mediates indirect defenses through the attraction of mutualist predatory insects to limit herbivory. Nearly 90% of all plant species, including 75% of domesticated crops, benefit from animal-mediated pollination, which is largely facilitated by FN. Moreover, EFN represents one of the few defense mechanisms for which stable effects on plant health and fitness have been demonstrated in multiple systems, and thus plays a crucial role in the resistance phenotype of plants producing it. In spite of its central role in plant-animal interactions, the molecular events involved in the development of both floral and extrafloral nectaries (the glands that produce nectar), as well as the synthesis and secretion of the nectar itself, have been poorly understood until recently. To date, a holistic and coordinated characterization of nectar secretion from a comparative genomic and molecular perspective has been lacking. Toward this end, we have evaluated the transcriptomes and proteomes of floral and extrafloral nectaries throughout development across twelve dicotyledonous species and identified core sets of genes and modules involved in the synthesis and secretion of nectar across species, as well as its regulation. Similarly, metabolite profiling coupled with transcriptomic data has identified specific loci responsible for different nectar characteristics that influence mutualist visitation.

**P40. Exploring the synergistic bioactivity of major constituents in plant essential oils against *Aedes aegypti***

**Maria Archevald-Cansobre**, *Iowa State University, Ames, IA, USA*

The abundant availability of plant essential oils worldwide facilitates the production of novel insecticidal formulations comprised of essential oils as starting materials. The development of high-quality insecticidal formulations is crucial for the control of mosquitoes, which is an important goal given their potential to vector disease agents. Mosquitoes are responsible for hundreds of thousands of deaths every year because of their ability to transmit fatal disease agents that cause malaria, dengue, yellow fever, and many more. Even more concerning is the rise in insecticide-resistant mosquito populations throughout the world, driving a critical need for new insecticidal formulations. Plant essential oils have demonstrated effective bioactivity against pest insects, mainly attributed to some of their major constituents. In the case of synthetic insecticide formulations, insecticidal activity is specifically attributed to the active component(s), but it is possible that less active components in plant essential oils might facilitate the insecticidal activity of the major constituents. Throughout this study, we assessed the individual efficacy of some of these major constituents against *Aedes aegypti* mosquitoes. Ultimately, our results yielded important insights into the synergy among various constituents within each oil. Despite the vast challenges facing the commercialization of plant oil based insecticides, it is important to consider and address these key questions regarding their bioactivity in order to produce optimal insecticidal mixtures in future formulations.

**P41. Herbivore Derived Fatty Acid Amide Elicitors Induce a Reactive Oxygen Species Burst in Plants**

**Anna Block**, *USDA-ARS-CMAVE, Gainesville, FL, USA*

The formation of a reactive oxygen species (ROS) burst is a central response of plants to many forms of stress including pathogen attack, several abiotic stresses, damage and insect infestation. These ROS act as a direct defense as well as signaling and regulatory molecules. Perception of microbe or damage associated



elicitors triggers an ROS burst in many plant species. In this study we show that the Lepidopteran derived fatty acid-amide elicitor N-linolenoyl-L-glutamine can induce an ROS burst in a diverse range of plants. Furthermore, in Arabidopsis, this ROS burst was partially dependent on the plasma membrane localized NADPH oxidases (RBOHD and RBOHF). Interestingly, an rbohD/F double mutant displays enhanced resistance to infestation with generalist caterpillars, indicating that induced ROS production may negatively regulate anti-herbivore defenses.

**P42. Unveiling the Synergistic Effect of Pyramiding Rag1 and Rag2 Aphid-resistance Genes in Soybean**

**Martha Ibore**, *Iowa State University, Ames, IA, USA*

Soybean aphids are phloem feeding insect pests of soybean. Aphids divert plant assimilates for their nutrition, causing yield losses of up to 50%. One of the management options for soybean aphids is cultivation of resistant soybean varieties. Aphid resistance in soybean is conferred by Resistance to Aphis glycines (Rag) genes with Rag1 and Rag2 being the most studied to date. Under insect attack, plants respond by inducing defenses which primarily consist of morphological, biochemical and molecular mechanisms. Previous studies showed that aphid populations were significantly lower in the double pyramid (Rag1Rag2) genotypes compared to soybean genotypes that contained the Rag1 or Rag2 gene alone. We hypothesized that pyramiding Rag1 and Rag2 in one soybean genotype had an additive or synergistic effect on aphid resistance at the molecular level. To test this idea, the transcriptional response of four near isogenic soybean genotypes (one aphid-susceptible and three aphid-resistant: Rag1, Rag2 and Rag1Rag2) to aphid feeding was measured at 6 and 12 hours, using RNA sequencing. Comparison of mock-treated samples versus aphid-treated samples for each soybean genotype showed that the transcriptional response to feeding by soybean aphids involved unique sets of genes and shared genes respectively at both time points. Biological processes modified due to aphid feeding were distinctive for each of the soybean genotypes at 6 hours but similar among them at the 12 hour time point. The transcriptional response of the Rag1Rag2 soybean genotype to feeding by soybean aphids at both time points involved unique sets of genes that were absent in the Rag1 or Rag2 response; suggesting that pyramiding the Rag1 and Rag2 genes in one soybean genotype has a synergistic effect on aphid resistance at the molecular level. This research will build the knowledge base on aphid resistance, contributing to better management of soybean aphids and optimization of soybean yields.

**P43. A High Oil Soybean Mutant Contains a 300 kb Deletion on Chromosome 14**

**William Serson**, *Ave Maria University, Naples, FL, USA*

Since the dawn of agriculture humans have been genetically modifying crop plants to increase yield, quality and utility. In addition to selective breeding and hybridization we can utilize mutant populations and biotechnology to have greater control over crop plant modification than ever before. Increasing the production of plant oils such as soybean oil as a renewable resource for food and fuel is valuable. Successful breeding for higher oil levels in soybean, however, usually results in reduced protein, a second valuable seed component. We have screened a soybean fast neutrino population derived from M92-220 variety and found three high oil mutants with minimal reductions in protein levels. From 11 F2 plant populations, we quantitatively pooled the high oil and low oil plants and performed comparative genomics hybridization (CGH) of three of these lines which showed significant phenotypic variation for oil content, indicative of segregation of a trait linked to oil content. Of the three populations analyzed, two sib lines have a 300 kb deletion in chromosome 14, which contains twenty genes, including a putative enoyl-CoA hydratase. Enoyl-CoA hydratases catalyze a step in fatty acid beta oxidation, a reduction in which may be responsible for the increase in oil content. This large deletion event leads to an increase in oil content and has genes in it which could be implicated in oil biochemistry.

**P44. Evaluating Loss of Motility in Tomato-Infecting *Xanthomonas* Strains**

**Tanvi Majumdar**, *University of Illinois at Urbana-Champaign, Champaign, IL, USA*

Evaluating Loss of Motility in Tomato-Infecting *Xanthomonas* Strains Motility in plant bacterial pathogens plays a crucial role in pathogen virulence as structures such as flagella enable bacterial movement around their environment and within the plant host. However, flagella can also be recognized by the host and trigger host immunity. Bacterial species in the genus *Xanthomonas* commonly infect a wide variety of plant species across the globe, including causing bacterial spot disease in tomato and pepper plants. This research project seeks to characterize loss of motility in some tomato-infecting strains of *Xanthomonas* (i.e. *X. euvesicatoria*, *X. vesicatoria*, *X. gardneri*, and *X. perforans*) and evaluate how motility relates to pathogen virulence. Possible reasons for the loss of motility include: loss of flagellum functionality due to amino acid changes in the flagellin protein, absence of the flagellum structure, or changes in gene expression of flagellum-encoding genes. Using swim assays, the motility of 38 strains was characterized. DNA sequencing analysis of the *fliC* genes for these strains revealed six different alleles across the four species. However, we do not observe a correlation between the alleles and the motility status, suggesting another factor is likely responsible for the altered motility. Analysis of flagellin protein abundance for these strains also does not reveal a correlation with motility. We are using microscopy analysis to observe motility and visualize the flagellum structure for several motile and non-motile strains, and future experiments may include examining gene expression of flagellum-related genes under different experimental conditions. We also may examine the potential role of chemotaxis in controlling *Xanthomonas* motility. Overall, our results reveal that allelic differences may not be responsible for motility loss, suggesting that additional factors (such as environmental conditions) may play a large role in controlling *Xanthomonas* motility.

**P45. Reverse Genetic Approaches to Understanding the Role of Auxin in Maize Development**

**Joseph Struttmann**, *University of Missouri Columbia, Columbia, MO, USA*

The growth hormone auxin regulates nearly all aspects of plant development. Therefore, a better understanding of the genes controlling auxin biosynthesis, transport, and perception is fundamentally important to basic plant biology with applications in crop improvement. Previous phylogenetic and expression analyses have demonstrated both conservation and diversification of the role of auxin in maize and *Arabidopsis* development. We are using maize vegetative and reproductive development as a model to further understand how auxin regulates development using both forward and reverse genetic approaches. Reverse genetic analyses using transposon insertions and CRISPR technology coupled with higher order mutant phenotyping are revealing a more complex network than previously expected. Results from the *ZmPIN* and *ZmTIR/AFB* gene families, involved in auxin transport and perception respectively, will be presented.

**P46. Investigating Catalytic Site of a Plastidial Putative Rhomboid Protease**

**Oliva Baylis**, *Michigan State University, East Lansing, MI, USA*

The plant species *Arabidopsis thaliana* uses two pathways equally when synthesizing glycerolipids; one pathway utilizes the endoplasmic reticulum (ER) where the other synthesizes lipids in the chloroplast. Rhomboid-like protein 10 (RBL10), a chloroplast localized protein, was previously investigated because of its potential role in *Arabidopsis thaliana* flower development. More recently, alterations in galactolipid metabolism of *rb110* mutants was uncovered (unpublished). RBL10, is a six-pass transmembrane protein, possessing conserved domains characteristic of an active rhomboid protease, as well as a predicted serine-histidine catalytic dyad. Although it is known that this protein is predicted to be a rhomboid protease, this

still remains to be confirmed with in vitro activity assays. Site-directed mutagenesis (Q5 Mutagenesis Kit, NEB) was used to create a serine 240 and histidine 293 to alanine double mutant as well as a histidine 293 to alanine single mutant of the RBL10 protein. These two residues are the predicted active residues; therefore, altering the residues would result in a lack of RBL10 function. One overarching goal of protein mutagenesis in RBL10 is to produce both the double mutant and the single histidine mutant proteins in the *rb110* mutant background. From this, we will be able to see if these mutant proteins will restore the galactolipid phenotype of the *rb110* mutant plants. Additionally, both constructs will be expressed in *Nicotiana benthamiana* for protein purification and in vitro protease activity assays. So far, a successful histidine 293 single mutant along with a successful serine 240 and histidine 293 double mutant have been generated, but have yet to be tested. Upon confirming the proteolytic nature of RBL10, this study will be the first description of a true rhomboid protease participating in plant lipid metabolism.

**P47. Analysis of COP9 Signalosome Mutants Reveals a Role of the CSN in Ethylene Sensitivity in *Arabidopsis thaliana***

**Steven McKenzie**, *Grand Valley State University, Allendale, MI, USA*

Plants use ethylene for a variety of hormonal functions, including leaf senescence and abscission, fruit ripening, and seedling development. The COP9 signalosome (CSN) is a metalloprotease involved in E3 ubiquitin ligase deactivation. Under normal circumstances, the CSN cleaves NEDD8 proteins covalently attached to cullin-based E3 ubiquitin ligases, resulting in their deactivation. In many CSN mutants, pleiotropic developmental phenotypes are observed that are seedling lethal. Although these severe phenotypes are observed in these plants, a distinct auxin related phenotype is observed in the redundant CSN catalytic subunit mutants. We have uncovered some evidence that the redundant CSN catalytic subunits may play a role in ethylene responses. In CSN5b subunit mutant *Arabidopsis thaliana* plants, we have observed ethylene insensitivity in dark grown seedlings. We are also testing several CSN inhibitors on *Arabidopsis* growth and ethylene responses in Col-0 wildtype seedlings. To further analyze the role of these subunits, we plan on assessing the phenotypes in double mutants (*csn5/csn6* mutants). With this research we hope to further understand the role of the CSN in plant development and specific plant hormone responses.

**P48. Probing the Biochemistry of an Unusual Fatty Acid Desaturase**

**Montgomery Smith**, *Michigan State University, East Lansing, MI, USA*

Photosynthesis is the natural biochemical conversion of sunlight into chemical energy. The photosynthetic thylakoid membranes within chloroplasts are comprised of galactolipids and the phospholipid phosphatidylglycerol (PG). Although researchers have extensively studied several of these lipid classes and individual species, we only have an elementary understanding of their roles and synthesis. A particularly unusual fatty acid, 16:1 $\Delta$ 3trans (16:1t), is synthesized by FATTY ACID DESATURASE4 (FAD4) and found only in chloroplast PG. We determined that, in planta, 16:1t synthesis requires both FAD4 and a thylakoid-associated redox protein, PEROXIREDOXIN Q (PRXQ). To determine the role of PRXQ in 16:1t synthesis we utilized biochemical and genetic approaches in *Arabidopsis thaliana* and *Saccharomyces cerevisiae* (yeast). In *Arabidopsis* and yeast, only when FAD4 and PRXQ are co-expressed did we see a trans fatty acid accumulate in the endogenous lipids, as confirmed by gas chromatography and thin layer chromatography. As the specific mechanism for FAD4-mediated 16:1t synthesis is unknown we tested several conserved FAD4 residues by site-directed mutagenesis for their contributions to 16:1t synthesis. These results suggest that FAD4 may require a disulfide bond between monomers to form an active dimer form of the enzyme and requires PRXQ for this dimerization process. As PRXQ's primary attributed intracellular role is the reduction of toxic hydrogen peroxide molecules to water we worked to determine whether FAD4 synthesis requires some form of reactive oxygen species in 16:1t synthesis. Understanding

the connection between these redox pathways and 16:1t synthesis will help further describe the synthesis and role of 16:1t in plants and enable future experiments engineering the photosynthetic membrane for improved agricultural production and sustainability.

