ASPB

Midwest Section

Midwest ASPB Meeting 2024

March 16-17, 2024

The Memorial Union

Purdue University, West Lafayette, IN

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ACKNOWLEDGMENTS

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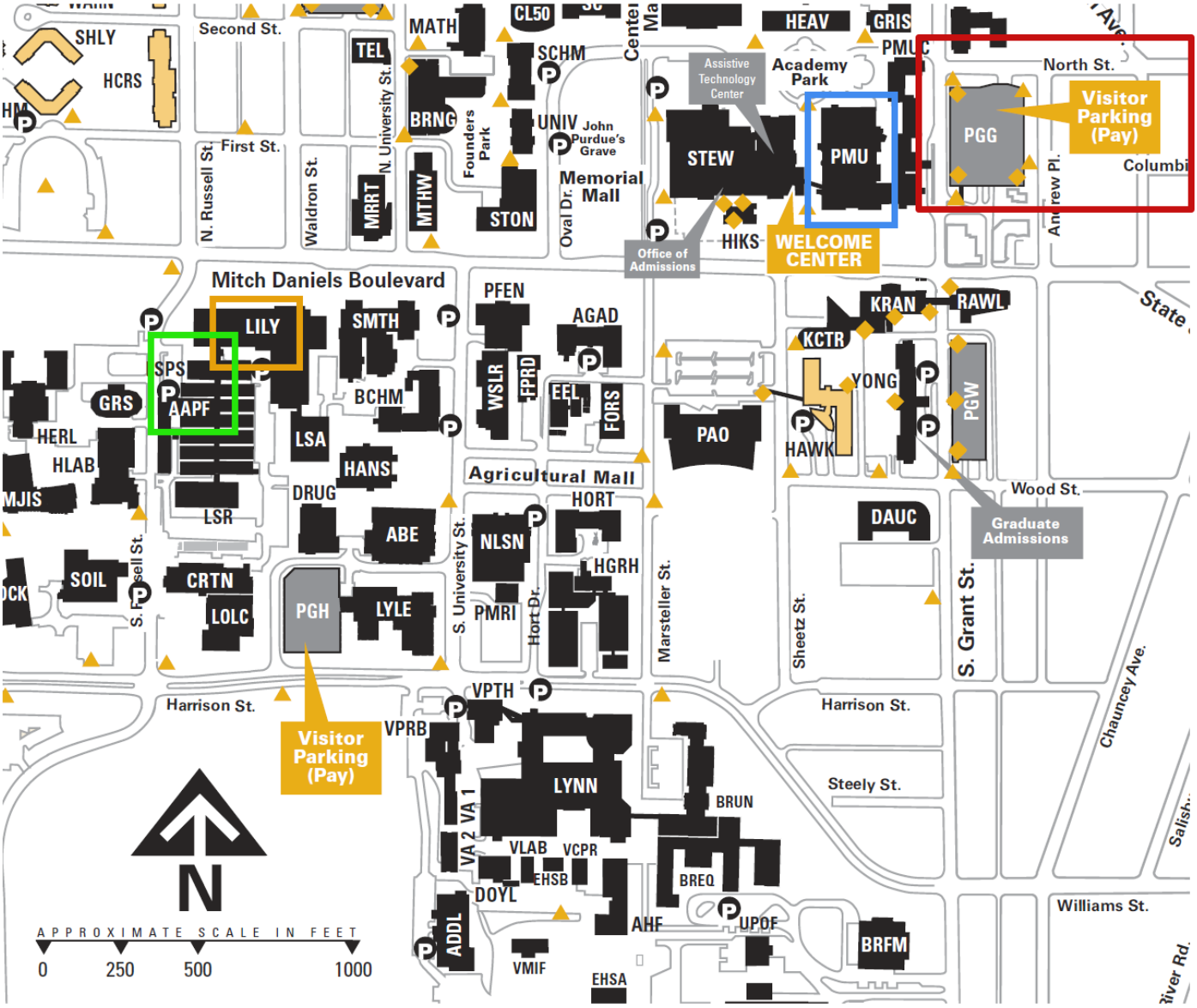
PURDUE CAMPUS MAP AND PARKING



Midwest ASPB Meeting Venue – Purdue Memorial Union (PMU)
Visitor Parking Garage – Grant Street Parking Garage (PGG)
Phenotyping Facility Tour - Ag Alumni Seed Phenotyping Facility (AAPF)
Purdue Herbaria Tour – Lilly Hall of Life Sciences (LILY)

MAP LINKS:

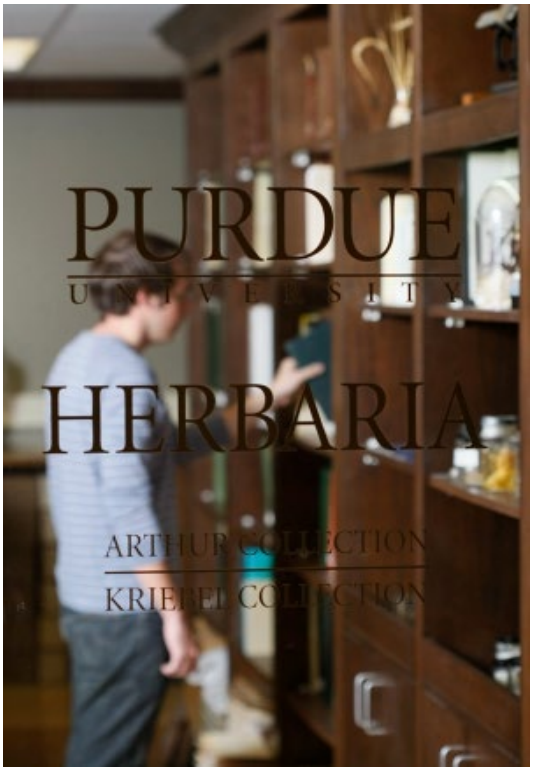
Purdue Memorial Union maps
Grant Street Visitors Garage

OPTIONAL TOURS

Friday, March 15th, 4:00-6:00pm

Meeting Point: Union Club Hotel Lobby @ 4.00 pm (Group 1) and 4.30 pm (Group 2)

Ag Alumni Seed Phenotyping Facility (AAPF)
160 S Russell St.
West Lafayette, IN 47907



Purdue University Herbaria
Arthur Fungarium and the Kriebel Herbarium
Lily Hall of Life Sciences, room G-447
915 Mitch Daniels Blvd.
West Lafayette, IN 47907

MEETING PROGRAM

Friday, March 15

4:00-6:00 pm	Tour of Ag Alumni Seed Phenotyping Facility (optional)
	Tour of Purdue Herbaria (optional)

Saturday, March 16

7:00 - 8:00 am	Registration/Check-in/Poster Set-up/Breakfast (provided)
8:00 - 8:05 am	Welcome Remarks
8:05 - 9:35 am	**Oral Session I**
8:05 - 8:35 am	Jianxin Ma, Purdue University Soybean Translational Genomics: From Natural Variation to Edited Mutation
8:35 - 8:50 am	Ryan Patrick, Purdue University (T1) Determining Regulatory Networks for Specialized Metabolism Using Natural Diversity
8:50 - 9:05 am	Rachel Rivero, University of Michigan (T2) Harnessing the Genetic Framework in "Mother of Thousands" Regeneration
9:05 - 9:20 am	Dilkaran Singh, University of Illinois Urbana-Champaign (T3) An Iterative Systems Biology Approach Reveals bZIP1 as Regulator of Primary Metabolism Under Elevated CO_2 in Arabidopsis
9:20 - 9:35 am	Nianyuan Hu, Michigan State University (T4) Identification and Characterization of Suppressor Mutant in a Jasmonic Acid Accumulating Arabidopsis Line
9:35 - 10:35 am	Poster Session I (Even #s)/Refreshments
10:35 - 12:05 pm	**Oral Session II**
10:35 - 11:05 am	Kathleen Greenham, University of Minnesota Leveraging a *Brassica rapa* Pan-Genome to Investigate Diel Transcriptomic Responses to Cold Stress
11:05 - 11:20 am	Amanpreet Kaur, Purdue University (T5) Transcriptomic Analysis of Semi-Dwarf Mutant Identifies a Role of GRAS42 Transcription Factor in Brassinosteroid Signaling
11:20 - 11:35 am	Lichun Zhou, University of Kentucky (T6) The Polyadenylation Factor CPSF30 is Important for Root Development and Plant Response to Abiotic Stresses
11:35 - 11:50 am	Samantha Fedoush, Ohio University (T7) The Role of Select E3 Ligases in Plant Gravitropism
11:50 - 12:05 pm	Jacob Kunkel, University of Wisconsin (T8) Arabidopsis ACT Domain Repeats 11 (ACR11) Knockout Mutants Hyperaccumulate Amino Acids Under High Ammonium Conditions
12:05 - 1:00 pm	Lunch (provided)
1:00 - 2:00 pm	Panel Discussion: "Priorities, Challenges, and Emerging Areas of Plant Physiology"

2:00 - 3:45 pm	**Oral Session III**
2:00 - 2:30 pm	**Peter Lundquist, Michigan State University** Dynamic Plastoglobules Under Environmental Stresses
2:30 - 2:45 pm	Coffee break
2:45 - 3:00 pm	**Jaspreet Sandhu, Donald Danforth Plant Science Center (T9)** Growth Hormones BR and GA Modulate Spikelet Meristem Identity Through the RAMOSA1 Pathway in *Setaria* and Maize
3:00 - 3:15 pm	**Steven McKenzie, Purdue University (T10)** Phosphorylation and Oxidative Damage Mediate the Disassembly of Photosystem II in *Arabidopsis thaliana*
3:15 - 3:30 pm	**Murtaza Barkarar, Michigan State University (T11)** Visualizing Acetylation-Induced Changes in Plant Secondary Cell Wall Structure and Dynamics through Molecular Simulation
3:30 - 3:45 pm	**Eric Fritschi, University of Missouri (T12)** Role of VESICULAR TRAFFICKING 5 (VES5) Protein in Iron Accumulation and Photosynthetic Compounds in *Arabidopsis thaliana*
3:45 – 4:30 pm	**Clint Chapple, Purdue University** (Plenary talk) Phenylpropanoids As Potential Regulators of Plant Growth
4:30 - 5:30 pm	**Poster Session II** (Odd #s)/Appetizers

Sunday, March 17

7:00 - 8:00 am	**Registration/Check-in/Breakfast** (provided)
8:00 - 9:45 am	**Oral Session IV**
8:00 - 8:30 am	**Tessa Smith, Danforth Plant Science Center** An Uncommon View: 3D Imaging of Plasmodesmata
8:30 - 8:45 am	**Deepak Bhandari, Michigan State University (T13)** Logistics of Defense: How TGNap1 Mediates Secretion of Antimicrobial Proteins
8:45 - 9:00 am	**Shawn Thomas, University of Missouri (T14)** Duplications Influence Abiotic Stress Tolerance in a Mustard Crop Wild Relative
9:00 - 9:15 am	**Xi Yang, Purdue University (T15)** Single Cell-Derived Multicellular Meristem: Insights into Male-to-Hermaphrodite Conversion and *de novo* Meristem Formation in *Ceratopteris*
9:15 - 9:30 am	**Ajay Gupta, University of Missouri (T16)** Multiplexed Prime Editing in Rice for Multi-Trait Improvement
9:30 - 9:45 am	**Shannon Stirling, Purdue University (T17)** Volatile Sesquiterpene Communication in Plants Relies on a KAI2-Mediated Signaling Pathway
9:45 - 10:00 am	Coffee break

10:00 - 11:45 am	Oral Session V
10:00 - 10:30 am	**Million Tadege, Oklahoma State University** Molecular Insight into the Mechanism of Leaf Blade Development in *Medicago truncatula*
10:30 - 10:45 am	**Erik Amézquita, University of Missouri (T18)** The Early Dodder Gets the Host: Decoding the Coiling Patterns of *Cuscuta campestris* with Automated Image Processing
10:45 - 11:00 am	**Meenu Singla-Rastogi, Indiana University (T19)** Plants Secrete Diverse Species of RNA onto their Leaf Surfaces with a Potential Role in Plant-Microbe Interactions
11:00 - 11:15 am	**Arkadipta Bakshi, University of Wisconsin (T20)** Unlocking Flooding Resistance: The Role of CAX2 in *Arabidopsis thaliana*'s Flooding Induced Low-Oxygen Survival
11:15 - 11:30 am	**Dalen Fultz, Indiana University (T21)** Sequence and Epigenetics of Active and Silenced Nucleolus Organizers in Arabidopsis
11:30 - 11:45 am	**Kumar Shrestha, University of Nebraska (T22)** Disruption of the Sorghum Circadian Clock Impacts Sorghum-Sugarcane Aphid Interaction Dynamics and Aphid Feeding Behavior
11:45 - 12:15 pm	**Awards, Announcements and Closing Remarks**

INVITED SPEAKERS

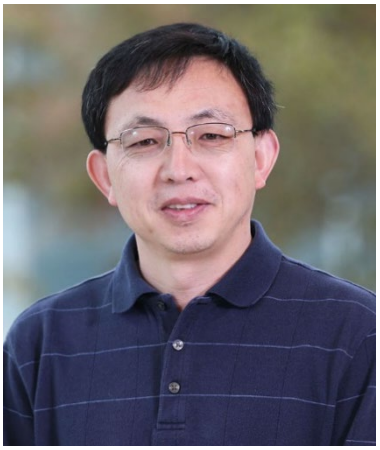
PLENARY TALK

Clint Chapple
Purdue University

Phenylpropanoids As Potential Regulators of Plant Growth

Lignin has been a target for cell wall engineering because it is a major determinant of biomass recalcitrance. Of all cell wall biopolymers, it can most easily be manipulated because its composition is dictated only by the levels of its component monomers, hydroxycinnamyl alcohols, produced by the plant. Plants engineered to produce lignins derived predominantly from sinapyl alcohol (S) monomers or hydroxycinnamaldehyde subunits grow normally, and their tissues exhibit superior saccharification properties. Unexpectedly, when these engineering strategies are combined in Arabidopsis, the resulting plants are extremely dwarfed, have fewer lateral roots, and have aberrant root hairs. Because both of these strategies would be predicted to decrease pools of the lignin precursor coniferyl alcohol (CA), we tested the hypothesis that supplementing these plants with CA or one of its downstream derivatives might complement these growth defects. Indeed, CA and its 8-8 dimer pinoresinol both complemented the growth and lateral root phenotypes. Because both of these compounds can be incorporated into the lignin polymer, we also tested the effects of dimethylpinoresinol which cannot. Dimethylpinoresinol complemented the lateral root phenotype, suggesting that CA and pinoresinol or a downstream metabolite derived from them may have an impact on plant development independent of their role in lignin biosynthesis.

INVITED TALKS



Jianxin Ma
Purdue University

Soybean Translational Genomics: From Natural Variation to Edited Mutation

Soybean ranks second, after corn, among the most-planted field crops in the United States. However, the genetic improvement of cultivated soybeans is hindered by a low level of genetic diversity, primarily due to the domestication bottleneck, which accounts for a ~50% reduction in diversity. To facilitate the utilization of untapped genetic variations in the wild ancestors for soybean improvement, it is essential to understand the genetic and molecular bases underlying the suite of domestication-related traits and to identify the 'neglected treasures in the wild' for enhancement of elite cultivars. Now, we are in the age that we have the ability to use genome editing technologies to create targeted changes within a particular gene, for a particular trait, in a precise and efficient way. In this seminar, I will present some recent progresses made by my lab and collaborators regarding the history and dynamic process of soybean domestication and discovery of genes or other genetic elements underlying domestication-related traits and other traits of agronomic importance such as plant architecture and root nodulation. I will also share our recent effort on soybean transformation and gene editing pipeline critical functional genomics studies and for translation of basic knowledge into sustained soybean improvement.

Kathleen Greenham
University of Minnesota

Leveraging a *Brassica rapa* Pan-Genome to Investigate Diel Transcriptomic Responses to Cold Stress

The genus *Brassica* offers a rich source of morphological diversity containing crops with leaf, flower, and root vegetables for consumption, oil production, and fodder. *B. rapa* captures much of this diversity in one species with Chinese cabbage, pak choi, oilseed, turnip, and leafy vegetable varieties. As with many crops, *B. rapa* is polyploid, diverging from *Arabidopsis thaliana* roughly 24 million years ago and undergoing genome triplication followed by extensive gene fractionation. Consequently, the genome architecture has diverged from Arabidopsis leaving open many questions as to how the expansion of the *B. rapa* genome has influenced the interaction between metabolic and physiological processes and the environment. The coordination of internal processes with the environment is dependent on the time of day. The ability to tell time is provided by an internal circadian clock found in all kingdoms of life. We are interested in how the circadian clock coordinates plant responses to environmental stressors throughout the day. We leverage the natural diversity in *B. rapa* and Arabidopsis species to decipher this temporal regulation with the goal of manipulating the timing of certain processes to improve stress tolerance. Following cold stress, we find large scale rewiring of the transcriptome that is likely driven in part by alterations to the pace of the clock. Surprisingly, we find extensive intraspecific variation in time-of-day transcriptional responses to abiotic stress across *B. rapa* morphotypes indicating divergence in gene regulation within the species. Through network analysis, we are uncovering candidate regulators of temporal cold responses that are associated with the more tolerant genotypes.

Peter Lundquist
Michigan State University

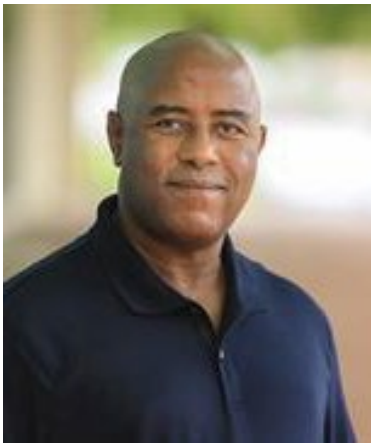
Dynamic Plastoglobules Under Environmental Stresses

Plastoglobules are ubiquitous lipid droplets of plant plastids that consist of a lipidic core that is enclosed by a monolayer membrane surface that is studded with structural and enzymatic protein. The dramatic changes in plastoglobule morphology in response to environmental stresses, including more than 10-fold increases in droplet diameter, suggest a crucial role in plant stress tolerance. However, in the absence of an understanding of protein or lipid changes of plastoglobules under stress, it has been difficult to connect plastoglobules to specific functions. We have used proteomic and lipidomic profiling of plastoglobules isolated from three different photosynthetic species, *Zea mays*, *Arabidopsis thaliana*, and *Synechocystis* sp. PCC 6803 experiencing permissive growth and a stress condition to unveil conserved and distinct features of plastoglobule composition and their dynamic changes. Our results point to common roles in lipid metabolism and regulation of photosynthesis that extend from the cyanobacterial ancestor through the plant lineage, with additional functions in plastid metabolism, such as regulation of jasmonic acid biosynthesis, that have subsequently accrued in angiosperms. Building on our results, we propose a model of core plastoglobule function related to mediating membrane remodeling processes in response to stress adaptation.

Tessa Burch-Smith
Donald Danforth Plant Science Center

An Uncommon View: 3D Imaging of Plasmodesmata

A defining feature of plants is the presence of a cellulosic wall surrounding each cell, resulting in the need for novel strategies for the exchange of signals and metabolites between cells. Multicellular plants have thus evolved cell wall nanopores called plasmodesmata (PD), as one route for intercellular molecular trafficking. PD comprise cytoplasmic, endoplasmic reticulum (ER) and plasma membrane (PM) continuities and thereby, effectively render the plant a symplast, previously described as a supracellular highway. A typical cell wall is perforated by hundreds to thousands of PD that are often clustered into groups, and therefore the continuity between adjacent cells can be extensive. That PD are essential is evidenced by the early developmental lethality of mutants with defects in PD. How PD are formed in plant cell walls to allow the regulated intercellular trafficking of micro- and macromolecules is a question of extreme importance for understanding fundamental aspects of plant biology. We have adopted plasma focused ion beam-SEM (pFIB-SEM) to image large volumes of plant tissue, a form of volume electron microscopy (vEM). One major advantage of pFIB-SEM is that the thickness of a sample that can be analyzed is only limited by the time spent collecting images. Thus, vEM with pFIB-SEM can be used for 3D analysis of larger cell volumes and thicker specimens than TEM tomography. TEM imaging of PD occasionally identified unusual PD structures, for example, 'half-PD' that appears to terminate prematurely and not traverse the entire cell wall. We therefore adopted TEM tomography in an effort to better understand these unusual PD, focusing on clusters of PD as PD are more likely to form near existing PD. Based on these results, we propose a new model for secondary PD formation to allow connections for intercellular communication.



Million Tadege
Oklahoma State University

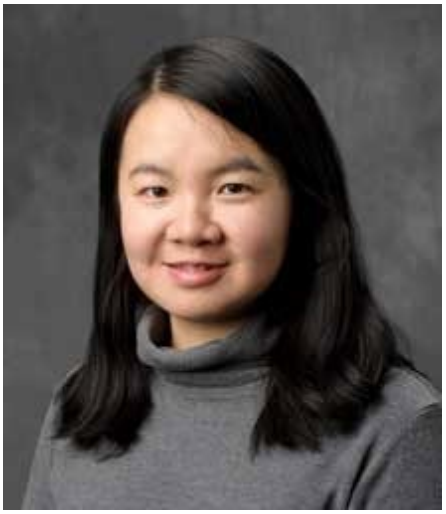
Molecular Insight into the Mechanism of Leaf Blade Development in *Medicago truncatula*

The leaf blade is a highly organized and differentiated photosynthetic apparatus, which develops from a small group of pluripotent stem cells in the shoot apical meristem (SAM). Once the leaf primordium initial cells are recruited from the peripheral region of the SAM, the primordium organizes itself into defined cell layers through highly regimented cell division and cell expansion patterns forming a flattened blade with functionally and structurally distinct adaxial (upper) and abaxial (lower) surfaces. The primordium then grows in both the length (proximodistal) and width (mediolateral) directions resulting in a species-specific leaf morphology. In addition, some cells at the basal portion of primary primordium acquire morphogenetic competence to initiate new leaflets in compound leaf species. For the past several years, we gave been studying the mechanism of leaf blade lateral expansion and leaflet initiation in the model legume *Medicago truncatula*. I will try to summarize our work focusing on two of the key regulatory factors in this process: *STF/LAM1/WOX1* and *SGL1/UNI/LFY*. *STENOFOLIA* (*STF)*, a WOX family transcription factor in *M. truncatula*, also called *LAM1* in *Nicotiana sylvestris* and *WOX1* in Arabidopsis, is required for lateral blade outgrowth and promotes cell proliferation at the adaxial-abaxial juxtaposition through a transcriptional repression mechanism. Lack of *STF* function strongly blocks blade width expansion in *M. truncatula* and results in a nearly naked midrib in *N. sylvestris*. Another *WOX* gene, *MtWOX9*, regulates blade outgrowth antagonistic to *STF,* worsening the *stf* and *lam1* mutant phenotypes, and functions via a transcriptional activation mechanism. On the other hand, leaflet initiation appears to be controlled by *SINGLE LEAFLE 1* (*SGL1*) in *M. truncatula*, also called *UNI* in pea and *LFY* in Arabidopsis. *SGL1* provides morphogenetic competence to the primary primordium to initiate leaflets but its signal is spatiotemporally controlled by BEL1-like homeodomain and C2H2 zinc finger transcription factors resulting in a characteristic trifoliate leaf in *M. truncatula* or pinnate compound leaf in chickpea. I will discuss how leaf blade expansion and leaf complexity pathways may operate independently to control leaf morphology.

PANEL DISCUSSION

"Priorities, Challenges, and Emerging Areas of Plant Physiology"

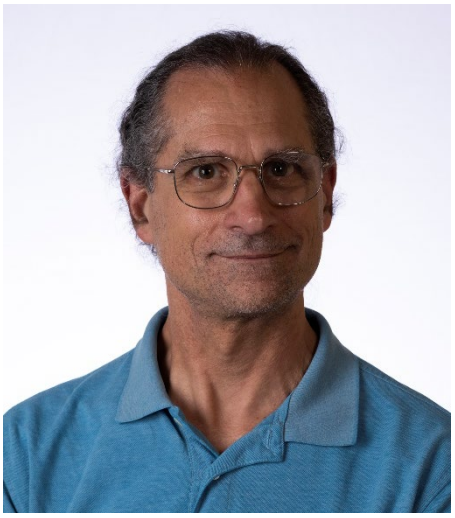
Yun Zhou
Associate Professor
Botany and Plant Pathology
Purdue University
Moderator



Ying Li
Associate Professor
Horticulture & Landscape Architecture
Purdue University
Panelist

Joseph Lynch
Assistant Professor
Plant and Soil Sciences
West Virginia University
Panelist

Tessa Burch-Smith
Principal Investigator
Donald Danforth Plant Science Center
Panelist



Mike Nuccio
Senior Director, R&D
INARI
Panelist

Kathleen Greenham
Assistant Professor
Plant and Microbial Biology
University of Minnesota
Panelist

ORAL PRESENTATIONS

T1 - Determining Regulatory Networks for Specialized Metabolism Using Natural Diversity (PD)

Ryan M. Patrick and Ying Li
Purdue University

The ability to obtain or generate large-scale sets of gene expression data, together with advances in computing approaches which can decipher underlying patterns in biological data, creates opportunities for using network-based analyses to understand the relationships between genes and traits. I hypothesized that using natural variation as an experimental system, together with machine learning algorithms, gene regulatory networks relevant for specific traits could be determined solely with a large set of transcriptomic data and measurements of the trait across related species with sufficient phenotypic diversity. As proof-of-concept, I applied a two-phase machine learning approach to determine underlying genes and regulators contributing to floral pigment variation (anthocyanins and flavonols) across a sample of petunia and related species. I was able to recapitulate essential genes and key regulatory networks for anthocyanin biosynthesis, along with intriguing subnetworks for known transcription factors and identifying potential novel regulators of floral anthocyanin levels, using only metabolite measurements and RNA-Seq data. For floral flavonols, a triad of transcriptional regulators acting coordinately was proposed by the analysis, which are undergoing evaluation. The results of this proof-of-concept project support the use of this approach for other plant traits for which the underlying basis or regulation is poorly understood.

T2 - Harnessing the Genetic Framework in "Mother of Thousands" Regeneration (G)

Rachel Rivero, Eun-Gyu No, Ping He, and Libo Shan
University of Michigan

Plant bioengineering is hindered by challenges in the recalcitrant tissue-culture regeneration process. The succulent *Kalanchoe laetivirens* (Kl), known as “Mother of Thousands,” proliferates by forming clonal plantlets along leaf margins. This phenomenon, involving efficient and cell-autonomous organogenesis and embryogenesis processes, provides a unique opportunity to understand regulatory networks of developmental regulators (DRs) and break the plant biomanufacturing bottleneck. To understand the genetic basis underlying the potent capacity of plantlet emergence, RNA-sequencing reads were collected from micro-dissected tissues at distinct developmental stages, culminating in a robust *de novo* transcriptome, validated for its capacity to identify DRs. Differential expression analysis revealed fundamental processes and re-categorized development. Transcription factors were uncovered with expression patterns parallel with stem cell maintenance, setting the framework for in-depth investigation. To understand genetic plasticity and overcome limitations of *de novo* transcriptomic analysis, we conducted whole-genome sequencing, aiming to assemble the genome. Furthermore, chromosomal counting confirmed that Kl is likely an allotetraploid, derived from the diploid parents *K. laxiflora* and *K. daigremontiana*, promoting us to genome assembly of *K. daigremontiana*. These data provide unprecedented resources to examine genome organization and development. Finally, transient expression using reporters through Agrobacterium-mediated manipulation was explored to generate genetically modified next-generation plantlets for biomanufacturing.

T3 - An Iterative Systems Biology Approach Reveals bZIP1 as Regulator of Primary Metabolism Under Elevated CO_2 in Arabidopsis (G)

Dilkaran Singh, Kavya Kannan, Andrew Leakey, and Amy Marshall-Colon
University of Illinois Urbana-Champaign

Elevated CO_2 (eCO_2) causes negative acclimation of photosynthesis capacity and shoot nitrogen (N) concentration via altered N allocation among photosynthetic enzymes, reduced Rubisco activity and reduced nitrate assimilation in Arabidopsis. This can result in lower-than-expected plant yield and compromised nutritional quality. Genetic factors regulating primary metabolism can be altered to produce phenotypes that have higher biomass and N concentration. We constructed a gene regulatory network of Arabidopsis leaf transcriptome grown under CO_2 and N treatments to identify such transcriptional regulators. We found bZIP1 as one of the regulators of CO_2xN-responsive genes. Next, we

analyzed the overexpression of bZIP1 TF experimentally. *bZIP1*-overexpression (*bZIP1*-OX) line accumulated more plant biomass under eCO_2. RNAseq analysis of *bZIP1*-OX plants revealed CxN responsive genes differentially regulated by *bZIP1*-OX are enriched in Jasmonic Acid (JA) related pathways. Further, co-expression network analysis revealed genes related to JA and structural carbohydrates processes are correlated with above-ground biomass. Next, we quantified total JA and 12-OPDA in both genotypes and found both metabolites are differentially accumulated under eCO_2 conditions. Our analysis showed that *bZIP1* overexpression can increase plant biomass under elevated CO_2 and it also differentially regulates JA synthesis and signaling, which likely regulates aspects of biomass accumulation in Arabidopsis under eCO_2.

T4 - Identification and Characterization of Suppressor Mutant in a Jasmonic Acid Accumulating Arabidopsis Line (UG)

Nianyuan (Sam) Hu[1], Yosia Mugume[1], Ron Cook[1], Linda Danhof[1], Abigail Proksch[2], Jordyn Flemming[1], Halle Purcell[1], Michael Beecher[1], Christoph Benning[1], and Jinjie Liu[1]
[1]Michigan State University, [2]Ferris State University

Jasmonic Acid (JA) and its derivatives are important plant hormones involved in plant growth and stress responses. Plastid Lipase 3 (PLIP3) cleaves 18:3 (number of carbons : number of double bonds) acyl groups from chloroplast membrane lipids which are then metabolized to oxylipins including JA. The *PLIP3* overexpression line (*PLIP3*-OX) showed increased levels of JA and its derivatives, altered leaf morphology, and stunted plant growth. We are interested in discovering novel components of JA synthesis and signaling processes. Toward this goal, we conducted a suppressor mutant screen in the *PLIP3*-OX line. One candidate, suppressor mutant 97, carries a recessive mutation leading in the homozygous state to a partial reversal of the *PLIP3*-OX phenotype in addition to yellow leaves and a lipid phenotype similar to *PLIP3*-OX, but with a reduced level of 18:3 acyl groups. Through next-generation DNA sequencing of bulk DNA from an F2 mapping population consisting of 93 homozygous suppressor mutant individuals, a set of mutated candidate genes likely including the one casually responsible for the observed phenotypes was identified. To ultimately determine the causal mutation, we are currently crossing independent T-DNA mutants disrupted in the candidate genes with *PLIP3*-OX and suppressor line 97.

T5 - Transcriptomic Analysis of Semi-Dwarf Mutant Identifies a Role of GRAS42 Transcription Factor in Brassinosteroid Signaling (PD)

Amanpreet Kaur[1], Norman B. Best[2], Thomas Hartwig[3], Josh Budka[1], Rajdeep Khangura[1], Steven McKenzie[1], Alejandro Aragón-Raygoza[4], Josh Strable[4], Burkhard Schulz[5], and Brian P. Dilkes[1]
[1]Purdue University, [2]Genetics Research Unit, USDA-ARS, Columbia, Missouri, [3]Institute for Molecular Physiology, Heinrich-Heine-Universität Düsseldorf, Germany, [4]North Carolina State University, [5]University of Maryland

Brassinosteroids (BR) and gibberellins (GA) regulate plant height and leaf angle in maize. The generation and analysis of mutants with defects in the biosynthesis or signaling of these hormones can enhance our knowledge about plant growth and development. We characterized two mutant alleles of *gras42*, the maize ortholog of rice *DWARF AND LOW TILLERING* (*DLT*) gene. These mutants exhibit a semi-dwarf stature with shorter, wider, and more upright leaves. Gene expression analysis of the *gras42-mu1021149* loss-of-function mutant indicated a weak loss of BR signaling. A parametric index calculated from the expression of experimentally determined BR-responsive genes demonstrated a coordinated increase in the transcript levels of BR-repressed genes and decrease in the transcripts of BR-induced genes in *gras42-mu1021149*. Double mutant phenotypes of *gras42-mu1021149* with *na1-1* and *d1* were consistent with *gras42* encoding a positive regulator of BR-responsive gene expression. Single cell expression data determined that *gras42* was expressed in cells in the G2/M phase of the cell cycle including nascent stomatal complexes, consistent with the previously demonstrated role of the Arabidopsis ortholog in cell-cycle-regulated gene expression. Expression-level GWAS of *GRAS42* transcript accumulation identified natural variation in multiple BR-pathway genes as regulators of GRAS42.

T6 - The Polyadenylation Factor CPSF30 is Important for Root Development and Plant Response to Abiotic Stresses (G)

Lichun Zhou and Arthur G. Hunt
University of Kentucky

Message RNA polyadenylation is an essential step for eukaryotic mRNA transport, translation and turnover. Alternative polyadenylation (APA) serves as an important role in regulation of gene expression. The *Arabidopsis thaliana* ortholog of the 30-kD subunit of the mammalian *Cleavage and Polyadenylation Specificity Factor* (*CPSF30*) gene encodes two proteins, CPSF30S and CPSF30L. Recent studies have shown CPSF30S is a calmodulin-regulated RNA-binding protein, regulates APA, and links with cellular signaling and stress response. To better understand how CPSF30 contributes to stress responses and connects to environment signals, we subjected a set of mutant and complemented Arabidopsis lines to salt, drought, and oxidative stresses. The set of lines included a mutant (*oxt6*) that does not express *CPSF30* as well as lines that express either *CPSF30S* or *CPSF30L* in the *oxt6* background. Seedlings were used to count lateral root number and collected to perform Poly (A) Tag Sequencing (PATSeq). The results show both CPSF30S and CPSF30L are functional in lateral root development. However, CPSF30S and CPSF30L have different functions in responses to stress treatments. Specifically, CPSF30L is required for APA in salt stress. More generally, after stress treatments, genes associated with stress responses tended to use proximal poly(A) sites.

T7 - The Role of Select E3 Ligases in Plant Gravitropism (G)

Samantha Fedoush, Madhura Yapa, Dr. Zihua Hua, and Sarah E. Wyatt
Ohio University

Gravity is a fundamental stimulus that directs plant growth; however, little is known about the regulatory mechanisms of gravitropic signaling. In a 2019 spaceflight experiment, a select group of E3 ligases were found to be differentially regulated in space. E3 ligases play an important role in protein degradation, they have the potential to inhibit or maintain the gravitropic signal depending on their substrate target. To narrow down these candidate E3 ligases to those involved in gravitropism, root and shoot reorientation experiments were conducted. Phloem Protein 2- A13 (PP2-A13) was shown to have a delayed gravitropic response. To characterize the biological importance of PP2-A13 within the gravitropic pathway, potential substrate binding partners of PP2-A13 were identified through yeast 2 hybrid analyses. To narrow the list of candidate binding partners, Arabidopsis tDNA insertion mutants were obtained for each potential binding partner, bred to homozygosity, and analyzed for gravitropic responses in root and shoot reorientation experiments. This work narrowed down candidate E3 ligases identified in a spaceflight study, identified substrate binding partners and explored the role of the substrates in plant gravitropism. This demonstrates the importance of ground-based experimentation in tandem with spaceflight studies to understand molecular players involved in plant gravitropism.

T8 - Arabidopsis *ACT Domain Repeats 11* (*ACR11*) Knockout Mutants Hyperaccumulate Amino Acids Under High Ammonium Conditions (UG)

Jacob Kunkel, Jorge El Azaz, and Hiroshi Maeda
University of Wisconsin

Plants, unlike animals, invest large amounts of energy and nutrients to produce all twenty amino acids. Therefore, these assimilation and biosynthesis pathways are highly controlled via feedback mechanisms. For this purpose, plants have a family of ACT domain containing proteins, named ACT domain repeats (ACR), of largely unknown function. In Arabidopsis, the ACR family consists of twelve genes, with ACR11 localized to the plastid where much of amino acid biosynthesis occurs. This makes ACR11 a particularly interesting model to investigate the role of ACR genes. To understand ACR11's role in the regulation of amino acid biosynthesis, *acr11* knockout mutants were grown at high and low nitrogen concentrations and under different nitrate:ammonium ratios. At high nitrogen levels, *acr11* plants exhibited reduced growth and elevated amino acid levels, most notably phenylalanine, tryptophan, alanine, aspartate and the aspartate-derived amino acids. Similar results were observed when *acr11* plants were grown under a low nitrate:ammonium ratio. In contrast, under high

nitrate:ammonium, *acr11* growth and amino acid content was similar to the wild type. These results suggest that ACR11 is involved in the regulation of amino acid biosynthesis and maintaining the carbon/nitrogen balance, particularly under high ammonium conditions.

T9 - Growth Hormones BR and GA Modulate Spikelet Meristem Identity Through the RAMOSA1 Pathway in Setaria and Maize (PD)

Jaspreet Sandhu, Jiani Yang, Max Braud, Edoardo Bertolini, and Andrea L. Eveland
Donald Danforth Plant Science Center

The degree of inflorescence branching, and position of grain-bearing spikelets are determined by timing of spikelet meristem (SM) identity, where an indeterminate branch meristem acquires determinate fate to initiate spikelet development. Here, we use *Setaria viridis* to dissect the mechanisms underlying SM identity and determinacy. Setaria's unique inflorescence offers an ideal system for this study; axillary branches terminate either in a spikelet or bristle. Using a mutagenesis screen, we isolated various meristem identity mutants. Among these, *bristleless1* (*bsl1*) and *spikeletless* (*spkl*) displayed homeotic conversions between bristles and spikelets. Genetic mapping indicated that brassinosteroids (BRs) and gibberellic acid (GA) were intimately involved in determining SM identity. The *bsl1* is an ortholog of rice D11, a rate-limiting enzyme in BR biosynthesis, and *spkl* is an ortholog of maize *ramosa1* (*ra1*). Genetic analyses in Setaria indicate that *bsl1* is epistatic to *spkl* and we propose that SPKL/RA1 regulates SM identity through interfacing with BR and GA pathways. We also isolated a mutant in maize *d11* and crossed it into *zmra1-R*, which suggested more complex genetic interactions in maize. Our results highlight conservation and divergence of pathways governing SM fate between Setaria and maize, knowledge that can be harnessed for developing higher yielding cereals.

T10 - Phosphorylation and Oxidative Damage Mediate the Disassembly of Photosystem II in *Arabidopsis thaliana* (G)

Steven D. McKenzie and Sujith Puthiyaveetil
Purdue University

Oxygenic photosynthesis is dependent on the bioenergetic reactions of the photosynthetic electron transport chain. Photosystem II (PSII), a large hetero-oligomeric pigment protein complex, utilizes radiant solar energy to oxidize water. In doing so, PSII produces protons and dioxygen as biproducts. Because of these highly energetic reactions, PSII is regularly subject to oxidative photodamage. To prevent photoinhibition due to oxidative damage, the damaged PSII undergoes a disassembly and repair cycle that results in the turnover (1 hour) of its damaged D1 subunit. Although this largely conserved process has been thoroughly studied across photoautotrophs, it is still unclear how PSII is turned over so rapidly. Previous research has demonstrated a role for phosphorylation of PSII in facilitating its turnover under high light intensities, however, the exact molecular mechanisms remain unclear. By examining several PSII phosphorylation mutants in *Arabidopsis thaliana*, we have demonstrated a role for PSII core phosphorylation in monomerization of the PSII homodimer. We have further demonstrated that oxidative damage is sufficient to induce the disassembly of the PSII monomer into smaller subcomplexes. Together, these results suggest at least two PSII disassembly pathways in plants, a more controlled phosphorylation-dependent pathway, and passive disassembly due to oxidative photodamage.

T11 - Visualizing Acetylation-Induced Changes in Plant Secondary Cell Wall Structure and Dynamics through Molecular Simulation (UG)

Murtaza Barkarar, Daipayan Sarkar, and Josh V Vermaas
Michigan State University

The secondary cell wall is essential to the mechanical properties of plants and particularly woody tissues. The cell wall is composed of hemicellulose and cellulose polysaccharides, as well as heteroaromatic lignin polymers, which represent a significant resource for a circular bioeconomy, such as construction materials. While wood has been used for millennia, modifying the structure of the underlying biopolymers for enhanced mechanical strength is far newer. By acetylating the cell wall, it has been demonstrated that wood degrades slowly, and resists

further treatment. In this work, we examine the structure and dynamics at the nanoscale induced by acetylation using a computational microscope. Overall, we observe diffusion decrease by 2-3x for hemicellulose, lignin, ions, and water as the degree of acetylation increases. The reduced diffusion is driven by molecular interactions between water molecules and the carbonyl oxygen on the acetyl acting as a hydrogen acceptor. The partial negative charge interacts strongly with cations and forms hydrogen bonds with surrounding water molecules. As a consequence, solvation pockets within the cell wall structure are smaller than in the unacetylated control. These observations provide detailed understanding on the chemo-mechanical coupling at the molecular level, providing a high-resolution view of wood as a building material.