**P49. Natural variation in boron content in *Zea mays* and its implications on seedling development**

**Skyler Kramer**, *University of Missouri, Columbia, MO, USA*

Boron (B) plays a significant role in species across all domains of life. In plants, B is essential for proper growth by forming dimers rhamnogalacturonan II (RG-II) in the cell wall. Other studies have proposed that B also influences several steps of the plant life cycle, including membrane-specific, hormonal, and reproductive processes in plants. Regardless, a lack of this essential micronutrient in soil negatively affects crop yield. The present study aims to help answer basic questions concerning B in maize. Plant growth is dependent on a relatively low B content, but that actual value varies between plant species. The natural variation of B content in several inbred maize lines will be obtained via the dry-ashing method on ear leaf tissue from mature plants. Because all plants were grown with the same B concentration in the soil, this analysis will help determine which of the tested maize lines most effectively take up B. A second set of experiments will analyze several inbred lines with different B contents, regarding their performance under conditions that mimic B deficiency. Phenylboronic acid (PBA) is known to compete with naturally occurring boronic acid for binding sites, mimicking B deficiency and preventing cross-links of RG-II in the cell wall. A series of seedling assays are currently being performed, and both the primary root and shoot growth of the seedlings will be analyzed. Pilot studies have already shown that elevated concentrations of PBA lead to shorter shoot and root lengths. The insights obtained by these analyses could be practically used to determine which lines best take up B from soil, aiding farmers in crop selection prior to the growing season. The next step would be to use a Genome-Wide Association Study (GWAS) to target the part of the genome that contains the genetic variations responsible for these differences.

**P50. Modulation of GGPPS11 Phenotypes**

**Toria Trost**, *Southern Illinois University Edwardsville, Edwardsville, IL, USA*

Geranylgeranyl diphosphate synthase (GGPPS) is an enzyme that operates in the isoprenoid pathway in plants. This pathway is important for producing precursors for carotenoids and chlorophyll. There are twelve members in the GGPPS family in *Arabidopsis thaliana*. Several of these enzymes are targeted to the chloroplast, while others reside in a different organelle, such as the mitochondria or the endoplasmic reticulum. The most ubiquitously expressed of these enzymes is GGPPS11. A specific point-mutation in this gene (ggpps11-1), leads to a variegated leaf phenotype with green on the edges and a white or yellow color in the middle, while insertion mutations within this gene are lethal. The goal of this work is to determine if the other GGPPS family members demonstrate the enzymatic activity required to replace GGPPS11. We are attempting to rescue the ggpps11-1 mutant by overexpressing other gene family members with a chloroplast targeting sequence. A gateway entry vector for each gene is being produced via cloning and will be transformed into the destination vector pEARLYGATE103. Transformed ggpps11-1 plants will be examined for phenotypic rescue. In addition, this work is also examining the impact of ABA on the severity of the ggpps11-1 phenotype. Because the variegated phenotype is temperature sensitive, we hypothesize that ABA-induced closure of stomata may exacerbate the size of the white sectors in the leaves. To test this, ggpps11-1 and wild-type plants were sprayed with ABA daily for 3 weeks. Chlorophyll and carotenoid levels, as well as the size of white sectors, was measured. Preliminary results indicate that exogenous ABA application does inhibit the activity of the ggpps11-1 enzyme.

**P51. Differential Expression of the Alfin Gene Family Members Among Maize Inbred Lines**

**Diane Janick-Buckner**, *Truman State University, Kirksville, MO, USA*

The Alfin gene family, first described in *Medicago sativa*, encodes transcription factors involved in salt tolerance. ALFIN proteins contain an Alfin domain at the N-terminus and a C terminal Zinc-finger. We have identified 16 Alfin gene family members in maize. Using the nomenclature described by Song et al. (2013) for Arabidopsis, our phylogenetic analysis separated the Alfin gene family members into three groups (Group I, II and III/IV). Using publicly available RNASeq data sets, we analyzed the expression of each Alfin gene family member in B73 and Mo17 root zones, as well as in B73, Mo17, and W22 ear, leaf, and stem. These analyses identified Alfin gene family members that were differentially regulated among inbred lines and tissues. Using quantitative reverse transcriptase-PCR, we also examined the expression of Alfin Group II members in B73, Mo17 and W22 14-day above ground tissues, as well as 5-day seedling primary root zones. These analyses further support that the Alfin Group II family members are differentially expressed among the inbred lines and tissues.

**P52. Evolutionary History and Expression of the LONESOME HIGHWAY Transcription Factor Gene Family in Maize**

**Terra Willard**, *Truman State University, Kirksville, MO, USA*

The LONESOME HIGHWAY (LHW) and LONESOME HIGHWAY-LIKE (LHL) genes encode atypical basic helix-loop-helix transcription factors involved in regulating vascular development in root and above ground tissues. This gene family was first identified in Arabidopsis and shown to have four paralogs. We have identified four orthologs of the AtLHW gene and two orthologs of the AtLHL3 gene in the maize genome. Using quantitative reverse transcriptase-PCR (qRT-PCR), the expression of all gene family members in maize were examined in above ground tissue of 14-day B73, Mo17, and W22 seedlings. Significant differences were found in the expression of these paralogs among the inbred lines. In addition, we performed qRT-PCR with cDNA prepared from primary root zones of dark grown 5-day seedlings and observed differential expression of ZmLHW and ZmLHL3 paralogs. Using publicly available maize RNAseq datasets, we have also analyzed the expression of the LONESOME HIGHWAY gene family members in leaf, stem and developing ear tissue, as well as 5-day primary root zones. These analyses further support that the LONESOME HIGHWAY gene family members are differentially expressed in the different tissues.

**P53. The Evolutionary History and Transcriptomic Analysis of the Phytoene Synthase Gene Family and Related Paralogs In Maize.**

**Megan Neveau**, *Truman State University, Kirksville, MO, USA*

In plants carotenoids are yellow-orange pigments that protect chlorophyll from photooxidation and serve as accessory light harvesting pigments. The phytoene synthase (psy) gene family encodes enzymes that catalyze the condensation of two molecules of geranylgeranyl pyrophosphate into phytoene, which is the first committed step in carotenoid biosynthesis. In maize, phytoene synthase is encoded by three subfunctionalized genes psy1, psy2 and psy3 that have been well characterized. The maize genome database identifies an additional 5 gene models as phytoene synthase. Our phylogenetic and bioinformatics analyses do not support annotating these 5 gene models as phytoene synthase. Using publicly available RNASeq data sets, we analyzed the expression of psy1, psy2 and psy3 as well as the other gene family members in various tissues and inbred maize lines. Significant differences in the expression of psy genes among the inbred lines and tissues were identified.

**P54. Syn-tasiRNA: One more step forward to unraveling the biogenesis of 22nt tasiRNAs**

**Jennifer Probst**, *Missouri State University, Columbia, MO, USA*

Small RNAs (sRNA) are key regulators of gene expression and control many biological processes, by mediating gene silencing at transcriptional or post-transcriptional level. sRNAs are 19-24 nt long and can

be divided in two classes, microRNAs (miRNAs) and small interfering RNAs (siRNAs), which are distinguished by their biogenesis and mode of action. It has been demonstrated that 22nt long sRNAs trigger the production of secondary siRNA, rather than the more typical siRNAs and miRNAs that are 21nt in length. Trans-acting siRNA (tasiRNA) direct RNA slicing in a sequence specific manner in trans. Analysis of eudicot tasiRNAs that consistently generate at least one 22-nt tasiRNA reveal that a 3' AC or AU causes DCL4 to generate a 22-nt tasiRNA. In order to experimentally test this observation, we design different synthetic tasiRNA (syn-tasiRNA) construct to produce 21 or 22nt tasiRNAs with the ability to target GFP-tagged viruses. In this study, we screened Arabidopsis thaliana lines that had been transformed with synthetic tasiRNA constructs from the TAS1c gene using Columbia-0 and ago2 backgrounds. We were able to transform, grow, and genotype 32 plants corresponding to 14 different constructs. We were able to experimentally determine, using Illumina sequencing, that the presence of a 3' AC produces a single nucleotide shift in the cut pattern of tasiRNA and production of a 22mer tasiRNAs. In the future, we will perform viral infection studies using lines characterized by this research to determine if the production of 22nt vs 21nt syn-tasiRNAs confers increased resistance to GFP-fused viruses.

**P55. How does Early Kernel Growth and Development Relate to Mature Kernel Size: A Case Study using Krug Divergently Selected Maize**

**Devon Leroux**, *Central Michigan University, Mount Pleasant, MI, USA*

Cereals grains including maize have global economic importance as they provide a large proportion of the world's calories. Although crop yield is determined by seed number and seed size, improvements to crop yield have often centered on seed size. The yellow dent cultivar Krug (KCO) was divergently selected for seed size to produce large seed (KLS30) and small seed (KSS30) populations that have a 5-fold difference in mature kernel size. Phenotypic and transcriptome analysis indicate that KSS30 kernels are smaller early in development and transition from the lag to linear phase earlier than KLS30 kernels. Moreover, KSS30 switches to starch biosynthesis and proceeds to seed maturation earlier than KLS30. In this study, we investigated how the kernel and its component parts, i.e. nucellus, endosperm, and embryo grow and develop during the early lag phase. KSS30 and KLS30 seeds were harvested at 0-12 DAP and kernel, nucellus, endosperm, and embryo areas were measured from median longitudinal sections of the kernel. KLS30 kernels were larger in early development and their nucellus was larger and persisted longer than KSS30 kernels. However, their endosperm did not differ from that of KSS30 kernels in size or rate of endosperm cellularization. KLS30 endosperm accumulated starch later. This work suggests that although at maturity kernel size in maize is driven by endosperm size (which accounts for 80% of kernel weight), during earliest development the size and growth of the nucellus generates the larger kernel. As this larger nucellus is depleted, the endosperm grows to occupy the nucellus space and produces the larger seed.

**P56. The role of maize mutant Suppressor of sessile spikelets 2 (Sos2) in meristem maintenance**

**Katherine Guthrie**, *University of Missouri, Columbia, MO, USA*

Plant development is driven by a group of undifferentiated stem cells, called meristems. During maize reproductive development, the shoot apical meristem (SAM), responsible for above ground growth, transitions into an inflorescence meristem (IM) which then produces a series of meristems along the periphery to give rise to the male reproductive structure called the tassel. The female reproductive structures, or ears, arise similarly from elongation of axillary meristems half-way down the stem. This developmental progression requires a fine balance of stem cell proliferation and differentiation, referred to as meristem maintenance. My research focuses on the semi-dominant maize mutant Suppressor of sessile spikelets 2 (Sos2), which has defects in meristem maintenance in all above ground meristem types, resulting in altered meristem size and timing of termination. Sos2 heterozygous plants have decreased branching in the tassel and suppression of spikelets, the short flower-bearing branches, in the tassel and the

ear. If a tassel is formed in *Sos2* homozygotes, it is short and bifurcated and ears are small and ball shaped. To determine the *Sos2* gene function, we have analyzed double mutants of *Sos2* with *CLAVATA* pathway mutants, previously shown to play a role in meristem maintenance. In addition, fluorescent microscopy of *ZmWUSCHEL:RFP* transgenic marker lines was used to characterize the stem cell niche of *Sos2* mutants. The region *Sos2* maps on chromosome 10 has been sequenced to identify candidate genes. Our results indicate that *Sos2* plays an important role in meristem maintenance throughout plant development.

**P57. Discovering Freezing Tolerance Genes from the Native Orchid *Aplectrum hyemale***

**Rasika Mudalig-Jayawickrama**, *University of Dubuque, Dubuque, IA, USA*

In our quest to find native orchids in Dubuque area, we came across an unusual orchid, *Aplectrum hyemale* (putty root), which show exactly the opposite of normal winter response. This unusual orchid produces a functional green leaf in mid-October and keeps this leaf viable until the next spring. Our main objective was to monitor the physicochemical changes of this plant and identify the genes that are upregulated during the cold-acclimatization period. We monitored the amount of chlorophyll, anthocyanin and the expression of cold-induced genes in putty-root leaves. Chlorophylls were extracted by immersing leaf samples in dimethylformamide (DMF) overnight. Anthocyanins were extracted by grinding 100 mg of leaf sample in liquid nitrogen and extracting with 1% HCl in methanol at -20°C in dark. The amounts of chlorophyll and anthocyanin was quantified using spectrophotometer. A cDNA suppressive subtractive hybridization (SSH) was performed between leaf samples collected before and after freezing. The results indicate that the amount of chlorophyll remain high throughout the winter even when the leaf is under the snow. The anthocyanin concentration is very high as the leaf emerges from the tuber in early October and slowly reduces to lower steady levels. We speculate that anthocyanin may protect the young leaf from high sunlight as the canopy opens up in fall. The results of SSH indicated six unique DNA bands in the cold-induced sample. Some of the key cold-induced genes code for glyceraldehyde-3 phosphate dehydrogenase (GA3PD) enzyme, C2domain/GRAM domain containing protein, MADS/FLC like protein, WD40 repeat containing protein and many chloroplast localized proteins. Their significance and future plans will be discussed.