T12 – Role of VESICULAR TRAFFICKING 5 (VES5) Protein in Iron Accumulation and Photosynthetic Compounds in *Arabidopsis thaliana* (UG)

Eric X. Fritschi, Alani Antoine-Mitchell, Nga T. Nguyen, and Antje Heese
University of Missouri

Worldwide, more than 1.7 billion people are affected by anemia caused by iron (Fe) deficiency, who are mostly women and children. As plants are a major food source through which humans acquire Fe, it is critical to improve our understanding of how plants accumulate Fe for human health. Fe is also a critical micronutrient to plants, serving as a cofactor to enzymes functioning in photosynthesis and chlorophyll biosynthesis. In plants, Fe-deficiency leads to chlorosis, decreased yield, and impaired nutritional value of crops. The Heese lab is interested in how vesicular trafficking proteins regulate stress responses, including Fe-deficiency responses, in *Arabidopsis thaliana*. So far, the roles of vesicular trafficking proteins, including VESICULAR TRAFFICKING5 (VES5), in Fe-deficiency responses, uptake and distribution remain largely unknown. Here, I provide evidence that *ves5* null mutants accumulated reduced Fe in roots using Perls' staining, correlating with reduced protein accumulation of the root-specific Fe uptake transporter protein IRT1. Further, my data showed that ves5 null mutants exhibited chlorosis and accumulated reduced chlorophyll content per leaf area and D2 protein, a critical Fe-containing protein of photosystem II. Currently, I am investigating whether the reduced accumulation of photosynthetic compounds may affect photosynthetic rate in *ves5* null mutants.

T13 - Logistics of Defense: How TGNap1 Mediates Secretion of Antimicrobial Proteins (PD)

Deepak D. Bhandari, Dae Kwan Ko, Sang-Jin Kim, Kinya Nomura, Sheng Yang He, and Federica Brandizzi
Michigan State University

Plant immunity depends on the secretion of antimicrobial proteins, which occurs through yet-largely unknown mechanisms. The trans-Golgi network (TGN), a hub for intracellular and extracellular trafficking pathways, and the cytoskeleton, which is required for antimicrobial protein secretion, are emerging as pathogen targets to dampen plant immunity. We identify Arabidopsis TGNap1, a TGN-associated and microtubule (MT)-binding protein as a critical component of plant immunity. A *TGNap1* loss-of-function mutant (*tgnap1-2*) is susceptible to biotrophic pathogens *Pseudomonas syringae* (Pst DC3000) and *Hyaloperonospora arabidopsidis*. Pst DC3000 infected *tgnap1-2* is capable of mobilizing defense pathways, accumulating salicylic acid (SA), and expressing antimicrobial proteins. The susceptibility of *tgnap1-2* is not due to a failure of immune activation but in efficient transport of antimicrobial proteins to the apoplast. TGNap1-mediated antimicrobial transport is partially MT-dependent but independent from SA and is additive to the pathogen-antagonizing MIN7, a TGN-associated ARF-GEF protein. Therefore, our data demonstrate that plant immunity relies on TGNap1 for secretion of antimicrobial proteins, and that Thus, TGNap1 is a key immunity element that functionally links secretion and cytoskeleton in SA-independent pathogen responses.

T14 - Duplications Influence Abiotic Stress Tolerance in a Mustard Crop Wild Relative

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Duplications provide raw material for the emergence of novel traits. Two mechanisms for duplicating genes are whole genome duplications/triplications (WGD, WGT) and small-scale duplications (SSD), and both are known to influence plant evolution. Mustard crops

like broccoli, cabbage, and canola (Brassica) share a relatively recent WGT. However, the placement and timing of this WGT is unclear, and little is known about how retained duplicates influence emergence of novel traits in mustard crop wild relatives. In this study we combine sequence and fossil data to place and date this WGT. Further we explore how duplications influence abiotic stress tolerance in a mustard crop wild relative sharing the WGT. Using a combination of ionomics, metabolomics, and comparative genomics, we characterize aspects of salt stress response in *Cakile maritima* and identify retained duplicate genes such as sodium transporters and genes in the Cytochrome P450 superfamily that have likely enabled adaptation to salt and mild levels of cadmium. We phylogenetically localize and date the WGT, and provide a putative understanding of duplicate genes influencing abiotic stress tolerance in C. maritima. Leveraging crop wild relatives, like *C. maritima*, can provide a better understanding of the evolution of environmental adaptation.

T15 - Single Cell-Derived Multicellular Meristem: Insights into Male-to-Hermaphrodite Conversion and *de novo* Meristem Formation in Ceratopteris (G)

Xi Yang, An Yan, and Yun Zhou
Purdue University

Typical of homosporous ferns, Ceratopteris develops haploid, free-living gametophytes with two distinct sex types: the meristic hermaphrodites and ameristic males. In the absence of the pheromone, Ceratopteris males convert to hermaphrodites with *de novo* formed meristems. To quantitively determine the cellular basis of *de novo* meristem formation and the conversion between the sex types, we performed long-term, non-invasive, time-lapse imaging of a ubiquitously expressed nuclear marker in Ceratopteris gametophytes. We captured the entire process of the male-to-hermaphrodite conversion, spanning from initiation to establishment of the *de novo* formed meristems at single-cell resolution. We re-constructed the lineage and division atlas of newly formed meristems through computational image segmentation, lineage tracing, and quantifying division activity and orientation. We identified one single non-antheridium cell in the male as the meristem progenitor, driving *de novo* formation and contributing all the daughter cells for a new meristem. We further found that cells in the marginal layer of newly formed meristems initiated from males displayed higher proliferation rates, suggesting the positional signal drives *de novo* meristem formation. This work elucidates the cellular mechanisms during the sex conversion in Ceratopteris and provides insights into the *de novo* meristem formation in land plants.

T16 - Multiplexed Prime Editing in Rice for Multi-Trait Improvement (G)

Ajay Gupta, Bo Liu, and Bing Yang
University of Missouri

We developed a high-efficiency multiplexed prime editing system and used it to edit multiple agronomically important genes in a single transformation event. Capable of precisely modifying genomes from single amino acid substitutions to small indels, this system enabled the editing of 2, 3, and 4 genes with an editing efficiency exceeding 40% for all targeted genes. Additionally, we simplified the guide RNA cloning protocol with a modular assembly system for wider use by the plant research community. Using duplex PE, we precisely edited two genes, *xa5* and *xa23*, related to bacterial blight (BB) of rice, rendering plants resistant to this major disease. Triplex PE allowed the editing of two BB-related genes (*xa5* and *xa13*) and one herbicide tolerance gene (*OsEPSPS1*). With quadruple PE, we edited two BB-related genes (*xa5* and *xa13*) and two herbicide tolerance genes (*OsEPSPS1* and *OsALS1*). In both triplex and quadruple PE, edited plants displayed high resistance to BB and herbicide tolerance across T0 and T1 generations. Further tests with quadruplex PE demonstrated similar editing efficiencies across different gene constructs. In conclusion, we have developed an easy-to-clone multiplexed toolkit for PE, promising significant contributions to crop improvement and functional genomic studies.

T17 - Volatile Sesquiterpene Communication in Plants Relies on a KAI2-Mediated Signaling Pathway (G)

Shannon A. Stirling[1], Angelica M. Guercio2, Ryan M. Patrick[1], Xing-Qi Huang[1], Matthew E. Bergman[1], Varun Dwivedi[1], Ruy W.J. Kortbeek[1], Yi-Kai Liu[1], Fuai Sun2, W. Andy Tao[1], Ying Li[1], Benoît Boachon[1,3], Nitzan Shabek[2], and Natalia Dudareva[1]
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Plants are constantly exposed to and utilize volatile organic compounds (VOCs) released during plant communication with their environment, neighboring plants, and within-plant-signaling. Over the last several decades, much research has gone into uncovering the biosynthesis and transportation of VOCs. However, the perception of VOCs and their downstream signaling remains largely unexplored. Using the hormone-like function of volatile terpenoids in the reproductive organ development of petunia as a system with a visual marker for communication, we demonstrated that a karrikin-insensitive receptor, PhKAI2ia, stereo-specifically perceives the (−)-germacrene D signal, triggering a KAI2-mediated signaling cascade and affecting plant fitness. This research uncovers the role(s) of the intermediate clade of KAI2 receptors, illuminates the involvement of a KAI2ia-dependent signaling pathway in volatile communication, and provides new insights into plant olfaction and the long-standing question about the nature of potential endogenous KAI2 ligand(s).

T18 - The Early Dodder Gets the Host: Decoding the Coiling Patterns of *Cuscuta campestris* with Automated Image Processing (PD)

Erik J. Amézquita, Max Bentelspacher, Supral Adhikari, Jaime Barros, and So-Yon Park
University of Missouri

Parasitic plants such as dodder (*Cuscuta* spp.) represent a major agricultural challenge due to their unique life cycle and host-seeking mechanisms. One intriguing aspect of parasitic vine weeds like *Cuscuta* spp. is their coiling movements, which are essential for locating and attaching the host. In this study, we aimed to elucidate the dynamics of the coiling patterns in Cuscuta campestris and examine the role of circadian rhythms in its host-seeking ability. Using time-lapse photography, we recorded the circumnutation and coiling movements of Cuscuta at different inoculation times (9 AM, 12 PM, and 4 PM) on non-living hosts. Subsequent image analyses were facilitated through an in-house python-based image analysis pipeline. Although no physiological changes were observed between inoculation times, we found that Cuscuta exhibited a prolonged resting stage when inoculated at 4 PM. These observations suggest that Cuscuta, despite lacking leaves and photodetectors, can discern photoperiod changes, which significantly determine its parasitic efficiency. Furthermore, our approach provides an automated image analysis tool for understanding the dynamics of plant movements, laying the foundation for future mechanistic studies aimed at mitigating the economic and ecological impacts of parasitic plants.

T19 - Plants Secrete Diverse Species of RNA onto their Leaf Surfaces with a Potential Role in Plant-Microbe Interactions (PD)

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We previously reported that plants secrete diverse RNA species into the leaf apoplast, and that the great majority of this extracellular RNA (exRNA) is located outside of extracellular vesicles (EVs) but protected from endonuclease degradation by RNA-binding proteins (RBPs). Our latest research findings suggest that plant leaf surfaces are coated with abundant RNA that differs from apoplastic and cellular RNA in both composition and size. Furthermore, leaf surface RNA is not protected from endonuclease degradation either by EVs or RBPs. Our preliminary data indicate that leaf surface RNA forms Ca^{2+} (cation)-dependent condensates, which possibly contribute to the stability of this naked RNA. We speculate that these RNA condensates play a role in plant-microbe interactions and believe that during the epiphytic phase of microbial infection, this naked RNA is released from the leaf surface, taken up by the microbes, and performs yet unknown regulatory functions.

T20 - Unlocking Flooding Resistance: The Role of *CAX2* in *Arabidopsis thaliana*'s Flooding Induced Low-Oxygen Survival (PD)

Arkadipta Bakshi[1], Won-Gyu Choi[2], and Simon Gilroy[1]
[1]University of Wisconsin, Madison, [2]University of Nevada, Reno

Flooding poses a significant threat to global agriculture and food security, impacting plant physiology through calcium ion transport. However, the specific role of transporters in regulating flooding-induced calcium signaling remains unclear. To understand this regulatory network, we, therefore, mined the publicly available transcriptomes of Arabidopsis plants subjected to flooding or hypoxic stress for rapidly upregulated, calcium-related transcripts, identifying two calcium transporters, *AUTOINHIBITED Calcium ATPase 1* (*ACA1*) and *CATION EXCHANGER 2* (*CAX2*) as potential candidates. Using a combination of physiological, biochemical, and molecular-based assays, our findings revealed that knockout mutants in *CAX2* but not *ACA1* showed enhanced survival to both short and long-term flooding. Additionally, *CAX2* knockout mutants lead to slightly elevated resting calcium levels along with larger and more sustained flooding-induced calcium signals, triggering the constitutive activation of some flooding-responsive genes, thereby preadapting them to flooding stress. These effects are consistent with the role of this vacuolar pump, exporting calcium ions from the cytosol whereas, when these pumps are inactive, it attenuates the calcium signals, which in turn, triggers adaptation to flooding. These observations suggest that the vacuolar CAX2 pump modulates the dynamics of flooding-triggered calcium signals as a rapid stress response system triggered at the onset of flooding.

T21 – Sequence and Epigenetics of Active and Silenced Nucleolus Organizers in Arabidopsis (PD)

Dalen Fultz, Anastasia McKinlay, Ramya Enganti, and Dr. Craig Pikaard
HHMI/Indiana University Bloomington

In eukaryotes, the 45S ribosomal genes occur in large repeat arrays called nucleolus organizer regions (NORs), which remain among the most difficult regions of genomes to assemble. *Arabidopsis thaliana* has two such regions, *NOR2* and *NOR4*, whose sequences remain undefined. Using ultra-long DNA sequencing combined with an unconventional variant-calling based assembly approach, we completed 5.5 and 3.9 Mbp sequences for *NOR2* and *NOR4*, revealing their structure and effectively finishing the *A. thaliana* genome for the reference strain, Col-0. *NOR2* is epigenetically silenced, and this assembly has allowed analysis of *NOR* silencing at individual gene copies, demonstrating correlations between *NOR* structure and activity. Identification of active genes through flow-sorting of nucleoli and RNA sequencing demonstrates that only the central region of *NOR4* is active in adult plants whereas most, but not all, *NOR2* genes are epigenetically silenced. These active regions overlap with long intervals of low cytosine methylation and 45S gene copy homogenization. Collectively, the data reveal the genetic and epigenetic landscapes of the *NOR*s and implicate transcription in 45S rRNA gene concerted evolution.

T22 - Disruption of the Sorghum Circadian Clock Impacts Sorghum-Sugarcane Aphid Interaction Dynamics and Aphid Feeding Behavior (PD)

Kumar Shrestha†, Prince Zogli†, Lise Pingault, Sajjan Grover, Juan Betancurt Cardona, and Joe Louis
University of Nebraska-Lincoln, †Co-first authors

Circadian clocks play a pivotal role in orchestrating metabolic rhythms in response to environmental stress. Sorghum, a multipurpose cereal crop, suffers severe growth and yield reduction due to feeding by sugarcane aphids (SCA), which are phloem-feeding pests. Sorghum utilizes a multitude of defense mechanisms to curb SCA colonization. However, our understanding on the impact of circadian rhythm on sorghum-aphid interaction dynamics is limited. To explore this, a time-series transcriptomics was conducted on sorghum plants in disrupted circadian rhythm with and without SCA infestation. Transcriptomic analysis revealed a total of 2,873 differentially expressed genes (DEGs) and WGCNA identified four modules with distinct expression patterns unique to night-time. Further, a total of 946 sorghum circadian genes were identified and among those, 328 circadian genes were unique to SCA-uninfested groups that belonged to defense responses. The circadian genes upregulated during the night-time after SCA infestation were related to MYB transcription factors, primary metabolism, and transporters, suggesting the modulation of host defenses during night-time. This is in alignment with the electrical monitoring of SCA feeding behavior

analysis, which revealed that SCA spent significantly more time in salivation phase during night-time feeding. Our study provides novel insights into the circadian response of sorghum-aphid interaction dynamics.

POSTER PRESENTATIONS

P1 - A Multi-Product Prenyltransferase Mediate Cytosolic Monoterpene Biosynthesis (PD)

Matthew E. Bergman[1], Anya E. Franks[1], Natalia Dudareva[2], and Michael A. Phillips[1]
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Monoterpenoids are responsible for various physiological functions in plants and are also used in medicines, pesticides, flavorings, and fragrances. These ten-carbon volatiles often accumulate in specialized tissue types such as glandular trichomes. The specialized metabolism for producing high-value monoterpenoid compounds is exemplified in rose-scented geranium (*Pelargonium* spp.) which is prized for its terpenoid-rich essential oil. This aptly named member of the Geraniaceae displays a rose-like scent due to an abundance of the acyclic monoterpene alcohol geraniol. Pelargonium can produce geraniol in two separate compartments: in the plastid through the action of a classical monoterpene synthase, or in the cytosol via a Nudix hydrolase mediated pathway. In the cytosol, a dual product farnesyl diphosphate synthase-like protein (PgFDL2) produces both geranyl diphosphate, the precursor for monoterpenes, and farnesyl diphosphate (FDP), a precursor in sesquiterpene and sterol biosynthesis. *PgFDL2* and the closely related gene *PgFDL1* encode proteins that are 83% identical at the amino acid level, but *PgFDL1* forms exclusively FDP. In this group of plants, the cytosol constitutes an important site for monoterpene biosynthesis in addition to those produced in plastids, emphasizing the plant species, tissue, and metabolic context specific contributions of the cytosol and plastid to monoterpenoid biosynthesis.

P2 - ATP-Dependent DNA Translocase Activity of DRD1 Facilitates Pol V Transcription (PD)

Feng Wang, Wei Zong, Akihito Fukudome, Vihbor Mishra, and Craig Pikaard
Indiana University

RNA-directed DNA methylation and transcriptional gene silencing in plants involves transcription by the nuclear DNA-dependent RNA polymerase, Pol V. Genetic evidence indicates that Pol V's association with chromatin and/or transcription of chromosomal loci requires the putative chromatin remodeling protein, DRD1. However, the mechanistic basis for the DRD1- Pol V partnership is unknown. We show that DRD1 possesses double-stranded DNA-dependent ATPase activity. A mutation that knocks out this ATPase activity largely abolishes Pol V association with target loci, genome-wide. *In vitro*, DRD1 is an ATP-dependent DNA translocase that, in the presence of *E. coli* topoisomerase I, converts relaxed double-stranded plasmid DNA into positively supercoiled DNA. DRD1 also renders the double-stranded DNA partially sensitive to cleavage by the single-stranded DNA-specific nuclease, P1, indicating transient DNA unwinding. Recombinant DRD1 alone, or in association with recombinant DMS3 and RDM1, facilitates Pol V transcription on plasmid DNA, suggesting that the transient DNA unwinding induced by DRD1's translocase activity facilitates Pol V transcription initiation.

P3 - Auto-Inhibition of the Arabidopsis DNA Methyltransferase DRM2 (PD)

Wei Zong, Feng Wang, Akihito Fukudome, and Craig Pikaard
Indiana University

In plants, an important pathway contributing to transcriptional gene silencing is RNA-directed DNA methylation (RdDM). Cytosine methylation in the RdDM pathway is achieved mainly by the *de novo* DNA methyltransferase, DRM2. We find that the cytosine methyltransferase activity of DRM2 is hindered by sequences within its N-terminal region, which include three UBA (Ubiquitin Associated) domains. *In vitro*, full-length DRM2 has negligible double-stranded DNA binding but DRM2 lacking the N-terminal region readily binds DNA. These observations suggests that the auto-inhibition of DRM2 activity by the protein's N-terminus is due to N-terminal inhibition of DNA binding. Through systematic deletion analyses of the N-terminal region, we identified an interval rich in acidic amino acids that is required for DRM2 autoinhibition. We speculate that this acidic interval may interact with the DRM2 DNA-binding domain, preventing DNA binding unless the auto-inhibition is counteracted by an unidentified component of the RdDM pathway.

P4 - Forward Genetics to Discover Novel Components of Chloroplast Lipid-Derived Signaling (PD)

Yosia Mugume[2], Ron Cook[2], Jinjie Liu[2], Linda Danhof[2], Jordyn Flemming[2], Halle Purcell[2], Michael Beecher[1], Nianyuan (Sam) Hu[2], and Christoph Benning[1]
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The Arabidopsis chloroplast lipase PLIP3 releases 18:3 fatty acids that can be converted into 12-oxo-phytodienoic acid (OPDA) which is metabolized to oxylipins such as Jasmonic acid (JA). Overexpression of PLIP3 resulted in stunted plant growth due to the accumulation of JA. To identify new factors involved in OPDA metabolism and perception of its metabolites, we implemented a forward genetic screen in the background of transgenic PLIP3-OX (PLIP3 overexpression) plants. Four suppressor lines have been isolated, and their likely causal mutations narrowed down by bulk genomic DNA sequencing of mapping populations. For three, the casual mutations appear to be in genes with described functions, KEG, LOX3, and CDK8. The causative mutation in the fourth line has not been predictable based on annotation of the possible candidates and awaits further identification. KEG is involved in coregulation of abscisic acid and JA responses, and its mutation likely stabilizes JAZ12 a transcriptional repressor of JA response. The mutation in the lipoxygenase LOX3 slows the conversion of OPDA and provides a first example for a specific lox3 mutant phenotype. The kinase CDK8 affects transcriptional regulation of JA response genes. Work is currently ongoing to fully characterize these lines and additional suppressor mutants.

P5 - Have We Selected for Higher Mesophyll Conductance in Domesticated Soybean? (PD)

Elena A Pelech, Sam S Stutz, Yu Wang, and Stephen P. Long
University of Illinois Urbana-Champaign

Soybean (*Glycine max* [L.] Merr) is among the most important agricultural seed crop, and further yield improvements per unit land area are needed if we are to meet future demand and avoid destruction of more natural lands. Mesophyll conductance (g_m) in C3 crops quantifies the ease with which CO_2 can transfer from the sub-stomatal cavity to Rubisco within the mesophyll. Increasing g_m during light induction and at steady state would increase assimilation rate without impacting stomatal conductance, ultimately improving soybean productivity and possibly, water-use efficiency. However, is there variability in g_m that could be used in breeding? If so, indirect selection could be expected and seen in comparing wild ancestors of soybean to a domesticated high-yielding elite cultivar. Here, we compared the domesticated high-yielding elite LD11 with four accessions of its wild ancestor (*Glycine soja* Siebold & Zucc) collected from the assumed area of domestication by concurrent measurements of gas exchange and carbon isotope discrimination ($\Delta^{13}C$) using tunable-diode laser (TDL) absorption spectroscopy which allows for dynamic measurement of gm after an increase in light intensity. The results of this study will determine whether there is variation within germplasm to accelerate g_m dynamics during variable light conditions.

P6 - Identification of Candidate Genes Underlying a Large-Effect Fitness Trade-Off Across Environments in Locally Adapted *Arabidopsis thaliana* (PD)

Samuel Mantel, Gwonjin Lee, and Christopher Oakley
Purdue University

Understanding the genetic basis of fitness trade-offs driving local adaptation and biological diversification across environments is a key goal of evolutionary biology. However, despite well documented examples of such local adaptation, the phenotypes and genetic variants underlying them are rarely known. Cold acclimation, an adaptive plastic response to cool temperatures allowing for freezing tolerance over winter, and its costs when subsequent freezing temperatures are not experienced, are likely to induce fitness trade-offs in species native to temperate zones. In locally adapted populations of *Arabidopsis thaliana* from Sweden and Italy, a reciprocal transplant study, performed over three consecutive years, identified loci underlying genetic trade-offs for mean fitness, some of which colocalize with freezing tolerance QTL in the same populations. Here, we identify genes which are differential expressed before and after cold acclimation in Swedish and Italian populations, and in near isogenic lines containing the Italian haplotype at one of these loci in a Swedish background. In combination with predicted large-effect genetic variants between the populations, these differentially expressed genes contain multiple promising candidates,

allowing for future confirmation of the causal locus via gene editing, and connecting naturally occurring sequence polymorphism to divergent adaptive responses to seasonal temperature.

P7 - Natural Variation Linkage Hypothesis Testing (NaVaLigHT): A New Way to Look at Genomics X Environment X Mechanism Problems and its Application to Scientific Findings (PD)

Donghee Hoh and David M. Kramer
Michigan State University

In this presentation, we will introduce a new scientific approach called Natural Variation Linkage Hypothesis Testing (NaVaLigHT) and its application to scientific findings. NaVaLigHT is a powerful tool that can help address complex interactions between genes and mechanisms. It generates hypotheses based on experimental observations of natural variations in phenotypes and then tests these by mapping genetic and mechanistic linkages, as well as cause-and-effect relationships, onto variations in the genome. Briefly, the NaVaLigHT approach involves several steps: measuring a range of phenotypes that reflect processes that are potentially mechanistically related using high throughput tools, conducting statistical analysis with genetic data and mapping quantitative trait loci, generating unbiased hypotheses by comparing linkages and testing hypotheses using subsequent experiments. We applied this approach to a diverse panel of cowpea (*Vigna unguiculata*) and discovered a distinct photosynthetic chilling tolerance model that had not been revealed using traditional approaches and laboratory conditions. Furthermore, we identified a distinct role of thylakoid-specific fatty acid 16:1Δ3transphosphatidylglycerol involved in this mechanism. This approach provides us with insights into mechanistic and genetic bases, as well as possible applications to breeding efforts. NaVaLigHT can be applied to any condition and any model system, showing great potential for future research.

P8 - Phylogenomic Analysis of *Juglans* Species Emphasizing Abiotic Stressors using Nuclear, Plastid Genomes, and Cold-Tolerance Genes (PD)

Aziz Ebrahimi, Mojtaba Z. Faradonbeh, and Douglass Jacobs
Purdue University

Climate change significantly impacts woody species regeneration through severe weather events like freezing and heavy snowfall, notably in the Midwestern USA. This study explores the genetic diversity and phylogenetic relationships among twenty Juglans species, analyzing 165 samples via whole-genome sequencing, chloroplast DNA, and cold hardiness-associated genes from various regions including Eastern Asia, Central Asia, Europe, and South North America. The analysis delineates Juglans species by geography, highlighting J. regia's unique genetics and reduced diversity. *J. hindsii*, from California, closely matches species from southern America, indicating distinct adaptations to climate. Tajima's D analysis on cold-hardy genes reveals lower diversity in *J. regia*, *J. ailantifolia*, *J. mandshuricha*, and *J. cathayensis*, contrasting with higher diversity in southern American species, while North American Juglans show balanced diversity (D=0). These insights into genetic underpinnings of cold hardiness offer guidance for silvicultural strategies and tree migration planning in response to climate change.

P9 - Evidence for Inside-Out Upregulation of Nucleolus Organizers in Arabidopsis (PD)

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Ribosomal RNA (rRNA) genes transcribed by RNA Polymerase I are repeated at multi-megabase Nucleolar Organizer Regions (NORs). In *A. thaliana*, genes of the NOR on chromosome 2, NOR2 are selectively silenced during development whereas genes at NOR4 are transcribed, an epigenetic phenomenon known as nucleolar dominance. How NORs are differentially regulated is unclear. As a first step, we recently used ONT sequencing to determine complete NOR2 and NOR4 sequences in the accession Col-0, revealing the positions of active and inactive genes and an inverse correlation between CG methylation and regional gene activity. By assembling NORs from ten Columbia strains, we show that NOR sequences are highly dynamic. Gene copy number varies considerably among the strains, with most variation occurring in the active NOR. However, methylation profiles suggest

that the number of demethylated, active genes remains relatively constant. Among the strains, we find that central regions of NOR4 are always active, with the extent of gene activity in the telomeric and centromere-proximal ends of the NOR being variable. Overall, the data suggest that NOR activation begins in the central regions of the NOR, rather than the edges, and is up-regulated by spreading from the center toward the edges of the locus.

P10 - Small Protein Facilitates Shikimate Export from Plastids (PD)

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In plants, shikimic acid is synthesized in the plastids and is a key intermediate in the biosynthesis of three aromatic amino acids. It is also utilized in the cytoplasm by hydroxycinnamoyl CoA:shikimate hydroxycinnamoyltransferase (HCT) in the phenylpropanoid pathway, which is responsible for the biosynthesis of several important primary and specialized metabolites. It has long been accepted that shikimate synthesized inside plastids is transported to the cytoplasm. However, the transporter exporting shikimate from plastids remains unknown. Using functional screening of *Petunia hybrida* flower cDNA library in *E. coli*, we identified a small protein PShT of 97 amino acids, that mediates the transportation of shikimate. Phylogenetic analysis revealed that the gene encoding this protein is conserved in all land plants. Subcellular localization analysis using fluorescent protein fusion and immunoblot analysis of sub-chloroplast fractions revealed that PShT is enriched in chloroplast envelope membrane fractions, supporting its role in controlling shikimate transport. Metabolic profiling of transgenic petunia with downregulated PShT and Arabidopsis T-DNA insertion lines showed respective decrease in emission and production of phenylpropene compounds which rely on the HCT-produced intermediate. Overall, our results suggest that this small plastid envelope membrane protein regulates shikimate homeostasis between plastidial and cytosolic compartments by controlling its export.

P11 - Transcriptome and Lipidome Dynamics of Soybean Floral Buds Under Heat Stress (PD)

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Soybean (*Glycine max* L. Merril) encounters elevated temperature (heat) stress during the reproductive stages of development, notably flowering, impacting its seed yield negatively. To unravel the molecular mechanisms underlying heat tolerance during flowering, we conducted an RNA-seq analysis of floral buds from two distinct genotypes, KS4520NS (heat-tolerant) and PI 603746 (heat-sensitive). Plants were exposed to optimal (30/20°C) and heat stress (38°C/25°C) conditions during flowering and flower buds were collected and analyzed for lipids. We identified numerous differentially expressed genes (DEGs) under optimum (8072 upregulated and 6044 downregulated) and heat stress (5501 upregulated and 2150 downregulated) conditions. Among these DEGs, several were associated with cell membrane lipids. Of the total 156 lipids analyzed and annotated with total acyl carbons: total carbon–carbon double bonds, 36:6, 36:5, and 34:3 phosphatidylcholine, and 34:3 phosphatidylethanolamine (all containing 18:3 fatty acids and decreasing under heat stress) were the most important lipids in differentiating the heat-tolerant from the heat-sensitive genotypes. The overall unsaturation index of KS4520NS was lower than that of PI 603746 under heat stress. Our results suggest that reducing lipid unsaturation levels, achieved by lowering 18:3 fatty acid amounts, is an essential acclimation mechanism to heat stress in soybean.

P12 - Unraveling the Maze of Gravity Sensing Cells in Maize to Discover Gravity Specific Transcriptional Networks (PD)

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We still have a relatively poor understanding of the molecular events linked to gravity perception in plants. To address this, we have harvested maize root cap cells that are enriched with gravity sensing columella cells and used RNA-Seq to detect differentially expressed genes after gravity stimulation by 90 degrees rotation. We have used a time series of 5 minutes, 10 minutes, 30 minutes, 1 hour and 2 hours to compare

vertical controls with roots subjected to gravity stimulation. We found that most genes were differentially expressed at 30 minutes, followed by 2 hours' time points. In contrast, no or minimal differentially expressed genes (DEGs) were observed at 5 minutes and 1 hour time points. Therein, we have selected candidate genes for gene function characterization during gravitropism in maize mutants and gene orthologs in Arabidopsis. Our results have uncovered that the lesions in *AUXIN SIGNALLING F-BOX-2* (*AFB2*) in maize and Arabidopsis delayed root gravitropic response. Further characterization of auxin redistribution in Arabidopsis *afb2* mutants with DII-VENUS revealed that auxin redistribution during root bending response in the root elongation zone was significantly altered, suggesting that root cap derived signals was transduced to the elongation zone during gravitropism to direct root bending.

P13 - Unveiling Novel Transcription Factors Orchestrating Lipid Biosynthesis in Arabidopsis Seeds (PD)

Rajeev Ranjan, Ying Li, Karen Hudson, and Kranthi Varala
Purdue University

Plant lipids are an essential component of our diet and also a source of renewable biofuels. It is derived from fatty acids and mainly stored as triacylglycerol (TAG) in the seeds. Extensive research has been done to understand fatty acid biosynthesis and TAG assembly. However, its regulatory network driven by transcription factors (TFs) is largely unknown. We inferred our recently developed Organ-Delimited Gene Regulatory Network (OD-GRN) to predict TF regulators of lipid biosynthesis in Arabidopsis seeds. The role of 11 predicted TFs was experimentally tested by analyzing fatty acid content in the knock-out mutant and overexpression lines. Results showed that the knock-out mutants of five TFs accumulated reduced lipid content in their seeds, while overexpression of three of them resulted in increased total lipid content. In addition, overexpression of four other TFs increases total lipid content. In an attempt to explore the molecular mechanism involved, we found that MYBS2 may induce the expression of purple acid phosphatase (*PAP*s) genes along with other lipid biosynthesis genes. Thus, this study has advanced our understanding of the regulation of fatty acid biosynthesis and provided new candidate genes to be used in genetic engineering or breeding programs to improve oil seed crops.

P14 - Dissecting PBR1-Mediated Recognition of AvrPphB Protease Activity in Barley (PD)

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We previously identified a barley NLR gene, *AvrPphB Response 1* (*Pbr1*), whose protein product confers recognition of the *Pseudomonas syringae* effector protease AvrPphB. In Arabidopsis, recognition of AvrPphB occurs through proteolytic cleavage of PBS1 that then activates the immune receptor RPS5. Though both Arabidopsis and barley have evolved a system for recognizing AvrPphB, it is unclear which barley receptor-like cytoplasmic kinases are responsible for activating PBR1- dependent immune responses. Here, we generated decoy versions of several barley PBS1-like proteins that could be cleaved by the TuMV Nla protease, with the expectation that proteolytic cleavage of the barley decoy proteins would activate PBR1. Our results revealed that proteolytic cleavage of multiple barley PBS1-like proteins activate PBR1-dependent immune responses. We also show that cleavage of barley PBS1-like orthologs from wheat, rice, maize, and sorghum also activate PBR1, revealing PBR1 can detect cleavage of PBS1-like proteins from other monocot crop plant species. Collectively, our results suggest PBL-based decoys may be used to expand the effector recognition specificity of PBR1.

P15 - Rules of RNA Polymerase V Transcription Elongation (PD)

Akihito Fukudome, Wei Zong, Jered M. Wendte, Feng Wang, and Craig S. Pikaard
Indiana University

In plants, the silencing of transposons and repetitive elements through RNA-directed DNA methylation (RdDM) relies on nuclear DNA-dependent RNA polymerase Pol V producing non-coding transcripts, to which AGO4/6/9-associated 24-nt siRNAs can basepair. The precise length of the Pol V transcripts *in vivo* and the mechanism behind their generation remain unclear. To address this knowledge gap, we first determined the length of *in vivo* Pol V transcripts by circular RT-PCR, allowing the detection of 5'- and 3'-ends of each transcript

simultaneously. The inferred Pol V transcription units range from 150- to 200-nt in length, with variable 5' and 3' ends. We then recapitulated Pol V transcription elongation *in vitro* and show that it only occurs in the context of double-stranded DNA. Pol V transcription on single-stranded DNA template is non-processive, yielding short abortive products. However, the presence of the nontemplate DNA strand allows Pol V to maintain elongation with the transcript remaining associated with Pol V throughout the process. Template-nontemplate DNA base mismatches at the upstream edge of the transcription bubble disrupt Pol V elongation severely, indicating that transcription bubble closure at the upstream edge is essential for bubble translocation and Pol V transcription elongation.

P16 - Ethylene-Triggered Subcellular Trafficking of CTR1 Enhances the Response to Ethylene Gas (PD)

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The phytohormone ethylene controls plant growth and stress responses. Dark-grown Arabidopsis seedlings exposed to ethylene exhibit dramatic growth reductions, yet rapidly return to their basal growth rate upon removal of the gas. However, the underlying mechanism governing this acclimation to ethylene in dark-grown seedlings remains enigmatic. In this study, we report that ethylene triggers translocation of the Raf-like protein kinase CONSTITUTIVE TRIPLE RESPONSE1 (CTR1), a negative regulator of ethylene signaling, from the endoplasmic reticulum to the nucleus. Nuclear-localized CTR1 stabilizes the ETHYLENE-INSENSITIVE3 (EIN3) transcription factor by interacting with and inhibiting EIN3-BINDING F-box (EBF) proteins, enhancing the ethylene response, and delaying growth recovery. Furthermore, Arabidopsis plants with enhanced nuclear-localized CTR1 exhibit improved tolerance to drought and salinity stress. These findings uncover a mechanism of the ethylene signaling pathway that links the spatiotemporal dynamics of cellular signaling components to physiological responses.

P17 - Phloem-Specific Expressed *AtROXY* Regulates Plant Responses to Low-Phosphate Growth Conditions (PD)

Jing Huang and Cankui Zhang
Purdue University

Phosphorus (P) is an essential macronutrient for plant growth and development. P deficiency is becoming one of the most limiting factors for crop productivity. In addition to local signaling, it has been discovered that vascular tissue-mediated systemic signaling plays important roles in plant responses to P deficient growth conditions. TRAP-Seq was used to specifically study molecular alterations in phloem companion cells in response to P deficiency. *AtROXY*, one of the upregulated genes, was found to be specifically expressed in the phloem. The *roxy* mutant plants showed larger shoot, as well as longer primary and lateral root than wild type plants. Overexpression of *AtROXY* by a phloem-specific promoter led to curly leaves and necrosis of leaf edge, shortened root, smaller shoot and root, with a higher concentration of phosphate in both shoot and root tissues. Molecular analysis in the over-expression and mutant plants showed that genes related to hormone metabolisms and root architecture establishment might be the major players enabling plants to cope with low P. The discoveries from this study may be used to implement strategies for the production of crops with increased P uptake efficiency.

P18 - Unravelling Metabolic Networks Involved in Tomato Acylsugar Biosynthesis (PD)

Varun Dwivedi and Craig Schenck
University of Missouri

Acylsugars are trichome enriched defense metabolites found across the Solanaceae family. These compounds consist of sugar cores decorated with acyl groups, which are connected through ester linkages. In tomato four acylsugar acyltransferases (ASATs 1-4) sequentially add acyl chains to specific hydroxyl positions on a sucrose core leading to tri- and tetraacylated sucroses. Although tomato ASATs are known,

the subcellular localization of acylsugar biosynthesis and how flux through the pathway is regulated remain unknown. To address these questions, ASAT1-4-YFP fusion proteins were expressed in *N. benthamiana* and Arabidopsis protoplasts and found to mainly localize to the cytosol, but ASAT2 also localizes to the nucleus. Additionally, we explored protein-protein interactions of ASAT1-4 using bimolecular fluorescence complementation and predicted a putative ASAT1-4 metabolic complex using structural modeling. We observed potential interactions using different combination of ASATs, which was confirmed through the predicted structure. Potential ASAT interactions are being confirmed through pull-down assays. The knowledge gained from this study is crucial for understanding the regulation and coordination of the biosynthesis of chemical defenses.

P19 - Plants Secrete Diverse Species of RNA onto their Leaf Surfaces with a Potential Role in Plant-Microbe Interactions (PD) (Also T19)

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We previously reported that plants secrete diverse RNA species into the leaf apoplast, and that the great majority of this extracellular RNA (exRNA) is located outside of extracellular vesicles (EVs) but protected from endonuclease degradation by RNA-binding proteins (RBPs). Our latest research findings suggest that plant leaf surfaces are coated with abundant RNA that differs from apoplastic and cellular RNA in both composition and size. Furthermore, leaf surface RNA is not protected from endonuclease degradation either by EVs or RBPs. Our preliminary data indicate that leaf surface RNA forms Ca^{2+} (cation)-dependent condensates, which possibly contribute to the stability of this naked RNA. We speculate that these RNA condensates play a role in plant-microbe interactions and believe that during the epiphytic phase of microbial infection, this naked RNA is released from the leaf surface, taken up by the microbes, and performs yet unknown regulatory functions.

P20 - The Early Dodder Gets the Host: Decoding the Coiling Patterns of *Cuscuta campestris* with Automated Image Processing (PD) (Also T18)

Erik J. Amézquita, Max Bentelspacher, Supral Adhikari, Jaime Barros, and So-Yon Park
University of Missouri

Parasitic plants such as dodder (*Cuscuta* spp.) represent a major agricultural challenge due to their unique life cycle and host-seeking mechanisms. One intriguing aspect of parasitic vine weeds like *Cuscuta* spp. is their coiling movements, which are essential for locating and attaching the host. In this study, we aimed to elucidate the dynamics of the coiling patterns in Cuscuta campestris and examine the role of circadian rhythms in its host-seeking ability. Using time-lapse photography, we recorded the circumnutation and coiling movements of Cuscuta at different inoculation times (9 AM, 12 PM, and 4 PM) on non-living hosts. Subsequent image analyses were facilitated through an in-house python-based image analysis pipeline. Although no physiological changes were observed between inoculation times, we found that Cuscuta exhibited a prolonged resting stage when inoculated at 4 PM. These observations suggest that Cuscuta, despite lacking leaves and photodetectors, can discern photoperiod changes, which significantly determine its parasitic efficiency. Furthermore, our approach provides an automated image analysis tool for understanding the dynamics of plant movements, laying the foundation for future mechanistic studies aimed at mitigating the economic and ecological impacts of parasitic plants.