**P58. Identifying Carbohydrate Partitioning Defective 28/47**

**Kyle Conner**, *University of Missouri, Columbia, MO, USA*

The process of converting inorganic carbon dioxide into carbohydrates predominantly occurs in photosynthetic leaves (source tissue). These carbohydrates are allocated to various non-photosynthetic sink tissues (e.g., roots, stems, flowers, and fruits) for proper growth and development, in a process termed carbohydrate partitioning. While much is known about the physiology of carbon transport, we have little knowledge of the genetics that govern it. Therefore, we are taking a genetic approach to identify mutants unable to properly transport sugars, which we call carbohydrate partitioning defective (cpd). To identify cpd mutants, we screened for plants displaying visible phenotypes suggestive of inhibited sugar transport: leaf chlorosis, starch accumulation in leaves, and decreased plant growth. Two mutants displaying these phenotypes, cpd28 and cpd47, were found to allelic through complementation testing. In addition to the visible phenotypes, cpd47 exhibits increased lignin deposition in the phloem of major and minor veins, and decreased leaf chlorophyll levels, stomatal conductance, and photosynthetic rates. Through bulk segregant analysis (BSA), we determined that the mutations responsible for the cpd28/47 mutants were located on the long arm of chromosome 1. Genetic fine-mapping enabled us to narrow this region. To pinpoint the causative gene, whole genome sequencing was performed on pools of cpd28 and cpd47 mutants, and an analysis of the SNPs between each mutant and the B73 reference genome resulted in the identification of a single gene. Further research and understanding of the gene's function will help provide knowledge that will aid in future advancements in increasing crop yield and growth.

**P59. Data Analysis of Maize Edited by CRISPR Systems**

**Jonah Miller**, *Iowa State University, Ames, IA, USA*

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) proteins are naturally-occurring defense systems found in bacteria. SpCas9 from *Streptococcus pyogenes* and CRISPR from *Prevotella* and *Francisella* 1(Cpf1) are the two most powerful RNA-guided nucleases that have been adopted for genome editing. Dr. Keunsub Lee has been editing the maize Glossy 2 gene (ZmGl2) with both SpCas9 and LbCpf1. To aid his research, I have analyzed the T1 generation of the transgenic maize plants to screen for mutations induced by SpCas9 and LbCpf1, using a variety of methods. These include simple phenotype screening, polymerase chain reaction (PCR) analysis, sanger sequencing, and Tracking of Indels by Decomposition (TIDE) analysis. These tests have given data on the genome of the edited plants at both the macroscopic and molecular levels. Current findings show that both SpCas9 and LbCpf1 are viable means of editing the maize genome.

**P60. Arabidopsis Plants Expressing a Fungal Pectin Methylesterase Exhibit Dwarfism and Resistance to Stresses**

**Lauran Chambers**, *Iowa State University, Ames, IA, USA*

Pectin is a major component of the plant cell wall and is involved in functions including growth of the plant cell, organ development, and defense against pathogens. Our lab has developed a suite of transgenic *Arabidopsis thaliana* plants expressing microbial-derived cell wall-degrading enzymes to study Cell Wall Integrity (CWI) responses. Plants expressing one such enzyme, *A. nidulans* Pectin Methylesterase (AnPME), exhibit dwarfism beginning with emergence of the first true leaves and affecting all specialized organs and structures throughout their lifetime. The cell wall analysis demonstrated that PME expressing plants have significantly reduced degree of methylation, lower content of galacturonic acid and higher content of arabinose in their pectins in comparison with wild type plants. The cell wall digestibility was analyzed using cellulase and pectinase assays, which showed no significant difference in cellulose digestion but a significantly higher digestion of pectins in the AnPME plants compared to the wild-type plant. These plants also possess reduced sensitivity to salt stress; while roots of AnPME plants are much smaller than wild-type roots under unstressed conditions, root growth was not inhibited by 100 mM NaCl treatment in contrast to wild type plants. Despite higher digestibility of pectins, the AnPME plants do not show difference in susceptibility to fungal pathogen, *B. cinerea*, which was corroborated with upregulation of several defense-related genes. Ongoing research aims to quantify the degree of cell wall de-methylesterification to correlate with degree of dwarfism, and further investigation of impact of this cell wall modification on plant stress responses. These results illustrate the importance of pectin methylesterification status in plant fitness and development, as well as response to both biotic and abiotic stresses, most likely through CWI response.

**P61. Salicylic Acid-Induced Freezing Tolerance in Spinach (*Spinacia oleracea* L.) Leaves Explored through Metabolite Profiling**

**Kyungwon Min**, *Iowa State University, Ames, IA, USA*

To investigate the effect of salicylic acid (SA) on plant freezing tolerance (FT) we applied 0.5 mM SA with sub-fertigation to two-week old *Spinacia oleracea* L. 'Reflect' seedlings grown under ambient (non-acclimated) conditions (NASA). A week later NASA and NA (fertigated controls) plants were subjected to 'whole-plant freeze-thaw' cycle and injury was assessed visually and by ion-leakage. NASA leaves accumulated ~60% higher SA and were more freeze-tolerant than NA. Comparative metabolomics revealed NASA leaves had higher trehalose, ascorbic acid,  $\gamma$ -tocopherol, proline, and leucine, whereas lower mannose and aconitic acid than NA. To investigate effect of SA on FT and metabolism at warm (NASA) vs. cold, plants were subjected to 9-d cold acclimation without or with SA-feeding (CA, CASA,



respectively). CASA leaves were most freeze-tolerant of four conditions followed, respectively, by CA, NASA, and NA. SA improved FT, measured as LT50, by 0.7 °C and 1.4 °C under warm and cold, respectively, and principle component analysis distinctly separated metabolic phenotypes for four conditions, indicating SA differentially affected FT and metabolism at warm vs. cold. CASA leaves accumulated higher concentrations of SA, compatible solutes and antioxidants than CA tissues. Pair-wise comparisons of NASA / NA, CA / NA, and CASA / NA indicate changes in trehalose, ascorbic acid, and aconitic acid were SA-specific and to a greater extent in cold vs. warm. Additionally, 7 metabolites (5-oxoproline, fructose, glucose, maltose, proline, sucrose, and tartaric acid) were quantitatively associated with the FT levels across four conditions.

**P62. Analyses of Berberine Bridge Enzyme-like Family Genes Potentially Involved in Leaf Development**

**Allison Newton**, *Southern Illinois University, Edwardsville, IL, USA*

In plants, the synthesis of isoprenoids and isoprenoid-related compounds such as chlorophyll, carotenoids, tocopherols, phytoalexins, and gibberellins require the GERANYLGERANYL DIPHOSPHATE SYNTHASE (GGPPS) gene family. In *Arabidopsis thaliana* (At), a point mutation in one member of this family, *ggpps11*, results in rounded, variegated rosette and cauline leaves with irregular margins. The variegation patterning is typically reproducible, with the center of the leaves being albino and the periphery phenotypically wild type. We have compared the transcriptome of wild type, *ggpps11* white sectors, and *ggpps11* green sectors. This analysis revealed a group of twelve Berberine Bridge Enzyme-like (BBE-like) proteins which are differentially regulated between the two mutant tissues. Although At does not produce endogenous berberine, exogenous application of berberine leads to pointy leaves, likely resulting from inhibition of adaxial cell differentiation by berberine, causing the leaf-polarity defects. To identify how these genes are involved in leaf development and polarity, T-DNA lines with individual mutations in the BBE-like genes were identified and obtained as segregating populations from the Arabidopsis Biological Research Center. We have used PCR analysis to identify homozygous mutant lines for all family members except BBE-like23, which appears to be embryonic lethal. We have determined phylogenetic relationships between BBE-like family genes and are using the information to create double mutants between the most closely related pairs. Because *Atbbe28* mutants show reduced salt tolerance and biomass, and BBEs in other plants are upregulated in response to stress and pathogenic attack, leaves from *bbe*-like mutants grown in normal conditions as well as salt stress conditions will be examined. Leaves are being examined for 1) overall leaf architecture using the open access LAMINA program and 2) differential RNA expression of known leaf-polarity determining genes using qRT-PCR. Mutants are also being examined for differences in chlorophyll and carotenoid levels.

**P63. Identification of BBE-like Double Mutants in *Arabidopsis thaliana***

**Peyton Robinson**, *Southern Illinois University, Edwardsville, IL, USA*

*Arabidopsis thaliana* contains 28 members of the BERBERINE BRIDGE-LIKE ENZYME (BBE-like) family of genes. Although named for structural similarity to the enzyme that catalyzes Berberine production in the genus *Berberis*, their function in *Arabidopsis* is unclear because *Arabidopsis* is not known to produce Berberine. RNA-seq analysis of the variegated mutant geranylgeranyl diphosphate synthase 11-1 (*ggpps11-1*) revealed that 12 of the 28 BBE-like genes showed altered regulation. In addition to variegation, this mutant also shows altered morphology along the periphery of the leaf. The objective of this work is to determine if and how BBE-like proteins affect leaf development. Segregating individual T-DNA lines for each gene were obtained from the Arabidopsis Biological Research Center and confirmed homozygotes were identified using PCR. In addition to analysis of individual mutant phenotypes, we plan to analyze higher-level mutants as well. A phylogenetic tree was created to determine the relationship among the BBE-like genes to find the most closely related pairs. These were crossed, and PCR was used to

isolate homozygous mutants in the F2 generation. To date, four homozygous double mutants have been isolated.

**P64. Identification of the Causative Mutant Locus in *Gravity Persistent Signal 5***

**Erica Periandri**, *Southern Illinois University, Edwardsville, IL, USA*

Gravity is a constant stimulus that affects plant growth and development. When reoriented by 90 degrees with respect to gravity at room temperature, plants rapidly alter their growth direction. If this reorientation is performed at 4°C, the plant fails to immediately respond. After return to room temperature in a vertical orientation results in delayed curvature. This is known as the gravity persistent signal (GPS) response. The gravity persistence signal 5 (gps5) mutation in *Arabidopsis* causes hypergravitropism after exposure to a cold gravity reorientation. The goal of this research is to identify the gene within the gps5 mutant that causes this phenotype. Genome sequencing and microarray analysis have identified 4 candidate genes with potential insertions. To determine which of the candidate genes causes the gps5 mutation, we are taking a multi-step approach. First, we are using PCR to screen gps5 mutants to determine if any of the four candidate mutant genes contain a T-DNA insert. We have also identified, and are screening, additional T-DNA lines obtained from ABRC. They are being examined for GPS response abnormalities. Finally, wild-type constructs for each gene are being assembled for transformation rescue of the gps5 mutant phenotype.

**P65. Meristem-specific Expression of Geranylgeranyl Diphosphate Synthase 11 (GGPPS11) to Rescue the *ggpps11-1* Mutant**

**Tessa England**, *Southern Illinois University, Edwardsville, IL, USA*

The biosynthesis of isoprenoids is an important pathway involved in the production of various products such as carotenoids and chlorophyll. Although much is known about chlorophyll, there are still some questions remaining on how plants prioritize the partitioning of chlorophyll and carotenoid precursor molecules. Within the plastidial MEP pathway, geranylgeranyl diphosphate synthase 11 is a major producer of the branchpoint compound GGPP. A point mutation in GGPPS11 in *Arabidopsis thaliana* (*ggpps11-1*) causes a variegated leaf phenotype. The leaf morphology of the mutant is also altered in a temperature dependent manner. We hypothesize that this temperature dependent variegation is caused by a temperature gradient within the shoot apical meristem that leads to differential activity of GGPPS11 in meristem layers. To test this, we will attempt to rescue the *ggpps11-1* mutant by expressing wild-type GGPPS11 within individual meristem areas using the promoters for WUS, AtML1 and STM. Variegation patterns will be observed and analyzed to determine if rescue in a specific layer of the meristem will restore the wild type phenotype and provide more insight into the regulation of variegation in *ggpps11-1*.

**P66. Transformation rescue of *ggpps11-1* using *GGPPS8***

**Lauren Davis**, *Southern Illinois University, Edwardsville, IL, USA*

Geranylgeranyl diphosphate (GGPP) is a key branchpoint compound for the downstream production of many isoprenoid-related compounds, including hormones such as gibberellic acid, and critical pigments including chlorophyll and carotenoids. The synthesis of GGPP is catalyzed by the enzyme GERANYLGERANYL DIPHOSPHATE SYNTHASE (GGPPS). In *Arabidopsis thaliana* there are 12 members of the GGPPS family of genes. Some, such as GGPPS11 and GGPPS12, are expressed liberally throughout the plant, while most are found only in specific tissues in limited amounts. The family also shows a variety of sub-cellular localizations, including the chloroplast, mitochondria, endoplasmic reticulum, and cytosol. Evidence suggests that the chloroplast-localized GGPPS11 enzyme is mostly, if not entirely, responsible for providing the pool of plastidial GGPP used for chlorophyll production. A point mutation resulting in a single amino acid change (*ggpps11-1*) leads to a variegated leaf phenotype, while more severe T-DNA insertions produce embryo lethal or seedling albino phenotypes. The goal of this

project is to determine if the other GGPPS family members retain the enzymatic ability to produce the GGPP necessary for chlorophyll production, but are limited by sub-cellular localization or tissue specific expression. Specifically, this work will focus on transformation rescue of *ggpps11-1* with GGPPS8, which is normally localized to the mitochondria. A truncated version of GGPPS8, lacking the predicted mitochondrial targeting sequence, has been amplified using PCR and ligated into the Gateway entry plasmid PCR8/GW-Topo. A chloroplast targeting sequence has been amplified using PCR and will be ligated in frame, upstream of the GGPPS8 open reading frame. This construct will then be transferred to pEARLEYGATE 100, which contains a 35S promoter for overexpression. This construct will be transformed into *ggpps11-1* and wild-type *Arabidopsis* to determine if it can rescue the variegated phenotype of the mutant.

#### **P67. Quantifying the Impact of Changes in Atmospheric Vapor Pressure Deficit on Maize and Soybean Yields**

**Kelsie Ferin**, *Iowa State University, Ames, IA, USA*

Vapor pressure deficit (VPD) is expected to increase under several global change scenarios. Increasing VPD will increase the atmospheric demand for water vapor and potentially water usage by crops. As a result, changing VPD and crop water use may increasingly affect crop yields in the future. Therefore quantifying the relationship between VPD and yield is critical to improve crop production scenarios in the context of global change. We hypothesize 1) as the growing season average VPD increases, yields will decrease and be negatively correlated and there will be a stronger negative correlation for cumulative VPD than averaged VPD. We also hypothesized that 2) soybeans will be less sensitive to VPD relative to maize due to CO<sub>2</sub> stimulation. To address these hypotheses, simulations were conducted using a mechanistic model Agro-IBIS (Integrated Biosphere Simulator – Agricultural version). Simulations were forced by meteorological data from multiple models from the North American Regional Climate Change Assessment Program. The interacting effects of climate and CO<sub>2</sub> were compared for past (1979-1998) and future (2049-2068) periods. Results show that both maize and soybean yields are negatively correlated with VPD. There is also a negative correlation to both yields in VPD-CumI, but this value does not differ from averaged VPD, like we hypothesized. Soybean yields were less sensitive to VPD under elevated CO<sub>2</sub> in future years than maize which supports hypothesis 2.