P21 - Agrobacterium-Mediated *Cuscuta campestris* Transformation as a Tool for Understanding Plant-Plant Interactions (professor)

So-Yon Park
University of Missouri

Cuscuta campestris, a stem parasitic plant, has served as a valuable model plant for the exploration of plant-plant interactions and molecular trafficking. However, a major barrier to C. campestris research is that a method to generate stable transgenic plants has not yet been

developed. Here, we describe the development of a Cuscuta transformation protocol using various reporter genes (GFP, GUS, or RUBY) and morphogenic genes (*CcWUS2* and *CcGRF*/GIF), ultimately leading to a robust protocol for Agrobacterium-mediated *C. campestris* transformation. The stably transformed and regenerated RUBY *C. campestris* plants produced haustoria, the signature organ of parasitic plants, and these were functional in forming host attachments. Transformed *C. campestris* also produced flowers and transgenic seeds exhibiting betalain pigment, providing proof of germline transmission of the RUBY transgene. The integration locations of T-DNAs were confirmed through TAIL-PCR. Furthermore, the RUBY reporter is not only a useful selectable marker for the Agrobacterium-mediated transformation but also provides insight into the movement of molecules from *C. campestris* to the host during parasitism. Thus, the protocol to generate transgenic *C. campestris* reported here overcomes a major obstacle to Cuscuta research and opens up new possibilities for studying parasitic plant interactions.

P22 - A Chemical Genetic Screen to Identify Novel Interactors of the Exocyst Complex (G)

Xiaohui Li, Christopher J. Staiger, and Chunhua Zhang
Purdue University

Accurate secretion through exocytosis is key to normal plant development and responses to biotic and abiotic stresses. During exocytosis, an octameric protein complex named the exocyst facilitates the tethering of secretory vesicles to the plasma membrane. Despite some understanding of the molecular aspects of the exocyst through use of reverse genetics and direct interaction tests, knowledge about its upstream regulators and genetic interactors remains limited. To fill this gap, an unbiased forward genetic screen for enhancers of exocyst defects is needed. Traditional genetic screens, however, encounter practical issues in exocyst mutant backgrounds. Addressing these challenges, this study employed Endosidin2 (ES2) - a synthetic inhibitor of EXO70 - for a large-scale chemical genetic mutant screen in Arabidopsis. This approach led to the identification of 70 ES2-hypersensitive mutants, named *es2s*. Through whole-genome sequencing–based mapping strategies, 16 *es2s* mutants were mapped and the candidate mutations reported. In addition, T-DNA insertion lines were tested as alternative alleles to identify the causal mutation. This research not only offers new genetic resources for systematically identifying molecular players interacting with the exocyst in Arabidopsis, but also enhances understanding of exocyst regulation. Moreover, it establishes a chemical genetic mutant screen and mapping pipeline with potential applications in future studies.

P23 - Acylsugar Chemical Diversity and Biosynthesis in the *Physalis* Genus (G)

Lillian N. Nowack and Craig A. Schenck
University of Missouri

Plants produce specialized metabolites that are structurally diverse and enable interactions with their environment. Acylsugars are specialized metabolites produced in glandular trichomes of plants in the Solanaceae family and play roles in defense. They consist of a sugar core decorated with acyl chains of various lengths and branching patterns. These acyl chains are attached to the core at various hydroxyl groups by enzymes called acylsugar acyltransferases (ASATs). Physalis is an understudied genus of Solanaceae with genomic resources and variation in metabolic traits, making it a good system to explore acylsugar biosynthesis. Our objectives are to characterize acylsugar structural variation across *Physalis*, identify ASATs responsible for acylsugar biosynthesis and validate the pathway by knocking out candidate ASATs. Preliminary acylsugar profiling via LC-MS has found that some species make acylsugars on the fruit surface in addition to trichomes on the leaves. Comparison of leaf and fruit acylsugar profiles shows that different acylsugars accumulate on different tissues, suggesting tissue-specific pathways. Using comparative genomics and biochemistry, two ASATs have been identified that catalyze consecutive steps in acylsugar biosynthesis in *P. grisea*. In the future we will characterize acylsugars from across the genus to better understand the evolution of chemical diversity in *Physalis*.

P24 - Altering Seed-Oil Content of Cover Crops with CRISPR/Cas9 Knockout Mutations (G)

Dexter White, Linah Alkotami, and Timothy P. Durrett
Kansas State University

Cover crops are an auspicious source of sustainable biofuel; however, the uncertain economic feasibility of cover-cropping remains an obstacle to widespread adoption by farmers. Cash cover crops offer an incentive whose value can be enhanced with gene editing. Two species from the Brassicaceae family, *Camelina sativa* and *Thlaspi arvense* L. (field pennycress), are oil crops amenable to genetic modification. The main component of seed oil, triacylglycerols (TAG), can be modulated by genetic alteration. Knockout of lipases, which break down TAGs in late-stage seed development, could increase overall seed-oil content. Knock-out of enzymes in the TAG-synthesis metabolic pathway could generate a platform to produce valuable specialty oils. *C. sativa* presents a unique challenge for gene editing with its hexaploidy genome. A deleterious mutation must occur in all six copies of a gene to achieve a full knock-out. To facilitate screening for DNA mutations, CRISPR/Cas9 constructs with multiple synthetic guide RNAs were designed to generate large deletions detectable by PCR amplification and gel electrophoresis. This research characterizes the efficacy of this method, providing a valuable tool for researchers working with *C. sativa* and CRISPR/Cas9, with *T. arvense* method and the mutations' effects on seed-oil content expected soon.

P25 - Bacterial-Derived Peptides Elicit Distinct Pattern-Triggered Immune Responses in Tomato Roots Through Intracellular Signaling Cascades (G)

Rebecca (BeKa) Leuschen-Kohl[1], Robyn Roberts[2], Silas Buchanan[1], and Anjali Iyer-Pascuzzi[1]
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Pathogen-associated molecular patterns (PAMPs) are semi-conserved sequences found throughout a range of microbes, from soilborne bacteria to foliar fungi. To recognize and respond to PAMPs, plants exhibit pattern recognition receptors (PRRs) that trigger downstream defense responses (ROS Burst, Ca^{2+} fluxes, MAPK activation) known together as pattern-triggered immunity (PTI). As the need for food security increases, research is shifting from R-gene-mediated resistance towards the implementation of broad-spectrum resistance, including the use of PTI-mediated defense. Interfamily and interspecies transfer of PRRs has been shown successful in Arabidopsis, *N. benthamiana*, and tomato. However, to sustainably use PTI-mediated strategies, we must first understand PTI in compatible host/pathogen combinations. In this study, we characterized PTI responses associated with two genera of bacterial pathogens of tomato: *Pseudomonas syringae* DC3000 – a foliar pathogen, and *Ralstonia solanacearum* – a soilborne pathogen. The aim of this study was to characterize below-ground response to PAMPs, including changes in ROS species formation and growth inhibition. Our results show that tomato PTI responses vary from those in Arabidopsis, including a lack of growth inhibition for PAMPs csp22 (RsK60) and flgII-28 (Pto) and differential regulation of ROS by SISERK3a/3b. We also find that root-mediated PTI is primarily found in developing regions.

P26 - Beyond Partitioning Tables - Implementing Transport-Resistance Carbon Allocation in a Crop Growth Model (G)

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Accurately predicting how crops allocate carbon is crucial to predicting crop yields under forecasted climates. Most current crop models use partitioning models such as empirical partitioning tables or harvest indices. These models prescribe carbon allocation while overlooking essential physiological mechanisms, thus compromising their predictive capabilities under novel conditions. We incorporated an extended version of Thornley's utilization-transport-resistant (UTR) model into the Soybean-BioCro crop model to simulate the entire soybean life cycle. We calibrated the UTR model using organ-level biomass data from two cultivars, one with two years of data and one with one year of data plus total non-structural Carbohydrate (TNC) measurements, both at ambient [CO_2] level. The UTR model reasonably predicted the biomasses for two cultivars across six years at different CO_2 levels with root mean squared errors between 0.35 to 1.05 Mg/ha. The predicted leaf and stem TNCs were within the scale and followed the patterns of the measurements. This is the first known attempt to validate the UTR

carbon allocation model at the organ level for the whole life cycle. By incorporating a more physiologically based allocation model, we expect to improve our ability to identify and evaluate crop improvement strategies that enhance adaptation to evolving climates.

P27 - Changes in Moon, Mars, and Micro Gravity: A Meta-Analysis of Transcriptome Shifts in Responses to Gravity and Spaceflight (G)

Emma L.J. Canaday and Sarah E. Wyatt
Ohio University

Long duration spaceflights and habitation of the Moon and Mars will require plants as components in bioregenerative life support systems. Each of these environments presents a unique gravity intensity. Understanding how plants respond to micro and fractional gravity will enable the development of plants that can thrive in space. A meta-analysis of the available fractional gravity RNAseq datasets is capable of addressing these questions around gravity detection, signaling and response. This analysis includes 13 gravity levels and multiple ecotypes and mutants. From the differential expression, several genes shared patterns of expression at Martian gravity including genes associated with cytoskeletal modification, protein turnover, and pressure regulation. A sequential analysis highlighted a sudden change in transcription at intermediate gravities which suggests an additional gravity detection mechanism that, while often suggested, was not previously confirmed. Interestingly, while all spaceflight datasets were exposed to a consistent radiation environment, fractional gravity levels showed differential expressions of reactive oxygen species (ROS) related genes. In total, this meta-analysis has identified conserved responses to gravity at several intensities important for space exploration, found evidence for a secondary gravity detection mechanism and highlighted the effects of compounding stresses in spaceflight.

P28 - Characterizing the Phenylalanine Ammonia-Lyase (PAL) Gene Family in *Arabidopsis thaliana* (G)

Lizhi Cheng, Miray Simsek, Arunima Gupta, Prashant Anupama-Mohan Pawar, Claire Nowak, Max Boxell, and Clint Chapple
Purdue University

The phenylpropanoid pathway generates various specialized secondary metabolites important for plant viability. The first step of the pathway is the deamination of phenylalanine to trans-cinnamic acid by phenylalanine ammonia-lyase (PAL), which is encoded by four genes PAL1-PAL4 in *Arabidopsis thaliana*. Previously, T-DNA mutant lines of PAL had been generated. Surprisingly, analysis of two independent *pal1/2/3/4* mutants revealed that they exhibited 10% of wild-type PAL activity, suggesting that one or more of the pal T-DNA mutant alleles are leaky. As a result, the previous work on higher level pal mutants may not faithfully represent the roles of each PAL gene in phenylpropanoid metabolism. To characterize PAL function and regulation in *Arabidopsis thaliana*, we have generated exon-specific CRISPR-Cas9 mutants and have observed various differences that distinguish our CRISPR mutants from the T-DNA mutants. Although the project is still in its early stages, our novel CRISPR pal mutants as well as our experience in the phenylpropanoid pathway provides us with an advantageous position to fully characterize and elucidate the function and regulation of the PAL family in *Arabidopsis thaliana*.

P29 - Cold Truths: Miscanthus' Vibrant Response to Chilling Stress Through Reflective Indices (G)

Rabia Ahuja, Yufeng Ge, Frank Bai, and Katarzyna Glowacka
University of Nebraska-Lincoln, Lincoln

Chilling stress significantly limits the productivity and geographical distribution of C4 crops, such as Miscanthus species, which are pivotal for sustainable agriculture and renewable energy production. This study aims to elucidate the adaptive mechanisms of Miscanthus species to chilling stress, thereby informing strategies for breeding climate-resilient crops. Utilizing the SpiderCam system for high-throughput phenotyping, we collected hyperspectral images to measure the Photochemical Reflectance Index (PRI) as proxy for non-photochemical quenching (NPQ) and Anthocyanin Reflectance Index (ARI) for anthocyanin accumulation. Our investigation focused on three Miscanthus species with varying chilling tolerances: *M. sacchariflorus* (high), M. sinensis (low), and *M.* × *giganteus* (intermediate). Our findings reveal

that the *M. sacchariflorus* exhibited a significant decrease in PRI under lower temperatures, indicative of an enhanced NPQ mechanism for efficient excess energy dissipation. Concurrently, an increase in ARI during chilling conditions suggests an augmented anthocyanin production, crucial for protection against oxidative stress. In contrast, *M. sinensis* demonstrated higher PRI levels under similar conditions, indicating reduced photoprotection. *M.* × *giganteus* exhibited intermediate responses. These results underscore the complexity of the NPQ-PRI and ARI in response to chilling stress. This research contributes to the development of climate resilient C4 crops, supporting sustainable agricultural practices and bioenergy production.

P30 - Conservation of Signaling Components in Pollen Tube Reception and Root Hair Development (G)

Sienna Ogawa and Sharon Kessler
Purdue University

FERONIA (FER) is a receptor-like kinase that senses changes in cell wall integrity. During pollen tube reception, FER is required for the trafficking of the calcium channel NORTIA (NTA, MLO7) to the filiform apparatus (FA) where it contributes to pollen tube bursting. faNTA is a chimeric, constitutively FA localized protein that suppresses fer infertility. FER is widely expressed throughout the plant and mutants have pleiotropic phenotypes. To determine whether there is a conserved FER-MLO signaling module elsewhere in the plant, *faNTA* was expressed under the FER promoter in *fer*. In vegetative tissues, *faNTA* complements the reduced root hair length and quantity of *fer*, suggesting an MLO might function downstream of FER in root hairs. There are 15 MLO family members in Arabidopsis. An MLO was identified as a regulator of root hair growth rate. Mutants in this gene have shorter root hairs that elongate at half the rate of the wild type. We predict that FER-regulation of MLO activity in root hairs modulates the tip-focused calcium gradient needed for tip growth and that FER-regulation of MLOs might be a conserved signaling module throughout the plant.

P31 - Debottlenecking the L-DOPA 4,5-Dioxygenase Step with Enhanced Tyrosine Supply Boosts Betalain Production in *Nicotiana benthamiana* (G)

Soyoung Jung and Hiroshi A. Maeda
University of Wisconsin

Synthetic biology provides an emerging tool to produce valuable compounds in plant hosts. However, little is known about how supply and utilization of precursor is coordinated at the interface of primary and specialized metabolism, limiting our ability to produce high levels of target specialized metabolites in plants. Tyrosine is an aromatic amino acid precursor of diverse plant natural compounds. Arogenate dehydrogenase (TyrA) catalyzes the final step of tyrosine biosynthesis in plants and is typically feedback inhibited by tyrosine. Here, we studied the impact of enhanced tyrosine supply on the production of tyrosine-derived betalains. *BvTyrAα* from *Beta vulgaris* (table beet) showing relaxed feedback inhibition was used to "push" the tyrosine supply, which was then "pull"ed into the target betalain pathway in *Nicotiana benthamiana*. Co-expressing *BvTyrAα* and the betalain pathway drastically increased tyrosine and 3,4-dihydroxy-L-phenylalanine (L-DOPA) levels; however, the accumulated L-DOPA were not efficiently converted to betalains. An enhanced expression of *L-DOPA 4,5-dioxygenase* (*DODA*) catalyzing the second step of betalain biosynthesis boosted betalain production, indicating that DODA is the rate-limiting step of betalain biosynthetic pathway. Our data suggest that balancing between additional supply and effective utilization of precursor will be critical to produce high levels of target compounds in planta.

P32 - Differential Responses to Clipping Stress in *Andropogon gerardii*: The Role of Plant Memory, Enzymatic Activity, and Secondary Metabolite Production in Predicting Plant Photosynthetic Performance (G)

Shahla Mohammadi, Jesse Nippert, and Timothy Durrett
Kansas State University

Our investigation delves into *Andropogon gerardii*'s adaptive strategies to stress, emphasizing the role of plant memory on phenylalanine ammonia-lyase (PAL) activity, secondary metabolite production, and photosynthetic efficiency. Sourced from Konza Prairie, these plants

underwent a greenhouse-based factorial experiment with two clipping treatments to mimic grazing stress, forming groups based on memory and clipping status. Results demonstrate that plant memory significantly boosts PAL activity—an enzyme key for phenolic compound synthesis and plant defense. This enhancement in PAL activity in plants with stress memory correlates strongly with increased photosynthetic performance, showcasing the critical role of stress memory in enhancing photosynthetic efficiency and resilience. Utilizing statistical and machine learning analyses, such as Random Forest and Gradient Boosting Machine models, we identified phenolic content as a prime predictor of photosynthetic efficiency. This study not only underscores the complex interplay between stress memory, enzymatic activity in secondary metabolite biosynthesis, and photosynthesis in *A. gerardii* but also highlights the ecological and evolutionary importance of plant memory, offering profound insights into plant adaptive mechanisms in response to biotic stress.

P33 - Elucidating the Impact of Spaceflight on the Plant Immune System (G)

Denise Caldwell and Anjali Iyer-Pascuzzi
Purdue University

Understanding how plants adapt to the unique pressures of spaceflight is critical for growing food in deep space. Microbial plant pathogens were recently isolated from the International Space Station (ISS), and disease threatens crops in space. To sustain long-duration human space exploration, we need to grow and harvest edible crops and minimize crop loss from disease. This requires knowledge of plant immune responses during spaceflight and how spaceflight conditions impact pathogen colonization and virulence. The Advanced Plant Habitat (APH) is a plant growth chamber that provides improved growing conditions during spaceflight. We are investigating how the tomato immune system adapts to spaceflight when grown in the APH aboard the ISS. We will grow wild-type and immune-deficient tomatoes in the APH and elicit defense responses with a chemical elicitor. Upon return to Earth, genome-wide transcriptional profiling will compare the immune responses of space-grown tomatoes to ground controls in wild-type and immune-deficient plants. We are also investigating how tomato colonization by a fungal pathogen, *Fusarium oxysporum*, is altered by simulated microgravity on Earth. Our research will reveal key information on how plants respond to spaceflight conditions, enabling sustainable crop production and advancing NASA's human exploration efforts.

P34 - Galactolipids Regulate Systemic Immunity in Plants (G)

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Systemic acquired resistance (SAR) provides a long-lasting broad-spectrum protection against various biotic stresses to prepare for future infection. Pre-exposure to pathogen provokes the generation and the movement of SAR signals for priming defense responses. Multiple chemicals have been identified to date as important SAR activators. One of the SAR signals, reactive oxygen species (ROS) hydrolyzes C18 unsaturated fatty acids in chloroplast lipids to produce another SAR signal, azelaic acid (AzA). The major component of the chloroplast lipid is a galactolipid which contains a galactose sugar head group. Galactolipids are specifically divided into two types, mono- and di-galactosyl-diacylglycerol (MGDG and DGDG) which possess one- and two galactose sugar moieties. Previous studies have shown that mutants deficient in MGDG (mgd1) or DGDG (dgd1) synthesis, are impaired in SAR and gene expression responsive to another important SAR activator, salicylic acid (SA). In addition to that, *dgd1* mutant is incapable of translocating SA to systemic leaves after pathogen infection and exogenous SA application does not restore SA transport. My work elucidates the important roles of galactolipids in chloroplast underlying the modulation of molecular mechanisms in SAR.

P35 - Identification of Anthracnose Resistance Gene in *Sorghum bicolor* (G)

Pascal Okoye[1], Gezahegn Girma[2], Habte Nida[1], Adedayo Adeyanju[1], and Tesfaye Mengiste[1]
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Anthracnose disease, caused by *Colletotrichum sublineola*, results in considerable yield loss of sorghum globally, especially in wet and humid regions. Genetic studies of natural variants of sorghum have identified numerous anthracnose resistance loci. A nucleotide-binding

leucine-rich repeat receptor gene designated as *ANTHRACNOSE RESISTANCE GENE1* (*ARG1*) has been determined to underlie one of these resistance loci. Thus, the current investigation aims to identify additional loci underlying anthracnose resistance in a resistant sorghum variety. In this study, F4:5 recombinant inbred lines (RILs) resulting from the cross between the resistant line PML981488 and the susceptible genotype TAM428 are used to identify resistance loci. Four-week-old seedlings from 212 RILs were inoculated with 2 x 10^6 spores/mL of *C. sublineola* (strain Cs29) and screened for disease symptoms. Results showed a 1:1 ratio for resistant and susceptible RILs, indicating minimal segregation of alleles. Genomic DNA resequencing and bulked segregant analyses suggest that the causative SNP is located on the long arm of chromosome 4, between 50 and 60 megabase pairs (Mb). Recombination analyses using 20 single sequence repeat markers identified a total of twenty-four candidate genes within a 230 Kb locus. The findings of this investigation will contribute to the development of anthracnose-resistant sorghum cultivars.

P36 - Interrogating Protein-Lipid Droplet Binding Mechanics through Molecular Simulation (G)

Duncan M Boren, Peter K Lundquist, and Josh V Vermaas
Michigan State University

Plastoglobules are specialized lipid droplets of plant chloroplasts derived from the thylakoid membranes of photosynthetic organisms. Plastoglobules are appear to be intimately connected with photosynthetic capacity and stress tolerance but the mechanisms by which specific proteins associate with plastoglobules to dictate their proteome are unknown. It is believed that amphipathic helices are required for the association of the most abundant protein family of the plastoglobule, the Fibrillins. In *A. thaliana*, the protein Fibrillin 1a is suspected to bind to plastoglobule membranes to facilitate plastoglobule formation. To investigate binding mechanics, we perform protein-lipid binding simulations consisting of the Fibrillin 1A protein above simulated thylakoid and plastoglobule membranes to find favorable binding poses and investigate specific amino acid residues involved in membrane binding. We find that Fibrillin 1a binds to thylakoid and plastoglobule membranes with distinct and spatially segregated binding faces, indicating that Fibrillin proteins may play a role at the membrane interface.

P37 - Investigating the Impact of Cold Stress on the Circadian Clock in Arabidopsis and Tomato Plants (G)

Frederick Mildenhall, Tina Agarwal, and Kranthi Varala
Purdue University

Little is known about how cold stress affects the circadian rhythm of plant species outside Arabidopsis. The circadian clock, comprised of clock genes, drives the daily oscillation of circadian regulated genes, contributing to the overall plant circadian rhythm. This study uses Arabidopsis and tomato, a cold tolerant and cold sensitive species, to explore how cold tolerance influences the impact of cold stress on the circadian clock. Utilizing diurnal phase-specific cold stress treatments, we investigate the effects of four-degree cold stress on the expression of clock genes in Arabidopsis and tomato. Our findings reveal that cold stress disrupts the cyclic expression of the Arabidopsis clock genes when applied during the mid-day or evening phases. Interestingly, divergent responses are observed between Arabidopsis and tomato under morning phase-specific cold stress. In tomato, morning cold stress induces a stabilization of clock gene expression for the duration of cold stress, contrasting with Arabidopsis where no stabilization occurs, and oscillation continues undisrupted. Our results suggest that the enhanced cold tolerance of Arabidopsis, contributes to this maintenance of clock gene expression oscillation. This work provides insights into the interplay between cold stress and the circadian clock, within the context of species with differing levels of cold tolerance.

P38 - Kinetic Model for Enhanced Nitrogen Fixation in Soybean Nodules (G)

Rourou Ji and Megan L. Matthews
University of Illinois Urbana-Champaign

Soybean plants form symbiotic relationships with Bradyrhizobia bacteria, where the plant supplies carbon to the bacteria in exchange for nitrogen. There is a lot of interest in understanding this relationship to improve plant growth and decrease the need for fertilizers. To understand more about this exchange of carbon and nitrogen, we are developing a kinetic model of key metabolic reactions in soybean

nodules. To develop this model, we are collecting kinetic rate equations and parameters from the literature and databases. We are also exploring additional parameter estimation approaches, including machine learning algorithms that predict kinetic parameters from protein sequences and global optimization algorithms to fit against experimental data, to calibrate our model to soybean nodules. Once developed, this model will provide insight into potential bottlenecks that could be engineered to improve nitrogen fixation efficiency.

P39 - Kochia-Canola Interactions Under Field and Greenhouse Conditions (G)

Farhan Tanvir[1], Mukhlesur Raman[1], Barbara Dobrin[2], James V. Anderson[2], and David Horvath[2]
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Canola (*Brassica napas* L.) is a vital oilseed crop that plays a significant role in global demand for oil production. Kochia (*Brassica scoparia* L.) is a troublesome annual weed in canola fields. In our field study, we demonstrated that increasing planting density significantly reduces canola biomass (dry weight) per plant (intra-species interference). Additionally, increased kochia biomass in canola fields causes small but significant reduction in canola biomass per plant (inter-species interference). Canola seed yield per plot and 1000 grain weight per plot were not influenced by planting density. Additionally, planting density had a minimal effect on canola seed size in all treatments. In greenhouse experiment, 60% reduction of biomass in single kochia plant was observed when it was surrounded by as few as 2 canola plants. However, no significant reduction in single plant biomass was observed when canola was grown in proximity to as high as 6 kochia plants. Currently, we are working on RNAseq data collected from tissue samples of both species under field and green house condition. Canola showed differential expression in red light, wounding, oxidative stress, and auxin responses. We are working on identifying gene expression differences in kochia responding to canola.

P40 - Metanalysis on the Impact of Elevated CO_2 on Root and Tuber Crops (G)

Daniel Eneji Sani and Justin McGrath
University of Illinois Urbana-Champaign

Increasing atmospheric CO_2 concentration has raised concerns about global food security, particularly for the growing population. While much research has been devoted to the effects of CO_2 on cereal crops and legumes, root and tuber crops (RTCs) have received less attention despite their crucial role in providing daily calories, especially in developing countries. This study conducts a comprehensive meta-analysis of RTC responses to elevated CO_2 levels, reviewing literature from 1983 to 2021. Our analysis reveals a significant yield increase in RTCs under elevated CO_2 conditions, with radishes and potatoes showing the most and least growth enhancement at 75% and 31%, respectively. This indicates that RTCs may exhibit stronger responses to CO_2 enrichment than cereals and other groups, likely due to their greater sink capacity, which boosts below-ground yield. Additionally, we examined the effects of ozone and rooting space on yield, finding a yield decrease of up to 13% due to ozone exposure and a 27% increase in yields for pot-grown crops over field-grown ones. These results highlight the importance of RTCs in climate change adaptation strategies and food security, emphasizing the need for tailored approaches to mitigate the impact of environmental stressors like ozone on crop productivity.

P41 - Mutation of the Genes Encoding Mediator Subunits 5A and 5B Leads to the Accumulation of Camalexin in Arabidopsis (G)

Zhi-Wei Luo, Nicholas D. Bonawitz, Brian Dilkes, and Clint Chapple
Purdue University

Tryptophan (Trp) metabolism produces compounds that impact plant growth and provide tolerance to environmental stress. We have found that subunits 5A and 5B of the transcriptional regulatory complex Mediator (MED5A/B), which were previously characterized as regulators in phenylpropanoid pathway, also modulate Trp metabolism. Mutants defective in both *MED5A* and *MED5B* develop lesions where the Trp-derived phytoalexin camalexin accumulates. RNAseq analysis revealed that *med5a/b* double mutants exhibited increased transcripts of *CYP71B15*, a camalexin biosynthetic gene. Disruption of either *CYP71B15* or *CYP71A13*, another gene required for camalexin biosynthesis,

fully suppressed lesion formation, indicating that the lesion formation in the *med5a/b* background was dependent on camalexin. In a parallel experiment to identify loci associated with Trp metabolism using the Pathway of Origin Determination in Untargeted Metabolomics method coupled with metabolic Genome-Wide Association Study, multiple Trp-derived features (WDFs) were associated with the fitness-relevant locus, *ACCELERATED CELL DEATH 6* (*ACD6*). Plants carrying the hyperactive allele *acd6-1D* have improved pathogen resistance but grow slowly. *ACD6* transcripts elevated in *med5a/b* mutants, and WDFs accumulated in *med5a/b* were also abundant in *acd6-1D*, suggesting a link between MED5A/B and ACD6 in Trp metabolism. These observations indicate that MED5, like other Mediator components, may play a role in plant-pathogen interactions.

P42 - Novel Use of Cell Wall Degrading Enzymes as a Tool to Elucidate Cell Wall-Mediated Signaling During Pathogenesis (G)

Megan DeTemple, Sivakumar Swaminathan, and Olga Zabotina
Iowa State University

The cell wall is the plant's first line of defense against microbial pathogens. During infection, microbial pathogens produce a notable quantity and variety of cell wall degrading enzymes (CWDEs) to invade the plant cell. The plant has evolved plasma membrane receptors to specifically sense these cell wall modifications which activate signaling pathways, triggering the host immune system. Here we present a novel approach to study CWDEs and their role in pathogenesis by transforming the pathogen enzyme genes one at a time into the plant genome. We can then evaluate their resistance to infection and measure the changes in plant defense gene transcript levels before, and after infection. Our results show that not only are plant defense genes upregulated before infection, but also that some of these plants are more resistant to infection. We hypothesize that the resistant plants experience only a minor change in their cell wall that causes plant immunity to be elevated without compromising its normal growth and development. Here I present an evaluation of 17 CWDE in *Arabidopsis thaliana* against the fungal pathogen *Botrytis cinerea*. Additionally, we have transformed a few of these genes into maize to see if we observe the same effects.

P43 - Role of Minor Cannabinoids as Defense Chemicals in Response to Insect Herbivory in Hemp (*Cannabis sativa*) (G)

Bikash Deo and Michael Gutensohn
West Virginia University

Cannabis sativa is a multipurpose crop used for food, fiber, cosmetics, medicine, and as a recreational drug. Cannabinoids are secondary metabolites that are synthesized in glandular trichomes of cannabis plants. These cannabinoids are versatile chemicals with a wide array of uses. Cannabinoids are classified into four groups depending on the length of the acyl chain of the molecule: varinol-, butol-, olivetol-, and phorol-type cannabinoids. The olivetol type cannabinoids, including the well-known THC and CBD, are considered major cannabinoids as their concentration is high while varinol, butol, and phorol-type cannabinoids, such as THCV, CBDV, THCB, CBDB, THCP, and CBDP, are considered minor cannabinoids as these are generally found in trace amounts in Cannabis. Some previous studies have claimed insecticidal properties for cannabinoids, however, these studies have only used THC or CBD and have not considered other minor cannabinoids. Minor cannabinoids in general are only found in trace amounts but are significantly increased in hemp plants upon herbivory by beet armyworm (*Spodoptera exigua*). These results suggest that minor cannabinoids likely possess properties against insect herbivores. We have performed feeding experiments with *S. exigua* utilizing an artificial diet infused with individual pure cannabinoid compounds and have analyzed their effect on *S. exigua* larvae.

P44 - Temporal Profiling of Root Gravitropism: Correlating Physiological, Transcriptome, and Proteome Players (G)

Gbolaga Olanrewaju and Sarah E. Wyatt
Ohio University

Gravity is a fundamental driving force of plant evolution, exerting profound influences on plants' numerous developmental and growth processes. Since gravity is ubiquitous and constant on Earth, investigating plant gravitropism usually involves microgravity experiments in Space and various gravistimulation experiments on Earth. Despite numerous physiological insights on the gravitropic signaling pathway, the molecular players and their interconnectedness remain a grey cloud. Combining RNAseq and tandem mass tag-labeled LC-MS/MS analyses, we resolved the transcriptomic and proteomic response of 10-day-old Arabidopsis plants grown in the Biological Research In Canisters - Light Emitting Diode hardware aboard the International Space Station, with control samples on Earth. RNAseq yielded 195 upregulated transcripts and 761 downregulated transcripts in microgravity while the proteomics analysis yielded 2518 and 2332 differentially abundant membrane and soluble proteins in the root and shoot respectively. RNA transcripts had an extremely low correlation with the proteins expressed in the shoot ($r = -0.004$) and root ($r = 0.021$), suggesting the prevalence of post-transcriptional regulation in microgravity or that RNA transcriptome might be a reflection of the plant's immediate response to microgravity stress, while the proteome represents a more stabilized, adaptive response. Ongoing work will temporally correlate physiological and molecular elements in root gravitropism.

P45 - Transcriptomic Analysis of Higher Order Mutants of Hydroxyproline-O-Galactosyl Transferases (G)

Damilola Ayorinde and Allan M. Showalter
Ohio University

Arabinogalactan-proteins (AGPs) are essential for plant growth and development, exhibiting a composition of 10% protein and 90% sugar. Eight galactosyltransferases (GALTs), GALT2-GALT9, mediate galactose addition to AGP hydroxyproline residues in the AGP protein backbone. The Arabidopsis *galt* octuple mutants that result from the knockout of all the eight *GALT* genes displayed severe phenotypic changes, prompting our exploration of AGP mechanisms of action through a transcriptomic approach. RNA sequencing of the flowers and siliques from the *galt* octuple mutants yielded insights into the intricate interactive landscape of AGPs. Notably, in both flowers and siliques, 31 down-regulated genes were implicated in cell wall proteoglycan processes and protein O-glycosylation, and 187 up-regulated genes were linked to calmodulin binding with kinases and phosphatases in the signal transduction pathways and pathogen defense response regulation. These findings uncovered compensatory pathways in response to *GALT* genes disruptions and the resulting abnormal (underglycosylated) AGPs. This underscores the significance of AGPs in orchestrating vital cellular processes and offer a molecular framework for understanding the consequences of *GALT* gene knockouts and abnormal AGPs. This research enhances our comprehension of plant molecular responses to AGP dynamics and provides a foundation for future investigations into the underlying mechanisms of action of AGPs.

P46 - Understanding Carbon Storage and Allocation in Almond Trees in Response to Drought (G)

Shreya Veeravelli and Morgan Furze
Purdue University

With climate change shifting agroecosystems towards increased drought conditions, tree resilience strategies provide an opportunity to advance crop physiology. In long-lived and sessile trees, the allocation of nonstructural carbohydrates is essential to cope with environmental challenges like drought and can be useful for predicting tree resilience. By comparing the dynamics of carbohydrate reserves across crop tree varieties, we will advance our current understanding of crop physiology in the context of stress and enhance orchard management strategies. At two time points, branch, root, and stem samples were collected for nonstructural carbohydrate analysis from four almond (*Prunus* spp.) varieties growing in an orchard (Woodland, CA, USA). These measurements will elucidate the minima and maxima size of carbohydrate reserves in one calendar year and will be linked to other tree-level measurements such as annual growth rates and yield as well as orchard-level C flux tower data. In addition, greenhouse trials will be conducted to evaluate the relationship between aboveground carbon

stores and the root exudate profile to better understand how it functions as a belowground carbon sink during drought. These data will inform improved orchard management strategies that help to build more resilient agroecosystems in the face of a changing world.

P47 - Understanding the Genetic Basis of Heterosis Using a Panel of Near-Isogenic Lines (NILs) (G)

Juan Diego Rojas Gutierrez and Christopher G. Oakley
Purdue University

Studying genetic variation contributing to population differentiation is a central focus in evolutionary biology. While natural selection remains the primary driver of adaptation, the interplay with genetic drift introduces a layer of complexity. The potential of genetic drift to fix partially deleterious recessive alleles, thereby diminishing the efficacy of selection, raises concerns about population extinction. Crosses between individuals of different populations result in heterozygosity for these deleterious alleles and may exhibit heterosis, defined as the increase in fitness of F1 crosses relative to the mean of the parents. Our research delves into the basis of heterosis using natural populations of *Arabidopsis thaliana* from Italy and Sweden, alongside a genome-wide panel of homozygous NILs for each population. This enabled dissecting heterosis effects on the effect of individual genomic regions. Measurements of heterosis across three environments, including two simulated environments replicating the conditions of the parental ecotypes and one greenhouse setting, revealed significant genotype-by-environment interactions. Heterozygous NIL fitness appears influenced by the masking of deleterious alleles (dominance) and epistasis, highlighting the importance of non-additive effects in adaptation. Understanding heterosis holds the potential to offer insights into how genetic drift shapes deleterious mutation frequency, impacting genetic diversity in natural populations.

P48 - *VERNALIZATION INDEPENDENCE 3* (*VIP3*): The First Plant Gene Shown to Play a Role in Deacclimation Resistance (G)

Ashok Mandal[1], Barbara Dobrin[2], Mukhlesur Rahman[1], James V Anderson[2], and David P Horvath[2]
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Winter canola acquires increased freezing tolerance if first acclimated to low temperatures. However, if plants are exposed to warmer temperatures for several days, they will deacclimate. During late winter and early spring, short stretches of warmer temperatures could result in deacclimation, leaving the plants susceptible when freezing conditions return. Winter canola shows variation in response to deacclimation rates among genotypes. Genome-wide association mapping implicated a gene similar to *VERNALIZATION INDEPENDENCE 3* (*VIP3*) as being associated with deacclimation rate in winter canola. Two independent Arabidopsis lines with insertion mutations in *VIP3*, showed better deacclimation resistance than that of wild type. RNA-seq analysis indicated that no genes were significantly differentially expressed (0.05) following cold acclimation in both mutants compared to wild type. 243 genes were differentially regulated in both mutants following deacclimation. Interestingly, *COR78*, *COR15*, and *COR6.6* were up regulated in both mutants compared to wild type, as were other genes previously described as targets of the VIP3 regulation. *CBF* genes were not up regulated in both deacclimated *vip3* mutants. RNA degradation does not underlie differences in *COR* gene expression. A small peak of open chromatin in the *COR* gene promoters was observed by ATAC-seq analysis.

P49 - Alternative Splicing: Peeling Another Layer of Cold Stress Response in Tomato (G)

Jasjit Mangat, Tina Aggarwal, and Kranthi Varala
Purdue University

Cold stress is one of the major abiotic stresses that limits the productivity and geographical distribution of plants. Reportedly, cold stress in plants leads to an increased number of differentially alternatively spliced (DAS) genes which acts as another layer of stress response in addition to the differential expression (DE) of genes. Alternative splicing is a post-transcriptional modification which is responsible for providing functional diversity to proteins originating from the same gene. Previously, our group collected RNA-seq data from a time-series experiment on tomato (*Solanum lycopersicum*) which involved subjecting the plants to 4°C cold stress and sampling at 14 time points from

5 mins to 120 mins. Now, we analyzed this data using multiple bioinformatics tools to detect splice variants. The results from these tools were merged and filtered using custom parameters to get a DAS gene list. Then this gene list was passed on to functional annotation and GO term enrichment analysis. It was found that a significant number of genes were localized in chloroplast and were involved in circadian rhythm and photosynthesis. We plan to integrate this DAS gene list with DE gene list and gene regulatory network to deepen our knowledge about cold stress response in tomato.

P50 - Arabidopsis MLOs are Expressed in Discrete Domains in the Roots (G)

Sowmiya Devi Venkatesan and Sharon A. Kessler
Purdue University

The plant specific *MILDEW RESISTANCE LOCUS-O* (*MLO*) gene family has 15 members in *Arabidopsis thaliana* and are involved in diverse cellular processes such as powdery mildew susceptibility, root thigmomorphogenesis, trichome secondary cell wall composition, pollen tube guidance and reception. Recently, MLO proteins were reported to act as calcium channels. However, it still not known how the calcium channel activity of MLOs relate to their role in different cellular communication processes. Several *MLO*s were reported to be expressed in the roots. Hence, to determine the cell-type specific expression patterns of all the *MLO*s I examined the roots of 15 days old Arabidopsis seedlings expressing MLOpro::MLO-GFP reporters. Interestingly, I found discrete expression patterns of *MLO*s in the roots which was consistent with a previously published single-cell RNA sequencing data from root cells. Future studies will be directed towards determining the subcellular localization of MLOs in various cell types and their potential role in cellular communication processes.

P51 - Carbon Source-Specific Regulation of Nitrogen Fixation in *Paraburkholderia xenovorans* (G)

Trever L. Thurgood, Abigayle M. R. Simpson, and Roland C. Wilhelm
Purdue University

Paraburkholderia is a genus of metabolically diverse, diazotrophic (free-living, nitrogen-fixing) bacteria with potential use in the manufacture of biofertilizers due to their beneficial effects on plant growth. Previous research suggests that nitrogen fixation is dependent on carbon source, with aromatics, like plant-produced monophenols, producing higher rates of nitrogen fixation than carbohydrates. The aim of this study was to determine the effect of carbon source, nitrogen level, and oxygen concentration on the nitrogen fixing capability of *Paraburkholderia xenovorans* str. 4B. Herein, we report evidence of the ability of *P. xenovorans* str. 4B to fix nitrogen using a monophenol (p-hydroxybenzoic acid—pHBA) as a sole carbon source. *P. xenovorans* str. 4B exhibited higher rates of respiration when grown on pHBA without nitrogen, implicating an increase in the energy-intensive process of nitrogen fixation. Moving forward, we expect these methods will be used to evaluate the relationship between monophenolic exudate profile in various plant species and their influence on microbial gene regulation, with a focus on nitrogen fixing genes. This work can be used to develop effective biofertilizers and to provide insights into co-evolution of symbioses between plants and bacteria.