#### **P68. Role of Lipid-Binding Proteins Involved in Lipid-Mediated Signaling of Abiotic Stress**

**Amanda Koenig**, *Michigan State University, East Lansing, MI, USA*

Regulation of plant development in response to abiotic stress has direct and significant impacts on global food security. Increasingly harsh conditions for plant growth have generated a need for plants to more efficiently cope with drought and other abiotic stresses. Systemic signaling in the plant is necessary to coordinate these developmental responses. The phloem is thought to transport predominantly photosynthates from source to sink. However, studies have shown that the phloem is a dynamic system involved in trafficking nucleic acids, proteins, and lipids throughout the plant. To further explain the presence of lipids in the phloem, we theorize that small lipid-binding proteins (LBPs), identified in phloem exudate, act in lipid-mediated stress signaling. Various proteomics studies have identified the following predicted lipid-binding proteins (LBPs), among others, in phloem sap: Bet v1 Allergen, Annexin 1, Major Latex Protein 43, and Major Latex Protein 423. These proteins are of interest because of their lipid binding START-like domains as well as their small size, which would permit movement into and throughout the phloem. Additionally, we will test the expression of Phospholipase D $\alpha$ 1, Phospholipase D $\alpha$ 2, Phospholipase C 3, and Phospholipase D  $\delta$  in response to abiotic stress to better integrate intercellular and systemic signaling pathways. To understand the response of these LBPs, we subjected *Arabidopsis thaliana* seedlings to various abiotic stress mimetics using a hydroponic system. Total RNA from whole seedlings

was used to test for differential gene expression in response to drought, salt, and osmotic stressors using RT-qPCR. We will describe the gene expression of small lipid-binding proteins involved in developmental regulation in response to abiotic stress. After identifying the best candidates among the above proteins, we will characterize their interaction with phloem lipids and their involvement in systemic signaling. This project was funded by USDA NIMSS MICL04147 and USDA MICL02414 to SHB and USDA-NIFA NNF 2015-38420-23697 to AK.

**P69. Nitrogen metabolism and nectar secretion in *Cucurbita pepo***

**Erik Solhaug**, *University of Minnesota, Minneapolis, MN, USA*

Nectaries are the glands that produce nectar. Nectar synthesis and secretion is an extremely energetically- and biosynthetically-dependent process. While the phloem provides biosynthetic precursors to nectaries, the composition of phloem sap and nectar differ dramatically in most species. There is also strong evidence that most nectar solutes are synthesized de novo in the nectary prior to secretion, including many nitrogenous compounds, which play important roles in plant-pollinator interactions. For example, pollinators display a strong preference for nectars containing select amino acids. In this study we investigated nitrogen metabolism in nectaries of *C. pepo* flowers to evaluate its role in nectar synthesis and plant-pollinator interactions. The expression and activity of key enzymes involved in nitrogen fixation are dramatically up-regulated during secretion, including nitrate reductase (NR), nitrite reductase (NiR), nitrate transporters, and transaminases, among others. These findings strongly suggest that NO<sub>3</sub><sup>-</sup> is actively fixed in the nectary and used to synthesize amino acids de novo. Additionally, we have found that nitric oxide (NO) is actively produced by the nectary during the secretory stage and that it accumulates in the nectar. Given that NO plays a role in memory formation in honeybees, we propose nectars containing NO may enhance pollinator visitation. We are currently conducting hydroponics experiments in which the levels of certain micronutrients (e.g., NO<sub>3</sub><sup>-</sup> and tungstate) known to affect NR activity will be altered. The impacts of nutrient treatments on NR activity, total nectar sugar, amino acid composition, and nitric oxide levels will be assessed. The function of amino acids and nitric oxide in pollinator preference will also be examined. These results help us to better understand how plants produce nectar and interact with pollinators in order to maximize pollination efficiency.

**P70. Metabolite profiling of the terpenoid indole alkaloids in engineered *Catharanthus roseus* hairy root lines**

**Le Zhao**, *Iowa State University, Ames, IA, USA*

*Catharanthus roseus* (*C. roseus*) produces more than 130 different terpenoid indole alkaloids (TIAs), and so far is the only source for obtaining vinblastine and vincristine, which are currently widely applied in chemotherapies for many types of cancers. Those TIAs exist at very low concentrations in *C. roseus*, making direct extraction from plant laborious and costly. Hairy root culture is one of the alternatives to produce valuable secondary metabolites with high biochemical and genetic stability and capacity of large-scale fermentation. The TIA pathway is complex and highly branched. And the pathway branch from tabersonine to vindoline (one precursor of vinblastine and vincristine) is blocked in hairy root. In this study, the first two genes in vindoline pathway, tabersonine 16-hydroxylase (T16H) and 16-O-methyl transferase (16OMT), were co-expressed in hairy roots. The metabolites in the engineered hairy root lines and the control lines were profiled. And some novel TIAs were also identified and quantified in the genetically engineered lines.

**P71. Plant Essential Oils Differentially Control Two-Spotted Spider Mites on Distinct Host Plant Species**

**Jacob Johnson**, *Iowa State University, Ames, IA, USA*

The two-spotted spider mite (TSM) is an economically important phytophagous mite species. It is able to

feed on a wide array of plant species, thus making it a significant worldwide pest. Multiple synthetic acaricides (also known as miticides) have been developed, and while effective, these chemicals may pose significant health risks to humans and other animals. This has prompted investigations into the use of plant essential oils for TSM control. This study assessed the efficacy of plant essential oils in controlling spider mites on two separate host plants, soybeans and marigolds. Plant essential oils were applied via foliar application to whole soybean or marigold plants, and demonstrated a wide range of efficacy in controlling TSM populations. Unexpectedly, the efficacy of these plant essential oils differed between host plants. Leaf dip assays were subsequently performed to assess potential differences in the toxicity of plant essential oils between host plants as a means of exploring this differential efficacy. The LC50 values of plant essential oils were significantly different between host plants. This study illustrates the potential of plant essential oils and their constituents in controlling future TSM infestations. The differential toxicity observed among plant essential oils demonstrates the importance of characterizing their efficacy when used in specific agricultural applications.

**P72. Comparative Metabolite Profiling of Commercially Edible Peppers – An Insight into what Makes Peppers “Hot” or “Sweet”**

**Mark Heggen**, *Iowa State University, Ames, IA, USA*

Metabolomics has emerged as a powerful tool to study the comparisons of metabolites/small compounds in a given complex mixture. In this study, the metabolite profiles of several varieties of peppers are explored. Different colors of bell peppers, red, yellow, orange, and green, and several varieties of “hot” peppers, jalapenos, habaneros, Thai chilies, and ghost peppers were analyzed. A novel GC-MS method has been developed focusing on capturing data of low abundance metabolites in the context of a few highly abundant metabolites in a complex extract. This new GC-MS method uses “heart-cut” technology and allows analysis of highly abundant, as well as low abundant metabolites in the complex pepper metabolite extract. This method allows for compounds at smaller concentrations in the pepper, such as trace amino acids, to be detected in conjugation with much higher concentrations of sugars in the same extract. Initial investigations of the data revealed very high amounts of sugar monomers among the ripe non-green “sweet” bell peppers, and slightly lower amount of sugars and high abundance of capsaicin among the “hot” peppers. Differential distribution of various low abundance metabolites in the various peppers are also observed. Using this modified GC-MS method, our study gives more insight into pepper metabolic profiles and the significance of low abundance molecules contributing to unique pepper characteristics.

**P73. Mapping loci that modify the efficacy of Teosinte crossing barrier 1**

**Merritt Burch**, *South Dakota State University, Brookings, SD, USA*

Teosinte crossing barrier 1 (Tcb1) is a genetic cross-incompatibility factor that is responsible for blocking non-self-type pollen in silks. Originally found in teosintes, Tcb1-s (strong allele) has been introduced into modern maize varieties conferring resistance to tcb1 pollen. Previous studies using a similar cross incompatibility system, Gametophyte factor 1 (Ga1-s) suggest that the cell wall modification enzyme ZmPme3, a pectin methylesterase, along with multiple modifying QTL loci contribute to the effectiveness of silks at resisting foreign pollen types. In Tcb1, little is known about the genetic modifiers and, more importantly, what the underlying biological mechanism is for this cross incompatibility. Cross-incompatibility systems like Tcb1 and Ga1 can be beneficial to breeders and farmers when only certain pollen types are desired on specialty maize crops. It was observed that nearly all the F1's of various inbreds, including B73, crossed by W22 Tcb1-s demonstrate strong incompatibility with tcb1 pollen. One exception was Mo17, whose F1s had weaker resistance. In this study we used recombinant inbred lines (RILs) from the intermated B73-Mo17 (IBM) population crossed with homozygous W22 Tcb1-s plants to test the efficacy of the various F1s at blocking tcb1 pollen. The F1s were tested by first challenging the

Tcb1-s silks with R1 C1 tcb1 pollen and the next day pollinated the same silks with r1 c1 Tcb1-s pollen. The resulting ears were scored for the percentage of colored kernels. Six quantitative trait loci (QTL) were detected on chromosomes 1, 3, 5, and 7 that explained 28.9% of the phenotypic variability. Most modifying QTL loci showed simple additivity effects and epistatic interactions between loci. Further exploration into these genomic regions and the underlying candidate genes is underway, these results could shed light on the genetic and physiological mechanisms controlling Tcb1.

#### **P74. Distinct Cell Type-Specific Auxin/Cytokinin Ratios in Soybean (*Glycine max*) Nodules**

**Paul Gaillard**, *South Dakota State University, Brookings, SD, USA*

Legume-Rhizobium symbiosis results in root nodules where rhizobia fix atmospheric nitrogen into plant usable forms in exchange for plant-derived carbohydrates. Biological nitrogen fixation in legume nodules alleviates the use of energy-intensive, expensive, and environmentally hazardous chemical nitrogen fertilizers. The development of these specialized root organs involves a set of carefully orchestrated plant hormone signaling. In particular, a spatio-temporal balance between auxin and cytokinin appears to be crucial for proper nodule development. We used two photon induced fluorescence microscopy for quantitative 3-dimensional imaging of fluorescent markers to determine cellular level auxin and cytokinin outputs and ratios during root and nodule development in soybean. The relative auxin cytokinin ratios in root tips and lateral roots determined in this study were in agreement with previously reported outputs for each fluorescent reporter individually. The ratiometric method used here is shown to largely compensate for variations in individual outputs due to sample turbidity and scattering, providing quantitative measures of the relative hormone outputs. Importantly, distinct auxin/cytokinin ratios corresponded to distinct nodule cell types indicating a key role for these hormones in nodule cell type identity. Future applications of the method for time-course imaging of auxin/cytokinin outputs and ratios along the course of nodule development are expected to provide key insights on hormonal control of cell differentiation during nodule development.

#### **P75. Seed Mass is More Influential than Simulated Climate Change on Biomass of American Chestnuts and Hybrids After One Season**

**Brett Frederickson**, *Ohio University, Athens, OH, USA*

The American chestnut (*Castanea dentata*) has been driven to functional extinction in eastern deciduous forest by chestnut blight (causal agent *Cryphonectria parasitica*). The American chestnut was hybridized with Chinese chestnut (*Castanea mollissima*) to increase blight resistance with the goal being to reintroduce the species. However, physiological data to accurately predict responses of these hybrids to environmental conditions are lacking. Here we address two questions: What are the effects of simulated climate change on the carbon uptake of the American chestnut? Do hybridized BC3F3 chestnuts respond similarly to their pure American counterparts under simulated climate change? Our experiment grew chestnuts in environmental chambers for the equivalent of one growing season before harvest. We replicated this experiment with different genotypes the following year. In total nine hybrid genotypes (four D-genotypes and five W-genotypes) and four pure American varieties (NC, NH, ME, VA) were grown. Plants were randomly assigned to one of four treatments in a factorial design of elevated CO<sub>2</sub> and temperature. We found that biomass was similar among the four treatments but not among genotypes in year one. D-genotypes showed greater biomass than W-genotypes which showed biomass similar to the pure Americans. Average seed mass of D-genotypes was greater than other genotypes ( $p < 0.001$ ), which we conclude to be the central factor to chestnut growth and performance in the first growing season. There was an elevated CO<sub>2</sub> effect in the second year when genotype and seed mass were included in the model ( $p = 0.02$ ). Hybrid genotypes increased in biomass (26% for W-genotypes and 20% for D-genotypes). The VA pure Americans increased in biomass by 85%, while the ME Americans decreased in biomass by 36%.

Findings indicate that increased CO<sub>2</sub> under future climate scenarios will influence hybrid reintroduction, but not as much as the initial seed mass of the chestnuts.