P52 - Characterizing and Interrogating Drought Resilience in *Thlaspi arvense* (Pennycress) (G)

Liza Gautam[1], Nikhil Jaikumar[1], Carol Kiam Assato[1], Arjuman Lima[1], Ryan Bayliss[1], Maggie Marlino[1], Amanda Darcy[1], Abby Volmar[1], Winthrop Phippen[2], and John Sedbrook[1]
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Drought damage to crops is a major threat to food security and is becoming more prevalent due to climate change. Pennycress (*Thlaspi arvense*) is a member of the Brassicaceae family related to canola and Arabidopsis that is being rapidly developed as an oilseed-producing winter cover crop for the U.S. Midwest and other temperate regions. As part of our efforts in domesticating pennycress, we are focusing on further understanding how pennycress responds to drought and identifying genetic changes that can improve drought tolerance without negatively impacting plant growth and seed yields. To broaden knowledge, we developed assays to test pennycress seedling and plant responses to drought including water withholding, chemical treatments that mimic drought, Li-cor 6400 instrumental measurements of

photosynthetic assimilation, and enzymatic assays measuring levels of oxidative stress. Our preliminary analyses indicated that pennycress naturally has enhanced drought tolerance compared to its close relative, the model plant *Arabidopsis thaliana*. Using CRISPR-Cas9 mutagenesis, we generated pennycress single, double, and triple mutants targeting ten genes shown to be negative regulators of drought responsiveness in other species. Preliminary phenotypic analyses of these mutant lines also support our hypotheses that pennycress may have relatively higher drought tolerance. These data will be presented and discussed.

P53 - Construction of a Synthetic Extensin Gene to Probe Cell Wall Assembly (G)

Allan Kenneth Q. Regunton
Ohio University

Extensins (EXTs) are diverse hydroxyproline – rich glycoproteins (HRGPSs) that exert their functions through self-assembly and wall crosslinking. These structural proteins are thought to act like molecular scaffolds by which various biomolecules like pectin attach to form extensin networks deposited in the growing cell wall. Here, we used a synthetic gene approach to design and construct extensin analogs which will be used to understand EXTs self-assembly and to elicit the role of amphilicity in the cell wall assembly. The extensin analogs containing repetitive motifs of [Ser-Hyp-Hyp-Hyp-Hyp-Tyr-Try-Tyr-Lys]n, where n = 1 up to n = 20, were synthesized using the Golden Gate cloning technique. Varying lengths of these extensin analogs were prepared from a single repeat. The extensin analogs will be expressed transiently in *Nicotiana tabacum* leaves and the proteins will be subjected to biophysical characterization. These studies may contribute to the molecular-level understanding of the plant cell wall assembly, architecture, and function.

P54 - Effect of Natural Variation on Pollen Tube Sensitivity to Synergid Signals Mediated by NORTIA (G)

Iyanu Adedeji and Sharon A. Kessler
Purdue University

Successful sexual reproduction in flowering plants relies on the intricate communication between the male gametophyte (pollen tube (PT)) and the female gametophyte, (synergid cells). This communication is pivotal for essential processes like PT reception, rupture, sperm release, and double fertilization. In *Arabidopsis thaliana*, NORTIA (NTA), an MLO protein family member, is a key regulator in this communication network. Disruptions in genes governing this communication leads to PT overgrowth. While considerable advances have been made in deciphering the molecular mechanisms of PT reception, understanding natural variation impact on related physiological traits to pollen tube and synergid signals in pollen tube reception remains imperative. We investigate how natural variation impacts PT sensitivity to synergid signals mediated by NORTIA. Mutations in NORTIA disrupt PT-synergid communication by lowering calcium signal levels in synergid cells, culminating in PT overgrowth. Through Aniline blue staining, fifteen ecotypes of *Arabidopsis thaliana* were identified as suppressors of the *nta-1* PT overgrowth phenotype. We propose that the suppressor ecotypes may require a small amount of calcium signals from the synergid cells to trigger PT rupture and release sperm cells to permit double fertilization. These varieties may have the unique ability to fine-tune their response to the signals from the synergid cells.

P55 - Elucidation of Shoot to Root Signaling Involved in Sulfur Deficiency Responses in *Arabidopsis thaliana* and *Plantago major* (G)

Juliana Miranda and Cankui Zhang
Purdue University

Sulfur (S) plays a crucial role in plant metabolism and crop productivity. Its deficiency in agricultural soils leads to reduced growth and lower yields. Plants have complex signaling pathways to sense and adapt root architecture under varying nutrient levels. To understand the long-distance signaling molecules involved in sulfur deficiency, split-root experiments are used along with gene expression analysis in *Arabidopsis thaliana*. In these experiments, isolated root systems are exposed to different sulfur conditions. One side receives the regular sulfur requirement (S+), while the other side is deprived of any sulfur source (S-). Root samples are then analyzed to explore gene expression

changes. This approach helps identify systemic signaling molecules, revealing how these signals travel through the plant and affect physiological responses. Recent findings indicate a starvation phenotype on the S+ side, with longer lateral root growth observed on the S- side as plants explore the medium to compensate for the deficiency. These results suggest the existence of long-distance signals related to starvation. Further gene expression analysis aims to uncover plants' strategies for sensing sulfur deficiency in environments with varying nutrient availability. This research lays the groundwork for future studies aimed at enhancing sulfur uptake in crops.

P56 - Employing CRISPR Genome Editing to Improve Seed Meal Quality in the Oilseed Plant Pennycress (G)

Arjuman Lima[1], Liza Gautam[1], Xin Lu[2], Xu Lu[2], and John Sedbrook[1]
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Pennycress (*Thlaspi arvense* L.) is a winter-hardy Brassica species having a short-enough life cycle to fit in the offseason between corn and soybeans in the lower U.S. Midwest. Domesticated pennycress seed oil is well suited for producing "climate smart" renewable diesel and sustainable aviation fuel (SAF). Ongoing efforts aim to improve pennycress seed meal quality for uses in animal feed through reducing seed glucosinolate content and sinapic acid content. Studies in rapeseed have shown that sinapate esters with sinapoylcholine contribute to the bitter taste, astringency, and dark color of seed products (Husken et al., 2005, Molecular Breeding). During seed oil processing, sinapate esters get oxidized and form complexes with proteins, thus lowering the digestibility of the meal. To reduce sinapic acid content in pennycress, we employed CRISPR-Cas9 genome editing to produce loss-of-function mutations in two phenylpropanoid biosynthetic pathway genes, namely *Ferulic Acid 5-Hydroxylase* (*F5H*) and *Reduced Epidermal Fluorescence 1* (*REF1*). We found that pennycress *f5h* and *ref1* single mutants produced seeds with substantially reduced amounts of sinapic acid and grew indistinguishable from wild type. These and other data will be presented that explore genetic relationships between reductions in sinapate esters and pennycress seed meal quality.

P57 - Establishing Plantago as a Model Species for Plant Vascular Biology (G)

Hannah Levengood and Cankui Zhang
Purdue University

The study of plant vascular tissue is of interest to researchers due to its important role in plant growth and development. Our laboratory has been developing species in the genus Plantago as models for vascular biology studies, due to their unique attribute of having easily extractable vascular tissue. We developed two transformation methods for narrowleaf plantain, one method that uses a conventional tissue-culture based approach to achieve a transformation efficiency of ~20% and another that uses morphogenic genes to generate transgenic shoots in-vivo. Plantago species are unique in the aspect of transporting two types of carbohydrates: sucrose and sorbitol, although their phloem loading mechanisms have not been experimentally studied. We have generated transgenic narrowleaf plantains in which the phloem-expressed sucrose-transporter gene was knocked out via CRISPR/Cas9. The retarded growth of the knockout plant suggests that sucrose is loaded to the phloem via an apoplastic pathway. Similar CRISPR/Cas9 knockouts are being developed for the sorbitol transporter, which will be experimentally compared to the sucrose knockouts. This study will dissect the phloem loading pathway for sucrose and sorbitol and reveal the importance of the two different phloem mobile carbohydrates to plant growth.

P58 - Exploring Monolignol Derivatives as Potential Plant Growth Regulators (G)

Chase T. Hearn, Fabiola Muro-Villanueva, Zhiwei Luo, and Clint Chapple
Purdue University

Manipulating lignin biosynthetic genes in crops can improve saccharification but often results in dwarfism. We found that overexpressing *FERULATE 5-HYDROXYLASE* (*F5H*) in Arabidopsis does not impact growth of wild-type plants but leads to dwarfism in multiple lignin-modified mutants. In addition to their reduced stature, *CINNAMYL ALCOHOL DEHYDROGENASE* mutants overexpressing *F5H* have defective root hairs, and lateral root production. We found that the canonical monolignol, coniferyl alcohol (CA), and one of its lignan dimers, pinoresinol (PR), rescue these growth defects with PR being more potent than CA. Furthermore, we found that methylated PR is able to rescue defective

root growth in these mutants, suggesting growth is restored independently of lignin. Paradoxically, we detected higher levels of endogenous CA in these mutants compared to wild type, despite their positive response when supplied with exogenous CA. Here, we present our current efforts to elucidate the mechanism of CA and PR as plant growth modulators in Arabidopsis, as well as the potential for these metabolites to act in a conserved fashion in other plant species. Together, our findings offer new insight into the biology of lignans and other phenylpropanoid intermediates, as well as the nature of growth defects brought on by lignin modification.

P59 - Genetic Insights into Apple Fruit Mass (G)

Jairam Danao and Peter Hirst
Purdue University

Pedicels are the slender stalks that attach the fruit to the plant. They play a crucial role in fruit development. The characteristics of the pedicel comprise complex traits that are controlled by multiple genes. To study this, we used two unique hybrid apple populations: 'Twenty Ounce' x 'Prairie Fire' and 'Edward VII' x 'Prairie Fire'. Both 'Twenty Ounce' and 'Edward VII' produce large fruit over 200 g, whereas 'Prairie Fire' is a small-fruited crabapple with fruit size less than 2 g. These populations offer the potential to investigate how pedicel attributes relate to apple fruit size. Our previous work established a clear connection between pedicel characteristics and apple fruit mass. Specifically, pedicel length showed an inverse relationship, while pedicel diameter is directly related to fruit mass. We hypothesize that among the genes that control fruit mass, some govern pedicel characteristics. We recently identified Quantitative Trait Loci (QTLs) associated with fruit mass, pedicel length and diameter. Knowledge of QTLs and subsequently genes that affect pedicel characteristics in apple have potential applications in apple breeding and fruit production. The identification and manipulation of these genes holds the promise of developing new apple cultivars with improved pedicel traits and ultimately fruit mass.

P60 - Genetic Mechanisms Underlying Sex Determination in the Homosporous Fern *Ceratopteris richardii* (G)

Katelin Burow, Jody Banks, Brian Dilkes, and Jennifer Wisecaver
Purdue University

Homosporous ferns, unlike seed plants, produce sexually undifferentiated spores. Instead, sex in homosporous ferns is environmentally regulated where single spores will develop into female or hermaphroditic fern gametophytes. These gametophytes secrete the sex pheromone antheridiogen causing nearby undetermined gametophytes to develop into males. In the fern *Ceratopteris richardii* (Ceratopteris), several hermaphroditic (her) mutants have been characterized that develop as hermaphrodites even in the presence of antheridiogen, but the immense size of the Ceratopteris genome makes identification of the underlying genes a challenge. We performed bulked segregant RNA- seq to map two her mutants (*her14* and *her19*) to a 16 Mbp interval on chromosome 29 of the Ceratopteris genome. A brassinosteroid receptor-like kinase gene located within this mapped interval contained a deletion mutation in *her14* and a missense mutation in *her19*. Three additional co- segregating her mutants had independent missense mutations in the same gene, which we name *HER7*, providing strong support for *HER7* being required for antheridiogen perception and downstream sex determination in Ceratopteris. Interestingly, antheridiogens in other ferns have been characterized as gibberellic acids and not brassinosteroid derived hormones. This work is the first to implicate a BRL, and by extension brassinosteroids, in sex determination in a homosporous fern.

P61 - *In vitro* Characterization of DNA Binding Sites of ERF104 Transcription Factor During Arabidopsis Gravitropism (G)

Sara Anwar and Sarah E. Wyatt
Ohio University

Gravity significantly influences the life and growth of all living organisms. Plant organs reorient their position in response to both environmental and developmental factors. However, gravity is common on Earth, yet there are very few choices available for studying how plants respond to it. NASA has overcome this challenge by conducting experiments in a microgravity environment. Data obtained from RNA-

seq and proteomics analysis of Arabidopsis seedlings cultivated on International Space Station (ISS) have provided a better understanding of how plants respond to microgravity. Meta-analysis of datasets from three previously conducted gravitropism experiments identified three transcription factors (TF) at the intersection of the datasets, one of which is the ETHYLENE RESPONSE FACTOR 104 (ERF104). Phenotypic characterization of the *erf104* mutant established that the gene plays a role in gravitropism in Arabidopsis. Hence, as ERF104 is a transcription factor, the goal of this project is to identify the DNA binding sites using Chromatin Immunoprecipitation sequencing (ChIP-seq). As a result of this study, we can determine the role of ERF104 in regulating the gravity response and gain a better understanding of signaling pathways in Arabidopsis.

P62 - Investigating the Biochemical Mechanisms Underlying the Phytotoxicity of Juglone (G)

George W. Meyer, Amanda Navodani Hewa Maithreege, and Joshua R. Widhalm
Purdue University

Juglone is the allelochemical responsible for the notorious phytotoxic effects of the black walnut tree (*Juglans nigra*). Juglone is released from *J. nigra* roots, transported via mycorrhizal networks, and leached into the soil from litterfall before being taken up by the roots of other plants. Juglone and other allelopathic 1,4-naphthoquinones are known to cause oxidative stress in susceptible plants through redox cycling. Given the open C2 and C3 positions on its quinoid ring, however, juglone can also form adducts with the thiol groups of accessible cysteine residues in proteins and peptides. Whether this chemical reactivity contributes to juglone's phytotoxicity remains an open question. We are using a combination of biochemical, genetic, imaging, and systems biology approaches in Arabidopsis to test the hypothesis that exposure to juglone at environmentally relevant concentrations depletes cellular glutathione and elicits a proteotoxic stress response.

P63 - Investigating the Effect of TPLATE Complex Components on Intercellular Trafficking (G)

Samantha Nuzzi and Tessa Burch-Smith
University of Missouri

Plasmodesmata (PD) are pores that traverse cell walls between neighboring plant cells. PD facilitate the movement of small molecules and macromolecules from cell-to-cell and are essential for intercellular communication. PD permeability changes in response to a variety of stimuli, including exogenous factors such as light and temperature and endogenous factors including hormones and developmental signals. The molecular and cellular mechanisms that regulate PD permeability remain obscure. Studies with the protein SYNAPTOTAGMIN A during virus infection have implicated endocytosis in intercellular trafficking via PD. Since endocytosis has been connected to intercellular trafficking, we investigated endocytic proteins to assess if they play a role in intercellular trafficking. One set of candidates is the TPLATE complex (TPC) is a plant-specific endocytic adaptor protein that is essential for clathrin-mediated endocytosis. We use virus-induced gene silencing to probe the relationship between the TPC and PD-mediated intercellular trafficking. We use free GFP to assess intercellular movement and a PLASMODESMATA CALLOSE-BINDING PROTEIN 1 tagged with a fluorescent marker to assess the number of clusters of PD. By observing and quantifying cell-to-cell movement and the number of PD pitfields, we can examine how silencing components of the TPC plays a role PD-mediated trafficking.

P64 - Investigating the Genetic Mechanisms of Low Nitrogen Tolerance in *Lolium perenne* (G)

Samantha Barker, Cankui Zhang, and Yiwei Jiang
Purdue University

Nitrogen (N) is an essential macronutrient for plant growth and development. As a major component of chemical fertilizers, it is often over-applied leading to excess nitrogen polluting fresh water. Perennial ryegrass (*Lolium perenne*) is a diploid grass species subject to this overapplication as it is commonly found as a turfgrass, ornamental pasture, and as a forage. Therefore, it is necessary to understand the genetic control of nitrogen use efficiency (NUE) in perennial ryegrass. Field trials consisting of 300 accessions from across the world were subjected to a high and low nitrogen treatment with two replications per accession at two locations. Phenotypic traits related to NUE, such as

normalized differential vegetative index (NDVI), chlorophyll fluorescence, and plant diameter were measured. The first field trial is underway, with one season complete. Concurrently, work is being done to efficiently transform perennial ryegrass using callus as an explant with RUBY as a screenable marker. Future work will include a second year of field trials whose results will be used to conduct a genome wide association analysis (GWAS) correlating these traits with genotyping by sequencing (GBS) data. Understanding this genetic mechanism will be invaluable to better integrate nitrogen use efficient crops into production.

P65 - Investigating the Role of Epigenetic Regulators in Plant Nitrogen Use (G)

Tanvir Dutt, Rachel McCoy, Russel Julian, Mary Sara Albert, Zheng Rong Yee, Kranthi Varala, and Ying Li
Purdue University

Nitrogen (N) is a macronutrient required for plant growth and is a major constituent of nucleic acids and proteins essential for several life processes. Uncovering the epigenetic level regulation of plant N signaling and response is essential to improving our molecular understanding of nitrogen use efficiency (NUE). To fill this knowledge gap, we first intersected the published transcriptomic dataset of N-responsive genes in *Arabidopsis thaliana* with EpiNet, an extensive epigenetic regulatory network, to identify a list of 18 potential epigenetic regulators, predicted to control nitrogen response in plants. Next, to validate these in-silico predictions, we screened T-DNA insertional mutants for these epigenetic regulators for various shoot and root physiological traits, under high and low N conditions. Our experiments indicate, 15 out of 18 mutants do show altered nitrogen-responsive phenotypes in comparison to the wild type. One of the mutants ashr2 displayed a prominent root phenotype for primary root length and we are currently using RNA-seq to identify the DEGs that are induced or repressed by this epigenetic regulator to gain further insight into the molecular underpinnings of N-response in roots. Our study will reveal knowledge of important epigenetic regulators, which can be extended to crop species for enhancing NUE.

P66 - Investigating the Role of Repetitive Basic Module in Extensin (EXT) Glycoprotein Self-Assembly (G)

Abdul Hakeem, Allan Kenneth Regunton, and Michael Held
Ohio University

The plant cell wall is made up of well-known polymers like cellulose, pectin, and hemicellulose, which are carefully organized and assembled to maintain the integrity and stiffness of the cell wall at the molecular level. One of these components is the extensin glycoprotein network. Extensins are modular glycoproteins that contain repetitive hydrophobic and hydrophilic modules, rich in [-Ser-Hyp4-] peptide repeats. Individual extensin monomers self-assemble early in cell wall development to form a polymeric network. The extensin glycoprotein network is believed to serve as a scaffold for the assembly of other essential cell wall polysaccharides, including pectin. In our study, we will synthesize genes that encode extensin analogs which contains various repeating peptide modules, namely, basic (alkaline), hydrophobic, and hydrophilic glycomodule units. First, we will synthesize desired EXT analog genes. EXT analogs will then be expressed transiently in tobacco as fluorescent protein fusions. Synthetic EXT glycoproteins will be isolated and biochemically characterized for amino acid and monosaccharide composition. Finally, characterized EXT glycoproteins will be biophysically tested for self-assembly by atomic force microscopy and crosslinking properties.

P67 - Investigation of the Role of SV2 Protein in the Emission of Volatile Organic Compounds in Petunia Flowers (G)

Jihee Lee and Natalia Dudareva
Purdue University

Plants synthesize and release volatile organic compounds (VOCs) which serve various biological functions. However, it is not well understood how these VOCs move within cells to be released into the atmosphere. As VOCs are lipophilic, they are likely to partition into the hydrophobic environment, such as subcellular membranes, and get to the plasma membrane via vesicle-mediated trafficking. Genes involved in vesicle trafficking with an expression pattern similar to VOC emission were searched from RNA-seq data of *Petunia hybrida*. Three PhSV2s, homologs

of mouse synaptic vesicle protein 2A (MmSV2A), which is important for the fusion of vesicles to target membranes in mice, were identified in petunia. *PhSV2*s expression pattern exhibits a rhythmic cycle, with levels increasing during the night and decreasing during the day, corresponding to the VOC emission profile of petunia. Therefore, we hypothesized that PhSV2 is involved in VOC emission through vesicle-mediated trafficking. To examine the role of PhSV2 in VOC emission, the expressions of *PhSV2*s were downregulated. To further identify if PhSV2s localize to the secretory pathway, fluorescent protein fused PhSV2s were transiently co-expressed with Golgi marker protein. Here, we showed that the downregulation of PhSV2s results in reduced VOC emission, and PhSV2s are localized to the Golgi.