#### **P76. Structure, Function, and Protein-Protein Interactions of Xyloglucan Xylosyltransferase**

**Jacqueline Ehrlich**, *Iowa State University, Ames, IA, USA*

The plant cell wall is the largest source of biopolymers on earth and has numerous industrial applications such as biofuels and biomaterials. In addition, understanding the biosynthesis of the plant cell wall may aid in engineering plant performance, such as higher resistance to biotic and abiotic factors. The plant cell wall is made mainly composed of cellulose, pectin, lignin, and hemicellulose. Xyloglucan is the most abundant hemicellulosic polysaccharide in the primary cell wall of dicotyledonous plants. Xyloglucan Xylosyltransferases (XXTs) catalyze the transfer of xylose from UDP-xylose to form a  $\beta$ -1,6 glycosidic linkage on the glucan backbone. The x-ray crystal structure for XXT1 has recently been solved which reveals modes of substrate binding including with UDP-Xylose and cellobiose. We tested this hypothesis through point-mutations of the predicted residues involved in UDP-xylose binding. K382 mutation severely reduced activity and was to be important in substrate binding with UDP through H-bonds of both  $\alpha$ - and  $\beta$ -phosphoryl oxygens. K206, N268, D317, D318, and Q319 all interact with the xylose in the substrate and mutation of these residues to alanine significantly reduce XXT1 activity. K206, H337, and a D227/D229 double mutation result in a 50-75% decrease. Lastly, S228 and N268 do not exhibit a significant decrease in enzyme activity. The activity levels of each mutation allow us to conclude that mutations to residues involved in interaction with the xylose moiety of UDP-xylose have most severe impact on catalytic activity.

#### **P77. Plant science learning activities for biomedical students**

**Jessica Lucas**, *Southern University Illinois-Carbondale, Carbondale, IL, USA*

Southern Illinois Bridges to the Baccalaureate is a National Institutes of Health program that prepares underserved community college students for success at four-year universities by providing them personalized mentorship, research experiences and social capital. Here we present learning activities specifically designed for these biomedical students that are aligned with the shared core competencies and concepts for undergraduate biology education outlined by the American Association for the Advancement of Science, American Society of Plant Biologists, Botanical Society of America, and National Science Foundation. To engage in the scientific process, select community college students participated in an eight-week summer research experience and preparatory lab class held at Southern Illinois University Carbondale (SIUC). Through plant-based lab activities, students learned the significance of positive and negative controls in experimental design and data analysis. The relationship between structure and function was explored through student-designed experiments on Arabidopsis seed coat mucilage. Students used quantitative reasoning to analyze light-microscopy data to compare different Arabidopsis genotypes. They then leveraged online gene expression and interactome data sets to interpret their results. Students progressively strengthened their scientific communication skills throughout the program by routine oral presentations and discussions of plant genetics in societal terms. Collaboration skills were emphasized throughout the program as students worked in groups on learning activities. Independence was fostered through individualized research projects that culminated in a capstone scientific poster presentation at the SIUC Summer Research Forum. Student perceived learning gains, as determined by the Classroom Undergraduate Research Experience survey (Lopatto, 2004), show increased interest in earning advanced degrees in biological science.

#### **P78. Genetic Analysis of 5'-3' Exoribonuclease (Xrn) Mutants in Alga *Chlamydomonas reinhardtii***

**David Higgs**, *University of Wisconsin, Kenosha, WI, USA*

Eukaryotes have 5'-3' exoribonucleases (Xrn's) that control accumulation and processing of RNAs. There are two Types of Xrn's (1 and 2), based on sequence, and both degrade RNAs processively. Studies with Arabidopsis, animals, and fungi show that Xrn Types correlate with sub-cellular targeting, RNA substrates, and cellular functions. Type 1 Xrn's are found in the cytosol where they control mRNA stability and RNAi regulation. Type 2 Xrn's are found in the nucleus where they affect rRNA processing and transcription termination of pre-mRNAs. To determine the roles of Xrn's in algae we are investigating four predicted Xrn genes (CrXrn1, 2, 3 and 4) in the model green alga *Chlamydomonas reinhardtii*. CrXrn1 and CrXrn4 are predicted to encode Type 1 proteins while CrXrn2 and CrXrn3 are predicted to encode Type 2 proteins. Based on genomic and mRNA sequence, we hypothesize that CrXrn1 uses alternative splicing to generate two mRNA isoforms, one isoform (CrXrn1a) encodes a predicted Type 1 cytosolic protein and the other isoform (CrXrn1b) encodes what appears to be a unique chloroplast-targeted Xrn where it could control stability of chloroplast mRNAs important for photosynthesis. CrXrn2 and CrXrn3 have homology to Type 2 Xrn's, and we predicted them to be targeted to the nucleus where they could control rRNA processing and transcription termination for pre-mRNAs. To test predicted functions of CrXrn's we obtained ten mutant strains from the *Chlamydomonas* indexed mutant library project (CLiP) transformed with a paromomycin resistance gene. PCR, sequencing, and growth curves determined that four mutants have insertions affecting three of the CrXrn genes (CrXrn2, CrXrn3, and CrXrn4). The remaining six have insertions elsewhere. Growth phenotypes of mutants compared to WT in TAP media, low-nitrogen media, with or without acetate, and at different temperatures have determined that three CrXrn mutants have significantly reduced growth compared to WT.

#### **P79. Optimization of the Enzymatic Synthesis of UDP-Xylose for the Study of Hemicelluloses**

**Matt Cook**, *Iowa State University, Ames, IA, USA*

The plant cell wall is of interest for agriculture and biofuel production; understanding the biosynthesis of plant cell walls is critical for improving the viability of biorenewable energy as a replacement for traditional energy sources. Xylan, xyloglucan, and other xylosylated hemicelluloses are important cell wall constituents; however, studies of xylosylation of hemicelluloses and other biomolecules are hampered, partially due to the low supply of the primary substrate for xylosylation, UDP-xylose (UDPX). The goal of this project has been to optimize the known biological pathway for UDPX synthesis for laboratory use. The two-enzyme UDPX biosynthesis pathway produces UDPX from UDP-glucose (UDPG), a more readily available substrate. The project also characterized enzymatic properties of the enzymes UDP-glucose dehydrogenase (Ugd) and UDP-xylose synthase (Uxs), which perform the catalytic activities in the pathway of interest. These enzyme homologs have not been characterized previously. Homologs of Ugd and Uxs have been partially characterized before, but neither to the degree necessary for efficient product synthesis and the specific homologs used herein have never been reported. Enzymatic assays were used to determine the optimum conditions for each enzyme and spectrophotometry and high-performance liquid chromatography were used to measure the products of reactions. Biologically-relevant conditions including pH, salt concentration, reducing agent concentration, presence of metal ions, et cetera were optimized. Greater than 90% substrate conversion has been achieved for the individual enzymes, though combination of the two into a single-pot reaction has proven elusive and a consecutive series of reactions is being optimized. Additionally, kinetics data collected has provided interesting insights into the enzymatic properties of Ugd and Uxs. Thus, it was shown that Ugd experiences substrate inhibition, but this substrate inhibition can be relieved by the addition of ATP, despite ATP not being involved in the reaction.

#### **P80. Maize (*Zea mays*) Standing Variation Affects Sensitivity to Auxin Treatment**

**Jenna Bohler**, *University of Missouri, Columbia, MO, USA*

There is great genetic variation among inbred lines of *Zea mays* (maize). This variation could be easily



observed by comparing the primary root length of maize seedlings grown in a solution of dimethyl sulfoxide (DMSO) to the root length of seedlings grown in DMSO and 2,4-dichlorophenoxyacetic acid (2,4-D). The phytohormone auxin has been shown to regulate many aspects of plant growth and development, including the inhibition of root length in maize. Initially, the most abundant naturally occurring auxin indole-3-acetic acid (IAA) was used as the treatment for this research. However, IAA had minimal effects on maize root length. The seedlings were then regrown using 2,4-D, which produced visible effects. 2,4-D is a synthetic form of auxin that is commonly used as an herbicide on dicotyledonous plants. Maize, being a monocot, was normally expected to be insensitive to 2,4-D. However, the results of this research suggest that 2,4-D actually inhibited the growth of maize roots when compared to untreated seedlings. With the 2,4-D treatment, the average percent reduction was 40.10% when compared to the longer, untreated seedlings. In addition, there was a significant variation in response to 2,4-D. The inbred line Mo18W actually showed longer roots with the treatment. This was likely due to genetic variation among the inbred lines. The purpose of this research is to identify different loci that control root architecture using Genome Wide Association Studies (GWAS) to either increase or decrease root length, depending on the plant's need. Modifying root architecture could allow the plant to better anchor in the soil, mine for water and nutrients, and/or allocate the products of carbon fixation.

#### **P81. Hydrotropic Responses in Maize Primary Roots**

**Yafang Wang**, *South Dakota State University, Brookings, SD, USA*

Hydrotropism is a fundamental mechanism in plant biology that facilitates roots to effectively acquire water in the soil. Despite its significance, hydrotropism and its potential application in improving drought tolerance in crops has not extensively studied. We are examining hydrotropic response of primary roots in different genotypes of maize. Our study reveals a large variation in hydrotropic response among 16 genotypes. Transcriptomic analysis reveals an over-representation of genes in auxin- and ethylene-response/signaling, fat metabolism/membrane transport, and protein degradation among the genes showing significant changes in hydrotropic roots compared to well-watered non-hydrotropic roots. This study establishes a foundation to identify key players in hydrotropic response in maize roots.

#### **P82. The silk surface lipid metabolome responds to abiotic stress and offers protection against desiccation**

**Bri Vidrine**, *Iowa State University, Ames, IA, USA*

Maize silks are essential for pollen reception and hydration, pollen tube growth and fertilization, and thus corn yield. While large silk surface areas are advantageous for capturing wind-carried pollen grains, surfaces are also susceptible to water loss, especially under desiccating conditions. As a primary line of defense, the silk cuticle is coated with a hydrophobic barrier of extracellular surface lipids (SLs) that is exceptional in its high hydrocarbon composition (~90% of total SLs in inbred B73). To consider the effect of abiotic stress on surface lipid accumulation, B73 silks were harvested from growth chamber-grown plants exposed to water deficit treatments under two different temperature treatments (25 or 30°C) imposed after tassel emergence. Results of subsequent silk surface lipid profiling demonstrate that the concentration of unsaturated hydrocarbons increase in response to temperature stress, whereas saturated hydrocarbons increase in response to water deficit. In a separate experiment, we tested the protective capacity of the SL metabolome against desiccation by measuring rates of water loss for excised silks from four diverse inbred lines (including B73) subjected to four combinations of temperature and humidity treatments. When excised silks were subjected to 15% relative humidity, the rates of water loss from inbred (MO378) were ~1.5 to 2-fold faster than silks from B73. In contrast, water loss rates did not vary significantly between inbreds subjected to 85% RH. Statistical modeling incorporating both water loss rates and surface lipid composition suggest that saturated hydrocarbons do provide some level of protection from water loss.

Collectively, our initial experiments demonstrate that silk SLs respond to abiotic stress and that these SLs likely offer protection from desiccation, however, other potential causal factors will also be discussed.

### **P83. Study of the Xyloglucan Synthesizing Complex Formation**

**Kayla Uthe**, *Iowa State University, Ames, IA, USA*

Xyloglucan (XyG) is a component of plant cell walls and there is little known about the mechanisms of xyloglucan biosynthesis and post-synthetic modification during plant growth and development. Our previous studies demonstrated that the XyG synthesizing enzymes are localized in Golgi and form multiprotein complex via protein-protein interactions. Thus, we aim to test a hypothesis: XyG synthesizing enzymes initiate the complex assembling in cis-Golgi and the composition of the complex changes in the course of Golgi maturation. XyG synthesis is initiated in cis-Golgi and completed in trans-Golgi and trans-Golgi network (TGN), where the mature XyG is released and transported to cell wall. We propose that the enzymes are recycled from TGN back to cis-Golgi for another cycle of XyG synthesis. To test our hypothesis, we chose two approaches: (1) Fluorescence Recovery After Photobleaching (FRAP) to study the localization and mobility of enzymes. In FRAP, high intensity illumination irreversibly photobleaches fluorescent proteins in target area of plant cell and diffusion velocity of proteins can be calculated. (2) Fluorescence Resonance Energy Transfer (FRET) to study dynamics of protein-protein interactions. Result of FRET, co-localized with different Golgi markers, can reveal location and dynamics of protein-protein interactions between XyG synthesizing enzymes. Currently, we have generated multiple fluorescent constructs with all XyG synthesizing proteins fused with either CFP or YFP and initiated FRET experiments using transient expression in Arabidopsis protoplasts. First, we have confirmed that all constructs work and fusion proteins are expressed at detectable level and localized to Golgi. The analysis of XyG assembly mechanisms will serve as a model for understanding the complex cell wall polysaccharide biosynthesis in general. This project was supported by NSF-MCB grant #1121163 and USD-NIFA grant #015227-00005

### **P84. Identification of Arabidopsis Candidate Genes for Cold Stress Response Using a High Throughput Phenotyping System**

**Dipak Kumar Sahoo**, *Iowa State University, Ames, IA, USA*

To reveal the genetic mechanisms regulating plant adaptation to cold stress, we have developed a phenotyping system by equipping Arabidopsis Percival growth chambers (AR22LC9) with digital cameras (CropScore, Tubingen, Germany) to study the natural variants. We have developed a method to batch process digital images of hundreds of Arabidopsis seedlings to numerical data in a spreadsheet-ready CSV file. We have phenotyped different Arabidopsis ecotypes using this newly developed system for cold stress response. By studying genome-wide association of responses of 404 Arabidopsis ecotypes to cold stress with the genotypes of individual ecotypes, we have identified 33 candidate genes. Surprisingly, three of the cold stress-related genes encode NBS-LRR-type proteins. Two Myb proteins with high identity to previously characterized rice regulator of stress tolerance were also identified under cold stress.

### **P85. Functional Characterization of Three Mitochondrial Acyl Carrier Protein Isoforms in *Arabidopsis thaliana***

**Rachel Garlock**, *Iowa State University, Ames, IA, USA*

Acyl carrier protein (ACP) is an important and highly conserved protein-cofactor needed for fatty acid biosynthesis in all living organisms. In plants and bacteria, ACP is a small (9 kD) monomeric protein that shuttles acyl intermediates among individual enzyme components in the Type II fatty acid synthase system. *Arabidopsis thaliana* expresses several ACP isoforms, five are targeted to plastids and three are thought to be in mitochondria. Although many studies have revealed the specific roles for the plastidic ACPs, the

physiological functions of the three mitochondrial ACPs (i.e. mtACP1, mtACP2, and mtACP3) remain to be elucidated. The subcellular localization of the three mtACPs was confirmed using confocal microscopy of transgenic plants carrying the green fluorescent protein-tagged isoforms. To understand the roles of the three mtACPs, we characterized several T-DNA-insertional mutants for each mtACP-encoding genes. No alteration in the morphological phenotype was found in *mtacp1*, *mtacp2*, and *mtacp3* homozygous mutants, suggesting functional redundancy among the three mtACPs. Further genetic redundancy was revealed by the fact that the double mutant combinations of *mtacp1 mtacp3* and *mtacp2 mtacp3* show an unaltered growth phenotype, whereas the growth of the *mtacp1 mtacp2* double mutant was extremely delayed. These results suggest that mtACP1 and mtACP2 are the major ACP isoforms in the mitochondria and are required for normal plant growth and development. Metabolic profiles of these mutants will be analyzed to identify the metabolic roles of the mtACPs that are associated with these phenotypes.

**P86. Genome-Wide Association Study of Twelve Inflorescence Traits in the Sorghum Association Panel**

**Jacob Givens**, *University of Nebraska-Lincoln, Lincoln, NE, USA*

Sorghum [*Sorghum bicolor* L. Moench] is the fifth most important cereal crop in global production. Inflorescence architecture, or the number and arrangement of inflorescence branches, is an agronomically important set of traits that impact grain yield. Genome-Wide Association Studies (GWAS) are commonly used for genetic characterization of natural diversity panels. Previous GWAS studies in sorghum have been limited in the scope of inflorescence architecture traits they included, focusing on inflorescence length, inflorescence width, rachis length, number of nodes, and branch length. For a more comprehensive inflorescence architecture dataset, we phenotyped 12 inflorescence traits within the Sorghum Association Panel (SAP), which consists of 406 architecturally diverse accessions. Of these 406 accessions, we obtained phenotypes from three representative individuals from a total of 391 lines. The quantitative phenotypes were normally distributed with few outliers and four pairs of traits were found to be correlated ( $r^2 > 0.50$ ). Since 52 of the accessions did not have corresponding genotype data, we used a total of 339 lines and 255,892 SNPs for GWAS. Preliminary results include 100 significant SNPs for 11 of the 12 traits phenotyped, which may underlie some of the variation in developmental patterns in inflorescence architecture. In future, these candidate genes near significant SNPs will be investigated for their role in inflorescence development, and the phenotyping data will also be used as ground-truths for high-throughput phenotyping.

**P87. Determining the Effect of the *sbe1* Allele from *Z. mays parviglumis* on Maize Endosperm Starch Composition in an *ae1* Background**

**Prameela Awale**, *South Dakota State University, Brookings, SD, USA*

Starch is the main constituent of maize endosperm. Structurally, starch is divided between two main forms: unbranched (or less branched) amylose and highly branched amylopectin. Generally, amylose constitutes about 25% of maize endosperm starch. The amylose content in the endosperm is increased up to 50% when *ae1*, which encodes starch branching enzyme IIb (SBEIIb), is homozygous recessive. However, one variety of maize that is homozygous *ae1*, GEMS-0067, has up to 75% amylose in its endosperm starch. We have shown that this high amylose content is due to an allele of *sbe1*, which encodes for starch branching enzyme I (SBEI). The GEMS-0067 allele of *sbe1* translates into a protein with six amino acid polymorphisms relative to what is found in all Midwestern dents that have been surveyed. We have also found that the amino acid sequence for SBEI from GEMS-0067 is identical to what is predicted for *Z. mays parviglumis*. We are interested in whether the *sbe1* allele of *Z. mays parviglumis* has the same effect on maize starch composition as GEMS-0067. To test this, we will analyze the progeny of *Z. mays parviglumis*-maize hybrids. Instead of using a recessive *ae1* allele, we are employing Ae1-5180, which acts

in a dominant fashion to eliminate SBEIIb. We will present our methods of analysis as well as data on developing markers to distinguish the *sbe1* and *ae1* alleles.

**P88. Characterization and Genetic Mapping of the carbohydrate partitioning defective60 mutant in maize**

**Singha Dhungana**, *University of Missouri, Columbia, MO, USA*

Carbohydrate partitioning is the process by which sugars, primarily sucrose, synthesized in the photosynthetic source tissues (mature leaves) are mobilized to non-photosynthetic (sink) tissues, such as roots, seeds, and developing organs. To identify genes controlling carbohydrate partitioning, we identified numerous mutants, termed the carbohydrate partitioning defective (cpd) mutants, which overaccumulate starch and sugars within their leaves. One such recessive mutant, cpd60, hyperaccumulates starch in its leaves, and displays stunted growth, reduced fertility, chlorosis and accumulation of anthocyanins in the mature leaves. The gene responsible for cpd60 phenotype has been mapped to the lower arm of Chromosome 1 by Bulk Segregant Analysis (BSA) mapping. Fine mapping using polymorphic markers is underway and we have narrowed down the location of the causative gene to a 600 kb region. Furthermore, we have identified three more alleles of cpd60 that we are characterizing to elucidate the gene function. The identification of the gene responsible for the cpd60 phenotype will provide insights into the genetic regulation of sugar metabolism and allocation. With this knowledge, we can translate our understanding of carbohydrate partitioning to other crop species, like sorghum and sugarcane, for genetic improvements to increase food yield and biofuel production.

**P89. *bottomless* is a Novel Short Root Mutant Involved in Auxin and Nutrient Signaling**

**Yunting Pu**, *Iowa State University, Ames, IA, USA*

Auxin is an essential hormone for plant growth and development. Auxin signaling has been shown to regulate cell growth through both transcriptional and translational mechanisms, but descriptions on how auxin shapes proteomic changes are not well understood. Through proteomics analysis on auxin treated *Arabidopsis* seedlings, we identified an auxin responsive protein, BOTTOMLESS (BTM), which is up-regulated upon auxin treatment in *Arabidopsis* hypocotyls. Knock-down mutants of BTM have severe defects in root development when growing on medium without sucrose, but display normal root growth on sucrose-supplemented medium. In sucrose transfer assays *btm* seedlings can be partially rescued after 4-5 days of growth without sucrose, suggesting that this postembryonic defect is linked to nutrient sensing. Confocal imaging of *btm* primary roots shows that in the absence of sucrose the root apical meristem is abnormally differentiated, which is in contrast to wild-type roots which have normal root apical meristems under the same conditions. Additionally, *btm* seedlings display normal auxin responsive behaviors in both the root and hypocotyl under both sucrose present and absent conditions, implying that BTM functions downstream of auxin perception. Altogether these data suggest BTM is required for post-embryonic root development in *Arabidopsis* and this protein may function to link between nutrient sensing and auxin regulated growth in primary roots.

**P90. *Arabidopsis* Aminotransferase ALD1 Site of Action in Plant Systemic Acquired Resistance**

**Shang-Chuan Jiang**, *The University of Chicago, Chicago, IL, USA*

Systemic acquired resistance is a long-distance defense response in which a plant infected with a pathogen in a local leaf (the “immunization site”) becomes resistant to subsequent infections on distal leaves. *Arabidopsis* deploys an aminotransferase called AGD2-LIKE DEFENSE RESPONSE PROTEIN 1 (ALD1) to control basal and infection-induced metabolites that regulate diverse defense-related events. As ALD1 is essential for SAR, we investigated the site of action of ALD1 in SAR activated by the bacterial pathogen *Pseudomonas syringae*. We tested whether ALD1 transgene expression at the immunization site

was sufficient to rescue SAR-deficiency of an *ald1* mutant. We employed a mosaic approach that permitted precise control of ALD1 expression in an *ald1* mutant background by painting an inducer dexamethasone (DEX) onto local leaves (immunization site). Signalling was characterized by measuring the signal molecule salicylic acid in the immunization site after primary infection, and in the distal leaves after secondary infection. Plants expressing ALD1 only at the immunization site were fully restored for local disease resistance and signalling. However, in distal leaves, SAR rescue was partial when compared with wild type as evidenced by pathogen growth and signalling. Our model is that ALD1 is needed at the immunization site to get SAR activation, but it is also needed at the secondary infection site to fully suppress pathogen growth and activate plant signaling. ALD1 controlled metabolites and immune response signaling may be needed during local signal amplification to get over a threshold for systemic immunity.

#### **P91. Nodule Zone-Specific Gene Expression in Soybean**

**Sadikshya Aryal**, *South Dakota State University, Brookings, SD, USA*

Nitrogen is one of the limiting nutrients for plant growth and yield. Leguminous plants such as soybean (*Glycine max*) have developed the ability to form symbiotic association with N-fixing rhizobia. This symbiotic association results in the formation of unique structures called nodules that originate from root cortex via de novo cell differentiation. During soybean nodule development, two major nodule zones are formed: the nodule primordium (Npr) in the middle and the nodule parenchyma (Npa) in the periphery. Npr gives rise to N-fixation zone and the Npa holds vascular bundles. It is not clear what early signaling pathways drive the conspicuous development of these two nodule zones. To bridge this knowledge gap, we adapted two techniques, INTACT (Isolation of Nuclei Tagged in specific Cell Types) and TRAP (Tagged Ribosome Affinity Purification), to characterize the transcriptional and translational responses in Npr and Npa of soybean nodules. Evaluation of nodule zone-specific nuclear and ribosomal transcripts is expected to help identify key determinants of nodule zone identity. This knowledge can be used to devise biotechnological strategies to enhance nitrogen fixation or even potentially transfer N-fixation trait to non-leguminous plants, and reduce environmental pollution caused by excessive use of chemical nitrogenous fertilizer.

#### **P92. Required for Mla Resistance 3, Revisiting Our Old Friend *Sgt1*.**

**Antony Chapman**, *Iowa State University, Ames, IA, USA*

Barley alleles of Mildew resistance locus a (*Mla*) encode c. 30 variants of CC-NB-LRR resistance proteins. These recognise corresponding avirulence (AVR) effectors secreted by the obligate fungal pathogen, *Blumeria graminis* f. sp. hordei (Bgh). *MLA* proteins require the assistance of co-chaperones to function, such as Required for *Mla*12 Resistance 1 (*RAR1*) and Suppressor of G-two allele of *skp1* (*SGT1*). However, our knowledge of the molecular interactions between *MLA* proteins and their co-chaperones is very limited. The barley line m11526, derived from CI16151 (*Mla*6), is susceptible to Bgh isolate 5874 (AVR<sub>a6</sub>) due to a mutation in an unknown gene (*Rar3*, Required for *Mla*6 Resistance 3). Crossing m11526 to other mutant lines revealed that *rar3* susceptibility is not due to mutations in *Rar1* or *Mla*6. Genetic crosses to lines containing other *MLA* variants show that some *MLA* variants have a differential requirement for *Rar3*, which is distinct from the requirement of *Rar1*. Bulk segregant exome-capture and fine mapping delineated the *Rar3* mutation to a 9Mb, low recombination region on chromosome 3H. This led to the identification of a 6bp in-frame deletion in *Sgt1* which co-segregates with the susceptibility phenotype. This mutation deletes two amino acids within the SGS domain of *SGT1*, which has previously been shown to interact with the LRR domain of some *MLA* proteins. As *Sgt1* mutations are lethal in most higher organisms, this mutation may only affect disease resistance. Therefore, this mutation represents a novel opportunity to study how *SGT1* is involved in disease resistance by examining whether the two amino acids deleted in m11526 *SGT1* are required for interactions with disease resistance proteins.

Considering the lack of crystal structures for the complexes that SGT1 makes with MLA and other proteins, identifying specific points of interaction will greatly enhance our current models. Supported by NSF-PGRP grants 09-22746 and 13-39348.

**P93. Elucidating the roles of repressor ARFs by probing ARF and AuxRE interactions**

**Pratiksha KC**, *South Dakota State University, Brookings, SD, USA*

Auxin is one of the key plant hormones and regulates several developmental processes. Auxin inducible gene expression is mediated primarily by Auxin Response Factor (ARF) transcription factors. The ARF gene family comprises of both activator and repressor transcription factors. Activator ARFs bind as dimers to conserved Auxin Response Elements (AuxREs) in the promoters of auxin-inducible genes. Though the mechanism of action for activator ARFs is well described, the function and mode of action of repressor ARFs are yet to be fully understood. Our lab had previously identified that a repressor ARF, GmARF16-2, played a key role in determining stage-specific auxin sensitivity during soybean nodule development. To determine the potential mechanism of action of GmARF16-2, we evaluated whether GmARF16-2 competitively bound to AuxREs. A simple SYBR Gold-based fluorescent assay was developed. Binding assays were performed with conserved AuxREs and purified ARF protein DNA binding domains. Binding assays showed activator ARFs, AtARF5 or GmARF8, efficiently bound two different AuxREs with distinct affinities. However, GmARF16-2 had very low, but detectable binding to AuxREs, and did not reduce binding by activator ARFs in a competition assay. Our results suggest that GmARF16-2 does not bind AuxREs and might use a different mode of action for repression.

**P94. Endoplasmic Reticulum Stress Tolerance Genes in Arabidopsis**

**Savannah Jones**, *Iowa State University, Ames, IA, USA*

Plants respond to environmental stresses, some of which can lead to the unfolded protein response (UPR). The objective of this research was to identify the genes for stress tolerance. It was found in previous studies that tunicamycin successfully induces stress on the endoplasmic reticulum to create the unfolded protein response. The hypothesis was that through the utilization of phenotypic information from tunicamycin induced stress and Genome Wide Association study (GWAS), we would be able to identify genes that control stress tolerance in Arabidopsis. This was carried out using a low dose application of tunicamycin to seedlings that were then grown in a growth chamber for 6 days along with untreated seeds from the same ecotype. The root length was measured from day 2 to day 6 using photos analyzed in ImageJ. This data was collected for almost 200 ecotypes. The phenotypic data was input into easyGWAS which computes and annotates a GWAS. The program identified At1g69810 WRKY36 as a potential candidate. Knockout lines were obtained and screened to ensure homozygosity, and overexpression lines of the candidate genes will be generated. These plants will then be screened with tunicamycin to ensure they align with the gene obtained through GWAS. Preliminary results from these approaches will be presented.

**P95. A start on understanding boron resistance in salt cedar**

**Lawrence Davis**, *Kansas State University, Manhattan, KS, USA*

The salt cedar, *Tamarix* spp., is found commonly in the southwestern U.S., originally coming from Eurasia. A lot of beneficial properties are not widely known. As one example, *Tamarix* absorbs a large amount of boron without being damaged. It tolerates high salt concentration and boron up to 100 mg/L. *Tamarix* can help clear up boron contamination, that was created by burning fossil fuels, running power plants and landfills. *Tamarix* could be used to keep boron localized; preventing it from going into the ground water. *Tamarix* is a long-lived perennial, easily propagated vegetatively. In some locations with poor water quality it behaves as a major invasive species, out-competing less tolerant species. Our initial hypothesis was, that boron resistance may depend on altered root membranes. *Tamarix* proved to be resistant to boron

up to 200 mg/L (ppm) in hydroponic growth experiments with the duration of up to three months. The aim of this study was to explore routes of boron transport in *Tamarix*. First, we looked at root lipids including fatty acids (FA), sterols and phospholipids, analyzed by different methods. The root fatty acids were analyzed by gas chromatography. Lipid types were determined via thin layer chromatography (TLC). In another experiment lipid composition of phospholipids was determined by mass spectrometry (MS). There were no major differences in the root lipids between treatments, caused by boron. Composition of phospholipids was not altered in levels up to 200 ppm of boron in the nutrient solution. It appears that boron tolerance does not depend on changes of root lipids in *Tamarix*. The next step is to examine root membrane proteins.

#### **P96. Role of Lipid Signaling in Plant Development**

**Briaunna Murray**, *Michigan State University, East Lansing, MI, USA*

The goal of the experiments is to determine the effect of phloem lipid-associated family proteins (PLAFP) on plant development and stress signaling in *Arabidopsis thaliana*. We hope to obtain data that will support the idea that phloem lipid binding proteins affect plant development. Several different mutant strains were used in the experiments; three lines overexpressing PLAFP, two being “knock down” which express lower amounts of PLAFP, one wild type used as a control and a complementary strand where wild type phenotypes have been restored by transforming an overexpressing PLAFP line into a knock-down line. Preliminary data suggest that the overexpression lines have a higher seed yield, thicker stems, and possibly more vascular bundles when compared to the other mutant lines, all characteristics of enhanced growth and increased drought tolerance. For the seed yield experiment, approximately 16 plants of each line were planted and grown until seeds are matured. The seeds were then collected and weighed for comparison. For the second experiment, cross sections of primary bolts of each line were examined under a microscope. Length and width of each cross section were recorded, along with number and appearance of vascular bundle. As expected the overexpressing lines have displayed characteristics of enhanced vascular development due to PLAFP.

#### **P97. Characterization of Ammonium Transporters from the Model Liverwort *Marchantia polymorpha***

**Tami McDonald**, *St. Catherine University, St. Paul, MN, USA*

Transporters in the AMT/MEP/Rh family of proteins facilitate the uptake and translocation of ammonium (NH<sub>4</sub><sup>+</sup>) or ammonia (NH<sub>3</sub>). Organisms from most branches of the tree of life contain genes encoding transporters in at least one of these transporter subfamilies. Land plants have both AMT1 and MEP transporters (called AMT2 in plants), but lack Rh transporters; While most of the proteins in this large family are hypothesized to transport ammonia, only AMTs from flowering plants have been demonstrated to be electrogenic - to transport a charge across the membrane; To determine if AMT1 transporters from non-flowering plants are also electrogenic, we expressed AMT genes from the liverwort *Marchantia polymorpha* in the yeast *Saccharomyces cerevisiae* triple MEP mutant. AMTs that were functional in the yeast mutant were recombined with a *Xenopus* oocyte expression vector and the AMTs were assayed via electrophysiology to determine the substrate and uptake kinetics of each transporter; We demonstrate that AMT1 genes from the model liverwort *Marchantia polymorpha* are electrogenic and vary in affinity for ammonium from high affinity (7 μM at pH 5.6 and a membrane potential of -137 mV) to low affinity; This result suggests that the electrogenic transport mechanism may be more broadly distributed in the AMT/MEP/Rh family than previously thought, and may be a property of AMTs in streptophyte green algae, chlorophyte green algae, or AMTs generally.

#### **P98. Higher Order Knock-out Mutants of *Arabidopsis* Galactosyl-transferases: A Reverse Genetic Approach to Reveal**

**Functional Roles of O-galactosylation of Arabinogalactan-Proteins (AGPs)**

**Dasmeet Kaur**, *Ohio University, Athens, OH, USA*

Arabinogalactan-proteins (AGPs) are abundant extracellular glycoproteins implicated in a variety of growth and development processes in plants. AGP biosynthesis involves a series of post-translational modifications resulting in the addition of complex sugar chains to AGP core proteins. Eight

galactosyltransferases (Hyp-GALTs) namely, GALT2, GALT3, GALT4, GALT5, GALT6, HPGT1, HPGT2, and HPGT3, have been characterized so far for O-galactosylation of hydroxyproline (Hyp) residues in Arabidopsis AGPs, but it is unclear whether they exhibit genetically distinct or redundant roles. A reverse genetic approach is proposed to assess the functional roles of the GALT2-6 subfamily, the HPGT1-3 subfamily, and the combined GALT2-6 and HPGT1-3 subfamilies. The current hypotheses include: 1. Hyp-GALTs glycosylate all co-expressed AGPs, but their levels of activity and sites of activity vary. 2. Multiple higher-order genetic mutants for Hyp-GALTs, which are partially redundant or non-redundant, demonstrate more severe physiological phenotypes. Higher order knock-out mutants were produced by crossing existing mutants (*galt2galt5* and *hpgt1hpgt2hpgt3*), and the subsequent off-spring were genotypically screened using PCR analysis. To date, we have identified homozygous triple mutants (*galt5hpgt2hpgt3*, *galt2galt5hpgt2*, and *galt5hpgt1hpgt2*), a quadruple mutant (*galt2galt5hpgt1hpgt3*), and a quintuple mutant (*galt2galt5hpgt1hpgt2hpgt3*) which will be confirmed using RT-PCR. These higher order mutants will be examined by biochemical (AGP and Hyp-glycoside profiling by RP-HPLC) and physiological phenotype analysis (soil and media based growth measurements of roots, stems, inflorescences, seeds) and compared to wild-type Col-0 control plants to elucidate the functions of various Hyp-GALTs.

**P99. Auxin Mediates Cell-Cell Communication in Unicellular Microalgae, *Chlorella sorokiniana***

**Jithesh Vijayan**, *University of Nebraska-Lincoln, Lincoln, NE, USA*

Auxin has been studied extensively in higher plants for its important role in development. Although much is known about the mechanism and role of this tryptophan derived signaling molecule in higher plants, very little is known about the evolution of this hormone. Its prevalence and role in unicellular algae has been questionable. We have detected appreciable levels of indole 3-acetic acid, the most common form of auxin, in cell-free supernatant of axenic *Chlorella sorokiniana* cultures while that in the cell pellet has been barely detectable. This leads us to hypothesize that auxin functions as a cell-cell communication system in this organism and also indicates that the mechanism of auxin secretion existed in the common ancestor of microalgae and multicellular land plants. To further characterize auxin signaling in this organism we carried out a chemical library screen and identified five compounds whose growth inhibition is recovered upon addition of IAA. We studied the growth characteristics of *C.sorokiniana* under the influence of two of these compounds. Two compounds chosen for further studies had a similar backbone structure. These compounds inhibited the growth while addition of Indole 3-Acetic Acid recovered the growth. When treated with these inhibitors, diversity of cell size was limited to Large and very small cells. This is in contrary to the uninhibited cells where we observe a wide range of cell sizes. This leads us to hypothesize that auxin plays a role in cell size control in *C.sorokiniana*. We are currently in process of studying the protein target of these molecules and how auxin signaling is perturbed by these molecules. By studying the role auxin plays in growth of *Chlorella sorokiniana*, a microalgae, we intend to understand the ancestral function of this important plant hormone.

**P100. Impact of Future Climate on Soybean Breeding Objectives**

**Theodore Hartman**, *Iowa State University, Ames, Iowa, USA*

Historic crop yield increases have been associated with deliberate breeding objectives designed to optimize plant performance for the contemporary climate. However, changes in climate, e.g. temperature, precipitation, and carbon dioxide concentration, are predicted to occur in future years. This study identifies how changes in soybean physiological parameters, through different cultivars, have attributed to observed yield increases and how future climate changes will impact soybean yields with changing plant physiological parameters. Simulations using an integrated biosphere model, Agro-IBIS, were conducted to model soybean yields. A method for calibrating the model was developed, using observed data for crop yield, harvest index, interception efficiency, and conversion efficiency to set model allocation parameters.



The model was run with data representing a contemporary climate, 1983-2013, and a future climate, 2041-2071. Soybean yields increased with newer cultivars, described by specific changes in plant physiological parameters including initial and final carbon allocation to the roots, when fitted with crop yield. This study also found that certain combinations of plant physiological traits exhibit more variability in future climates, information which plant breeders can use to make selections for less variable, high yielding soybeans.

**P101. Unraveling the Metabolic and Biological Importance of Sphingolipid <sup>1</sup><sub>SEP</sub> Long-Chain Base  $\Delta$ 4 Unsaturation in Plants**

**Dongdong Zhang**, *University of Nebraska-Lincoln, Lincoln, NE, USA*

Long-chain bases (LCBs) are unique structural components of the sphingolipid ceramide backbone and also function in their free and phosphorylated forms as regulators of cellular processes, such as programmed cell death. A portion of the immense sphingolipid structural diversity arises from the C-4 hydroxylation and  $\Delta$ 4 and  $\Delta$ 8 desaturation of LCBs. In these studies, we are attempting to address why most tissues of Arabidopsis are deficient in LCB  $\Delta$ 4 unsaturation, a structural modification typically limited to the glucosylceramide (GlcCer) sphingolipid class, while tomatoes are highly enriched in LCB  $\Delta$ 4 unsaturation. To this end, we have generated Arabidopsis lines with constitutive up-regulation of the LCB  $\Delta$ 4 desaturase from Arabidopsis and tomato, and tomato RNAi lines with suppression of the native LCB  $\Delta$ 4 desaturase. Arabidopsis lines with up-regulated  $\Delta$ 4 desaturase expression accumulate the diunsaturated LCB d18:2 $\Delta$ 4,8 to levels of 60% in GlcCer, and display ~2-fold increase in total GlcCer levels. Of the two sources of LCB  $\Delta$ 4 desaturase genes tested, the strongest chemical phenotypes observed were achieved with the tomato gene, and these lines also displayed defects in flower, pollen and silique development. Conversely, RNAi silencing of the LCB  $\Delta$ 4 desaturase in tomato leaves resulted in a reduction of d18:2 $\Delta$ 4,8 to 1% of the GlcCer LCBs, a nearly 90-fold reduction compared to leaves of non-transgenic plants. Most notably, the total GlcCer content of leaves of RNAi lines was only 10% of that of wild-type leaves, but had no impact on levels of inositolphosphoryl ceramides. Despite this dramatic reduction of GlcCer content, no phenotypic differences were observed in the growth and development of the tomato RNAi lines. Overall, these results demonstrate the importance of LCB  $\Delta$ 4 unsaturation to ceramide channeling for GlcCer synthesis, and suggest that high levels of LCB  $\Delta$ 4 unsaturation can have deleterious effects on Arabidopsis reproductive capacity. The mechanism for this phenotype is currently under investigation.

**P102. RhizoDive: An education cum research pipeline on rhizobial biodiversity**

**Jesus R. Loya**, *South Dakota State University, Brookings, SD, USA*

The discovery of high efficiency nitrogen-fixing rhizobia strains has the potential to alleviate the use of man-made nitrogenous fertilizers that contribute to water pollution from run-off. The first step towards this goal is to determine the diversity of rhizobia present in native soils and evaluate them for plant competency and N-fixation efficiency. In conjunction with South Dakota schools, the Rhizodive project will seek to collect rhizobia samples from local unimproved land through teacher-guided lessons. The collected samples will be submitted to South Dakota State University for analysis. Inoculants of extracted rhizobia strains will be used with Robert variety Glycine Max seeds. The nodules will be examined using phenotypic and metagenomics analysis. Nitrogen fixation capacity will be examined using the Acetylene Reduction Assay. By comparing the sequence data of high-capacity and low-capacity fixing strains, we expect to see correlations in genes responsible for high efficiency nitrogen-fixation pathways.

**P103. Investigating a chromatin-based model for cell cycle regulation by the retinoblastoma complex in Chlamydomonas**

**Yi-Hsiang Chou**, *Donald Danforth Plant Science Center, St. Louis, MO, USA*

The Retinoblastoma (RB) tumor suppressor pathway is conserved in plants and green algae where it plays a role in gating the G1/S-phase transition and in cell size control. The green alga *Chlamydomonas reinhardtii* proliferates using a multiple fission cell cycle in which a long G1 phase is followed by a series of rapid alternating S and M cycles to produce 2<sup>n</sup> daughters. We previously found that mutants in the single copy genes (*MAT3/RB*, *DPI* and *E2F1*) encoding Chlamydomonas RB complex (RBC) subunits disrupt size control, but do not appear to affect cell cycle transcription which is thought to be a main function of the RB

pathway for driving S phase entry in other taxa. New global transcriptome data from RBC mutants also failed to support a transcriptional control model for the *Chlamydomonas* RBC since periodically expressed cell cycle genes were unaffected in the mutants, and most mis-regulated genes did not show an expression pattern consistent with the mutant cell cycle phenotypes (i.e. up in *mat3/rb*, down in *dp1* or vice versa). Our data suggest that the RBC instead may govern cell cycle progression through direct modification of chromatin via association with stage-specific cell-cycle proteins, chromatin modifiers, or replication factors. We describe here a proteomics approach for identifying RBC associated proteins. Using an affinity-tagged and complemented double mutant strain, *mat3/rb dp1::HA-MAT3/RB FLAG-HIS-DP1*, we can consistently co-purify the core RBC subunits. Ongoing work involves identification and validation of additional cell-cycle-stage specific and constitutively-associated subunits of the RBC that may help mediate its function as a chromatin-based cell cycle regulator.

#### **P104. Characterization of xylan synthase complexes (XSCs) in rice cultivar (*Oryza sativa* ssp *Japonica*)**

**Javaid Tasleem**, *Ohio University, Athens, OH, USA*

Hemicellulosic polysaccharides xylans represent the third most abundant polymer on Earth after cellulose and lignin. Xylans maintain the integrity of plant cell wall and play a significant role in plant growth and development. Xylans are made up of a linear backbone of  $\beta$ -(1,4)-linked xylosyl residues with side chains of  $\alpha$ -arabinofuranose and/or  $\alpha$ -glucuronic acid residues. Despite that many genes have been associated with the biosynthesis of xylans in grasses, the biochemical mechanism is not well characterized. For example, we still don't know whether these genes contribute to different Xylan Synthase Complexes (XSCs) needed for xylan synthesis in primary and secondary cell walls (CWs). To answer these questions, rice endosperm is used as model system because of its economic significance and the deposition of endosperm CW polysaccharides (including xylans) is tightly regulated during endosperm development, as they are sequentially synthesized over time. In this study, a co-expression based gene association network (GAN) approach was used to efficiently predict the gene regulatory network based on gene expression similarity, our analysis reveals the presence of three potential XSCs (OsXSC1-3). While OsXSC1 is associated with primary CW deposition, OsXSC2 and OsXSC3 are associated with secondary CW synthesis. The characterization of the biochemical and physiological functions of these putative OsXSCs is underway using the combination of genome editing (CRISPR/Cas9), bimolecular fluorescence complementation (BiFC), and biochemical approaches.

#### **P105. A Novel Maize Glycosyltransferase is Required for Carbon Export from Source Tissues**

**Tyler McCubbin**, *Division of Plant Sciences, University of Missouri*

As autotrophs, plants must transport carbon that is fixed in the photosynthetic source tissues, such as leaves, to the non-photosynthetic sink tissues, such as roots or reproductive tissues. This process, known as carbohydrate partitioning, is essential for plant growth and survival and requires coordinated action by many enzymes and transporters. Here we describe a mutant with carbohydrate partitioning defects, including reduced growth, reproductive defects, and carbohydrate hyperaccumulation in leaves. We identified three alleles of the causal gene, which were all single amino acid mutations that were mapped to a putative glycosyl transferase on chromosome 9. Little is known about the biochemical function of the predicted protein, but we present data suggesting it may function in cell wall biosynthesis or remodeling. To further characterize these mutants, we conducted a morphological analysis which revealed ectopic lignin deposition in the phloem of mature leaves. These deposits occurred in a developmentally progressive pattern. We hypothesize that these lignin deposits might act to perturb long distance transport of sugars, interfering with source to sink sugar transport.

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Last Name	First Name	email
Alexander	Liza	liza@iastate.edu
Arndorfer	Ryan	ria@iastate.edu
Aryal	Sadikshya	Sadikshya.aryal@sdstate.edu
Auger	Donald	donald.auger@sdstate.edu
Awale	Prameela	prameelaawale@gmail.com
Baert	Nicholas	nwbbgk@mail.missouri.edu
Bapat	Amruta	amruta03@iastate.edu
Bassham	Diane	bassham@iastate.edu
Baumbach	Jordan	baumbach@iastate.edu
Baylis	Olivia	baylisol@msu.edu
Becraft	Philip	becraft@iastate.edu
Benning	Christoph	benning@msu.edu
Best	Norman	bestn@missouri.edu
Block	Anna	anna.block@ars.usda.gov
Blythe	Amanda	amb4x2@mail.missouri.edu
Bohler	Jenna	jkv5f@mail.missouri.edu
Braun	David	braundm@missouri.edu
Braun	Ian	irbraun@iastate.edu
Brown	Reid	rbbrown@iastate.edu
Burch	Merritt	merritt.b.burch@sdstate.edu
Cao	Lijun	clj8903@gmail.com
Carino	Elizabeth	eicarino@iastate.edu
Carter	Clay	cicarter@umn.edu
Chaiprom	Usawadee	chaipro2@illinois.edu
Challa	Ghana	challa.ghanashyam@gmail.com
Chambers	Lauran	lauranc@iastate.edu
Chatt	Elizabeth	elizabeth.chatt.ec@gmail.com
Chen	Wei	wchen3@dow.com
Chen	Keting	kchen@iastate.edu
Chen	Changtian	cchen8@iastate.edu
Christians	Matthew	christmi@gvsu.edu
Conner	Kyle	krp7c@mail.missouri.edu
Cook	Mathew	mdcook@iastate.edu
Culbertson	Alan	culb@iastate.edu
Dannenhoffer	Joanne	danne1jm@cmich.edu
Davis	Lawrence	ldavis@ksu.edu
Davis	Lauren	laurdav@siue.edu
Deever	Dan	daniel.deever@omahazoo.com
Dhungana	Singha	srdm93@mail.missouri.edu
Ehrlich	Jacqueline	ehrlchj@iastate.edu
Ekanayake	Gayani	gemrb@mail.missouri.edu
England	Tessa	tenglan@siue.edu
Esfahanian	Malihe	mesfaha@ilstu.edu
Fekaris	Nick	fekarism@msu.edu
Ferin	Kelsie	kmferin@iastate.edu
Flyckt	Kayla	kayla.flyckt@pioneer.com
Fredericksen	Brett	frede368@umn.edu
Fu	Xinyu	xinyufu@iastate.edu
Gaillard	Paul	paul.gaillard@sdstate.edu
Garlock	Rachel	rgarlock@iastate.edu
Geisler Lee	Jane	geislerlee@siu.edu
Givens	Jacob	jake.givens12@gmail.com
Gontarek	Bryan	bryan.gontarek@monsanto.com
Gonzalez Solis	Ariadna	ariadnags718@gmail.com
Grambo	Sarah	segrambo@gmail.com
Gunn	Shayla	slgunn@siu.edu
Gutensohn	Michael	michael.gutensohn@mail.wvu.edu
Hartman	Theodore	theodore@iastate.edu
Heaton	Emily	heaton@iastate.edu
Heggen	Mark	mheggen@iastate.edu
Higgs	David	higgs@uwp.edu

Last Name	First Name	email
Hind	Sarah	srhind@illinois.edu
Hoffman	Teddy	Hoffmanthe@uwplatt.edu
Hoffmann-Benning	Susanne	hoffma16@msu.edu
Hohenstein	Jessica	jdhoven@iastate.edu
Holan	Katerina	holan2@iastate.edu
Horvath	David	david.horvath@ars.usda.gov
Ibore	Martha	mibore@iastate.edu
Jahan	Md Asraful	jahan.geb@gmail.com
Janick Buckner	Diane	djb@truman.edu
Jantes	Justin	justin.jantes@pioneer.com
Javaid	Tasleem	tj999716@ohio.edu
Jia	Meirong	meirongj@iastate.edu
Jiang	Shang-Chuan	jiangsc@uchicago.edu
Jiang	Hao	jianghao@iastate.edu
Jones	Savanah	savy15012@iastate.edu
Kanodia	Pulkit	pkanodia@iastate.edu
Kaur	Dasmeet	dk782516@ohio.edu
KC	Pratiksha	pratikshakc77@gmail.com
Kelley	Dior	dkelley@iastate.edu
Keppler	Brian	bkeppler@gmail.com
Kessens	Ryan	kessens@wisc.edu
Klee	Harry	hjklee@ufl.edu
Koenig	Amanda	koenigam@msu.edu
Kovinich	Nikola	nikovinich@mail.wvu.edu
Kramer	Skyler	stk7c9@mail.missouri.edu
LaBrant	Evan	evan.labrant@huskers.unl.edu
Lapham	Rachelle	rbuuck@purdue.edu
Larue	Clayton	clayton.t.larue@monsanto.com
Lavell	Anastasiva	lavellan@msu.edu
Lawrence-Dill	Carolyn	triffid@iastate.edu
Lee	Keunsub	leex1708@umn.edu
Lekai	Gloria	gloria.lekai@licor.com
Lemke	Cody	clemke@iastate.edu
Leroux	Devon	lerou1d@cmich.edu
Lewis	Clarissa	crissayeLewis@gmail.com
Li	Zhaoxia	zhaoxia@iastate.edu
Li	Wanlong	Wanlong.li@sdstate.edu
Liao	Ching-Yi	cyl@iastate.edu
Liu	Bing	bingl@iastate.edu
Liu	Yang	bluesun5198@gmail.com
Lucas	Jessica	jessicareganlucas@gmail.com
Luesse	Darron	dluesse@siue.edu
MacIntosh	Gustavo	gustavo@iastate.edu
Majumdar	Tanvi	tanvimajumdar@gmail.com
Matthes	Michaela	matthesm@missouri.edu
McCubbin	Tyler	mccubbin.t@gmail.com
McDonald	Tami	trmcdonald@stkate.edu
McKenzie	Steven	stevenmckenzie.sm@gmail.com
McLaughlin	Sean	Seanm1@iastate.edu
McNinch	Colton	cmcninch@iastate.edu
McReynolds	Maxwell	maxwellm@iastate.edu
Mertz	Rachel	mertzr@missouri.edu
Miller	W. Allen	wamiller@iastate.edu
Miller	Jonah	jdmler@iastate.edu
Min	Kyungwon	kwmin@iastate.edu
Mudalige Jayawickrama	Rasika	rmudalig@dbq.edu
Mugume	Yosia	ymugume@iastate.edu
Mukherjee	Thiya	thiya.usa@gmail.com
Nagel	Raimund	rnagel@iastate.edu
Neveau	Megan	mnn6412@truman.edu
Newton	Allison	anewton@siue.edu
Ngaki	Micheline	mnnngaki@iastate.edu

Last Name	First Name	email
O'Neill	Kelly	koneill@iastate.edu
Oyagbenro	Richard	gbenro@iastate.edu
Paul	Puneet	puneet6288@gmail.com
Periandri	Erica	eperian@siue.edu
Peters	Reuben	ripeters@iastate.edu
Petla	Bhanu	bhanuprakash360@gmail.com
Pluemer	Brett	pluemerbr@uwplatt.edu
Posekany	Tes	posekany@iastate.edu
Probst	Jennifer	jap91496@live.missouristate.edu
Pu	Yunting	ypu@iastate.edu
Robinson	Peyton	peyrobi@siue.edu
Roesler	Keith	Keith.Roesler@Pioneer.com
Rosenthal	David	rosentha@ohio.edu
Roy	Rahul	rroy@umn.edu
Russell	Andrew	andrew.russell@northern.edu
Sahoo	Dipak Kumar	dsahoo@iastate.edu
Schmidt-Dannert	Mark	marksd@iastate.edu
Schmitt	Anthony	schm2692@umn.edu
Schnable	Patrick	schnable@iastate.edu
Schrick	Kathrin	kschrick@ksu.edu
Sedbrook	John	jcsedbr@ilstu.edu
Serson	William	william.serson@avemaria.edu
Sigmon	Brandi	bsigmon2@unl.edu
Singh	Dilkaran	Dilkaran.Singh@sdstate.edu
Smith	Natalie	Venette658@live.missouristate.edu
Smith	Montgomery	smit2317@msu.edu
Solhaug	Erik	solha006@umn.edu
Sorkin	Maria	sorkinm21@gmail.com
Struttmann	Joseph	jwsgk9@mail.missouri.edu
Subhasappa	Raviraj	ravirajbanakar@hotmail.com
Subramanian	Senthil	Senthil.Subramanian@sdstate.edu
Suo	Taylor	tjsuo@ilstu.edu
Takeuchi	Tomomi	takeuch9@msu.edu
Tang	Jie	jietang@iastate.edu
Tarpley	Mason	mwt7316@truman.edu
Tejera	Mauricio	mauri@iastate.edu
Thu	Sandi	swthu@iastate.edu
Tran	Thu	tmtqk3@mail.missouri.edu
Trost	Toria	ttrost@siue.edu
Uthe	Kayla	kuthe@iastate.edu
Venkateshwaran	Muthusubramanian	venkateshwam@uwplatt.edu
Vidrine	Bri	bvidrine@iastate.edu
Vijayan	Jithesh	jitheshbt@gmail.com
Vollbrecht	Erik	vollbrec@iastate.edu
Wang	Ping	pingw@iastate.edu
Wang	Wei	oneway1985@gmail.com
Wang	Fumin	fw0003@mix.wvu.edu
Weingartner	Kyler	kaweingartner@ksu.edu
Welti	Ruth	welti@ksu.edu
Willard	Terra	tjw6676@truman.edu
Wimalanathan	Kokulapalan	kokul@iastate.edu
Winters Rozema	Elizabeth	ekwinters@eastcentral.edu
Wu	Yajun	vajun.wu@sdstate.edu
Wu	Hao	haowu@iastate.edu
Xu	Meimei	xumm@iastate.edu
Yandeau Nelson	Marna	myn@iastate.edu
Zabotina	Olga	zabotina@iastate.edu
Zebosi	Brian	bzebosi@iastate.edu
Zhang	Yuan	vz192814@ohio.edu
Zhang	Jiaoping	jiaopingzhang@gmail.com
Zhang	Juan	juanz@iastate.edu
Zhang	Jiaping	zhangjiaping0604@aliyun.com

Last Name	First Name	email
zhang	Ning	ningz@iastate.edu
Zhang	Dongdong	zhangdong081@gmail.com
Zhao	Le	lezhao11@iastate.edu
Zhou	Mian	mianzhou@iastate.edu
Zhu	Huilan	huilanz@iastate.edu