P68 - Multi-Omic Characterization of Seed Proteomic Rebalancing Mutant Reveals Major Translation Re-Modeling and Anti-Oxidants Increase (G)

Clement Bagaza, Huda Ansaf, Abou Yobi, and Ruthie Angelovici
University of Missouri

Major crop seeds are deficient in essential amino acids (EAA) that humans and livestock require for their diet due to the dominance of seed storage proteins (SSP) that are EAA poor in their composition. However, eliminating SSP does not result in any major improvement in EAA composition due to a phenomenon termed 'proteomic rebalancing' that 'resets' the seed's amino acid content and composition back to their original states. This phenomenon is highly conserved, but its mechanism remains elusive. Understanding the biological processes underlying this mechanism will explain how seeds can exert high resilience to large perturbations in their proteome and how they can be manipulated for biofortification. Toward this goal, we performed a comparative metabolic and proteomic analyses of a triple knock out mutant (*cruabc*) of the three main SSPs in developing and dry Arabidopsis seeds. This analysis uncovered two major responses to SSP perturbation: an elevation in the reactive oxygen species (ROS) scavenging system of seeds and an adjustment to the translational machinery. ROS suggests that storage proteins are essential for the regulation of redox homeostasis, and the latter suggests that translational regulation, and potentially the composition of its apparatus, are key to the plastic rebalancing response of seeds.

P69 - Phenotyping the Chloroplast Sensor Kinase in Arabidopsis (G)

Matthew Martin, Emily Fannin, and Sujith Puthiyaveetil
Purdue University

Two-component systems involving a histidine-sensor kinase and its cognate response regulator are evolutionarily conserved in most prokaryotes, including cyanobacteria. A singular, modified version of this two-component system has been reported in the chloroplast of eukaryotic plants such as *Arabidopsis thaliana*. In this system, Chloroplast Sensor Kinase (CSK) no longer contains the conserved histidine residue from bacterial lineages and does not appear to have a true cognate response regulator. Instead, it has been shown to control gene expression in the chloroplast by utilizing an iron-sulfur cluster to monitor the plastoquinone pool and responding to changes by phosphorylating trans-acting elements, such as sigma factors of the plastid-encoded polymerase. Regardless of the evolutionary importance of CSK in photosynthetic organisms, a clear phenotype for CSK deficient plants has yet to be reported. This project aims to characterize three separate CSK tDNA-insert mutants and discern an observable phenotype of CSK deficiency. Fluorescence data measured with a PhotosynQ Multispec v1 and verified with a PSI FluorCam imaging PAM suggests CSK mutants have altered NPQ and ETR. Establishing a reliable and repeatable phenotype of CSK in Arabidopsis will increase understanding of the mechanism behind this elusive sensor kinase and provide an important control point for downstream investigations.

P70 - Pre-Breeding Strategies for Durable Septoria Leaf Spot (SLS) Resistance in Tomatoes (G)

Inty O. Hernandez-De Lira, Estefania Tavares-Flores, Mannon Gallegly, Mahfuz Rahman, and Vagner A. Benedito
West Virginia University

Septoria leaf spot (SLS) is a fungal disease that poses a significant threat to tomato and has become a severe problem in West Virginia. Unfortunately, commercial varieties with effective SLS tolerance are not available. Our research study aims to introgress robust resistance

from wild tomato species into commercial cultivars, as well as identify the genetic locus or loci responsible for SLS resistance. To achieve these goals, we used local varieties as recurrent parents to produce five F1 interspecific hybrids via *in vitro* ovule culture with *S. peruvianum* and *S. arcanum* accessions identified as sources of SLS tolerance. We demonstrated that among the hybrids produced, the SLS-resistant level of confirmed F1 plants was the highest in Hybrid-4. However, due to the self-incompatibility of F1 hybrids, we performed a segregation analysis in 345 H6xH2 pseudo-F2 seedlings. Our results suggest that SLS resistance is oligogenic, potentially involving 3 loci. While we have already obtained plants highly resistant to SLS, research is ongoing to confirm the QTLs in the genome linked to this trait and provide genetic materials and molecular markers to tomato breeders. Our research also paves the way for research into resistance mechanisms applicable to various Septoria diseases affecting crops and forages.

P71 - Probing Nuclear DNA-Dependent RNA Polymerase IV Subunit Assembly and Function *in vitro* (G)

Zheng Tian, Akihito Fukudome, Jasleen Singh, Yuichiro Takagi, and Craig S Pikaard
Indiana University

All eukaryotes have three essential multi-subunit RNA polymerases to transcribe nuclear genomic DNA into RNA: DNA-dependent RNA polymerases I, II, and III. Plants have two additional multi-subunit RNA polymerases, both of which evolved from nuclear RNA polymerase II (Pol II) and function in the RNA-directed DNA methylation (RdDM) pathway, a process required, primarily for silencing transposable elements throughout the genome. Pol IV partners with an RNA-directed RNA polymerases, RDR2 to generate double-stranded RNAs that are then processed by DICER-LIKE 3 into 24-nt short interfering RNAs (siRNAs). The 24 nt siRNAs are then incorporated into ARGONAUTE 4 and basepair with longer RNA transcripts of Pol V to guide DNA methylation and histone modifying enzymes to the adjacent chromatin. In collaboration with the Takagi lab, we have established a system in which the twelve subunits of *Arabidopsis thaliana* Pol IV complex are co-expressed as recombinant proteins in insect cells, using baculovirus expression vectors. Recombinantly expressed Pol IV contains all twelve subunits and is transcriptionally active *in vitro*. Using recombinant Pol IV, we can biochemically test the roles of individual subunits in Pol IV assembly, enzymatic activities and interactions with RDR2 or other proteins.

P72 - Recent Harmful Algal Blooms of *Prymnesium parvum* in Texas Consist of Multiple Cryptic Species (G)

Nathan Watervoort and Jennifer Wisecaver
Purdue University

Prymnesium parvum is a type of unicellular, biflagellated, haptophytic algae. *P. parvum* forms toxic blooms around the world which are disruptive to local ecosystems. In the US, *P. parvum* blooms are most concentrated in Texas. *P. parvum* is a species complex with at least three member species which are referred to as A-, B-, or C-types based on their toxins. Previously, all known strains (n=3) of *P. parvum* from Texas were characterized as A-types. However, the genetic diversity of Texas *P. parvum* is not well understood beyond these cases. I have surveyed eight different *P. parvum* blooms in Texas that occurred from 2018-2020, and isolated 32 *P. parvum* strains from them. Each strain was then sequenced, and had their genome size, heterozygosity, and ploidy characterized. These genomes were then used to construct a phylogenetic tree. I found that both A-types and B-types are currently in Texas and can be found in the same bloom. Significant intraspecific genetic variation was also found in A-types, with multiple ploidy states identified, but was not found in B-types. Future work should be done on the behavioral and metabolic differences between the two cryptic species, and the effects that both species have on Texas blooms.

P73 - Receptor-Like Cytoplasmic Kinase TPK9 Functions in Fungal Resistance and Light Stress (G)

Sara Hailemariam, Carol Bvindi, Sanghun Lee, Chao Liao, Iskander Ibrahim, Namrata Jaiswal, Matthew Helm, Sujith Puthiyaveetil, Ying Li, and Tesfaye Mengiste
Purdue University

The ability to recognize and quickly respond to diverse environmental cues allows plants to cope with both biotic and abiotic stressors. This process requires highly regulated plant response signaling, which requires the activity of receptor-like cytoplasmic kinases (RLCKs), a protein

family known for their role in signaling pathogen responses. However, their role in responding to abiotic stressors such as light quality and quantity is poorly understood in models and crop plants such as Tomato. Here, we determined the function of TPK1b Related Protein Kinase (TPK09) in both pathogen infection and light stress responses. We found that CRISPR-cas9-generated tomato *TPK09* mutants are more susceptible to Botrytis and exhibited impaired defense gene expression and reduced accumulation of ROS in response to chitin and flg22. *TPK09* transcripts accumulate in response to Botrytis infection and exposure to light. Interestingly, the *TPK09* mutants exhibited an elongated hypocotyl, progressive cell death, and accumulation of H_2O_2 under light-emitting diode (LED) light. Furthermore, TPK09 mitigates damage in the photosynthetic system under light stress, as demonstrated by a decrease in the effective photochemical quantum yield of PSII and electron transport rate. These observations suggest that TPK09 restricts the severity of fungal diseases and stress caused by LED light.

P74 - Small Peptides, Big Impact: Identification of Novel Peptides Regulating Soybean Nodulation (G)

Bruna Montes Luz, Jae Hyo Song, and Gary Stacey
University of Missouri

Soybean's ability to establish a symbiotic relationship with nitrogen-fixing rhizobia reduces the need for nitrogen fertilizer in agriculture. Utilizing advanced functional genomics techniques, we investigate the role small secreted peptides (SSPs) in this interaction. SSPs act in intercellular communication and play various roles in biological processes, including symbiotic interactions. Using TRAP-seq and *in silico* predictions of SSP genes, we identified hundreds of SSP genes differentially expressed in the vasculature in response to rhizobial inoculation 3 and 21 days after inoculation (DAI). Among the upregulated genes were members of the Inflorescence Deficient in Abscission (IDA) peptide family. We found that the expression of many IDA-encoding genes is induced in both the xylem and the phloem in response to rhizobia. Interestingly, overexpression of an IDA peptide in soybean roots resulted in a significant reduction in nodule numbers. Though their specific function in nodule development remains obscure, we hypothesize that it is related to the previously described function of IDA peptides in cell separation during the emergence of lateral roots. Based on data from this work, we clearly demonstrate the necessity to investigate cell-type-specific processes, which aid in the identification of novel genes that have highly specific temporal and spatial expression patterns.

P75 - Study of the *in planta* Metabolic Role of Putative Cytosolic ncADH Enzyme in Tomato (G)

Monika Choudhary and Joseph H Lynch
West Virginia University

Tyrosine (Tyr) is essential for protein synthesis and serves as a precursor for numerous specialized metabolites crucial for both plants and animals. Its synthesis from prephenate, downstream of the shikimate pathway, involves two possible alternative routes catalyzed by distinct TyrA family enzymes: Prephenate dehydrogenase (PDH/TyrAp) or Arogenate dehydrogenase (ADH/TyrAa). While plants typically possess canonical ADH specific to arogenate in plastids, recent phylogenetic analysis has identified a non-canonical clade localized in the cytosol, potentially contributing to total tyrosine production in plants. The metabolic role and contribution of this cytosolic enzyme in an alternative Tyr biosynthesis pathway remains unknown till date. This study employs reverse genetic strategies to investigate the metabolic impact of non-canonical ADH loss of function and reduction of function in tomatoes. Through CRISPR-Cas9 and RNAi techniques, three mutant tomato plants have been generated for each construct. These mutants will be further developed into T3 homozygous plants. Quantification of Phenylalanine (Phe), Tyrosine (Tyr), and Tryptophan (Trp), along with targeted metabolic analysis in fruits and vegetative tissues, will be conducted. This research aims to enhance our understanding of the metabolic function of the putative cytosolic ncADH enzyme that will be applicable to metabolic engineering and breeding strategies to improve crop species.

P76 - The Influence of Maize Genotype on Aerial Root Mucilage Microbiome (G)

Trever L Thurgood and Roland C. Wilhelm
Purdue University

Mucilage is a gelatinous mixture of plant-produced compounds with diverse functions including lubrication of extending root tips, deterrent of pathogens, and promotion of beneficial microorganisms. Certain germplasms of *Zea mays* exhibit increased production of mucilage at the aerial root and are associated with diazotrophic (free-living, nitrogen-fixing) bacterial strains. Herein, we investigate different microbial community structures and functions associated with aerial root mucilage of different genotypes of *Z. mays*. We performed a 16S rRNA gene sequence analysis and show there are significant differences between microbial communities collected from genotypic variants of *Z. mays*. In addition, we isolated individual bacterial strains for metabolic analysis and report on their genomic features which may enhance growth of their host plant. This research can inform selection of crop genotypes which promote microbiomes with increased nitrogen fixing capacity, and the potential to improve crop performance by decreasing the requirement for nitrogen supplementation.

P77 - The Role of Biological Nitrogen Fixation in Plant Acclimation to Elevated CO_2 (G)

Yuchen Wang and Matthew D. Brooks
University of Illinois Urbana-Champaign

The rapid and accelerating increase in atmospheric CO_2 levels makes it crucial to understand plant responses, particularly how their internal metabolic pathways adjust under stress. As CO_2 levels rise, plants typically enhance their photosynthetic activity. However, this benefit is often limited by nitrogen availability in soil. In this scenario, legumes benefit from their symbiosis with rhizobacterium that help assimilate atmospheric nitrogen fixed in exchange for carbon. This ability enables legumes to offset the carbon-to-nitrogen imbalance under high CO_2. The goal of the research is to understand the regulatory pathways and transcription factor hubs that balance carbon and nitrogen across different plant tissues, which underpins the improvement of biomass, yield, and nutritional quality of future crops. We take advantage of mutants and natural variants with different nodulation capabilities in model (*M. truncatula*) and crop (*P. vulgaris*, *M. sativa*) legumes to investigate the role of biological nitrogen fixation in response to elevated CO_2. To understand their adaptive responses, we combine physiological and transcriptomic analyses for plants grown under various CO_2 and nitrogen levels. Key transcription factor hubs that integrate these environmental signals will be identified using biological network analysis. To validate transcription factor (TF)-gene interactions, we employ stable and transient transformation methods.

P78 - The Role of GAUT Proteins in *Arabidopsis thaliana* Root Development (G)

Allison R. Triebe, Dami Olatunji, and Dior R. Kelley
Iowa State University

Cell wall dynamics are regulated during root development through the activity of cell wall modifying enzymes. However, how cell wall composition is modulated in root stem cell populations to influence organogenesis is not well understood. The pectin modifying enzyme GALACTURONOSYLTRANSFERASE 10 (GAUT10) has been shown to be involved in both primary root elongation and cell division, *GAUT10* mutants have a short root phenotype that is sucrose dependent. GAUT proteins can form complexes and may work in concert with one another. Gene expression mining indicates that *GAUT3*, *GAUT8*, *GAUT10* and *GAUT11* are all enriched in root stem cell populations. Through BiFC assays, it appears that GAUT10 can interact with GAUT3, GAUT8, and GAUT11 in the Golgi apparatus. To test the function of these GAUT proteins during root development, mutant combinations of these four *GAUT* genes were made. Preliminary phenotyping has shown that these genes have non-redundant and epistatic interaction. *gaut11-3* roots are longer compared to wild-type and loss of *gaut3* and *gaut11* can suppress the short root phenotype of *gaut10-3*. Continued investigations will examine the impact of GAUT8 and cell wall composition changes in the absence of these *GAUT* genes in a combinatorial fashion.

P79 - Transcription Factors from bZIP and MYB Families Integrate Nutrient and Light Signals to Coordinate Photosynthesis and Nitrogen Use (G)

Kithmee De Silva and Matthew Brooks
University of Illinois Urbana-Champaign

Plants rely on a complex yet finely-tuned network of processes that swiftly respond to environmental cues. While most previous studies have focused on the effect of individual signals, plants exhibit a synergistic response when presented with these cues in concert. This study harnessed gene regulatory networks (GRNs) to identify potential transcription factors (TFs) responsible for tuning plant responses to nitrogen (N) and light (L) availability. Arabidopsis grown in a matrix of N and L treatments exhibited discernible responses at both physiological and transcriptomic levels. The expression profiles of N- and L-responsive genes were used to construct a GRN. The correlations between gene expression and physiological traits (photosynthetic efficiency, non-photochemical quenching and C:N ratio) were used to infer subnetworks for each trait. TFs with high connectivity within subnetworks were hypothesized to be potential regulators. Several TFs from bZIP and MYB families emerged as key regulators of genes associated with the pathways of photosynthesis, N assimilation and L sensing. Ongoing efforts involve characterizing mutant phenotypes to validate these candidate TFs' roles. This study unveils validated and novel TFs involved in coordinating plant response to N and L availability, offering new strategies for enhancing photosynthetic activity and nutrient-use efficiency in plants.

P80 - Uncovering the Proximal Proteome of CTR1 Through TurboID-Mediated Proximity Labeling (G)

Yuan-Chi Chien[1], Andres Reyes[2], Hye Lin Park[1], Shou-Ling Xu[2], and Gyeong Mee Yoon[1]
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Protein-protein interactions play a crucial role in driving cellular processes and enabling appropriate physiological responses in organisms. The plant hormone ethylene signaling pathway is complex and regulated by the spatiotemporal regulation of its signaling molecules. Constitutive Triple Response 1 (CTR1), a key negative regulator of the pathway, regulates the function of Ethylene-Insensitive 2 (EIN2), a positive regulator of ethylene signaling, at the endoplasmic reticulum (ER) through phosphorylation. Our recent study revealed that CTR1 can also translocate from the ER to the nucleus in response to ethylene and positively regulate ethylene responses by stabilizing EIN3. To gain further insights into the role of CTR1 in plants, we used TurboID-based proximity labeling and mass spectrometry to identify the proximal proteomes of CTR1 in *Nicotiana benthamiana*. The identified proximal proteins include known ethylene signaling components, as well as proteins involved in diverse cellular processes such as mitochondrial respiration, mRNA metabolism, and organelle biogenesis. Our study demonstrates the feasibility of proximity labeling using the *N. benthamiana* transient expression system and identifies the potential interactors of CTR1 *in vivo*, uncovering the potential roles of CTR1 in a wide range of cellular processes.

P81 - The Role of Select E3 Ligases in Plant Gravitropism (G) (Also T7)

Samantha Fedoush, Madhura Yapa, Dr. Zihua Hua, and Sarah E. Wyatt
Ohio University

Gravity is a fundamental stimulus that directs plant growth; however, little is known about the regulatory mechanisms of gravitropic signaling. In a 2019 spaceflight experiment, a select group of E3 ligases were found to be differentially regulated in space. E3 ligases play an important role in protein degradation, they have the potential to inhibit or maintain the gravitropic signal depending on their substrate target. To narrow down these candidate E3 ligases to those involved in gravitropism, root and shoot reorientation experiments were conducted. Phloem Protein 2- A13 (PP2-A13) was shown to have a delayed gravitropic response. To characterize the biological importance of PP2-A13 within the gravitropic pathway, potential substrate binding partners of PP2-A13 were identified through yeast 2 hybrid analyses. To narrow the list of candidate binding partners, Arabidopsis tDNA insertion mutants were obtained for each potential binding partner, bred to homozygosity, and analyzed for gravitropic responses in root and shoot reorientation experiments. This work narrowed down candidate E3 ligases identified in a spaceflight study, identified substrate binding partners and explored the role of the substrates in plant gravitropism. This demonstrates the

importance of ground-based experimentation in tandem with spaceflight studies to understand molecular players involved in plant gravitropism.

P82 - Phosphorylation and Oxidative Damage Mediate the Disassembly of Photosystem II in *Arabidopsis thaliana* (G) (Also T10)

Steven D. McKenzie and Sujith Puthiyaveetil
Purdue University

Oxygenic photosynthesis is dependent on the bioenergetic reactions of the photosynthetic electron transport chain. Photosystem II (PSII), a large hetero-oligomeric pigment protein complex, utilizes radiant solar energy to oxidize water. In doing so, PSII produces protons and dioxygen as biproducts. Because of these highly energetic reactions, PSII is regularly subject to oxidative photodamage. To prevent photoinhibition due to oxidative damage, the damaged PSII undergoes a disassembly and repair cycle that results in the turnover (1 hour) of its damaged D1 subunit. Although this largely conserved process has been thoroughly studied across photoautotrophs, it is still unclear how PSII is turned over so rapidly. Previous research has demonstrated a role for phosphorylation of PSII in facilitating its turnover under high light intensities, however, the exact molecular mechanisms remain unclear. By examining several PSII phosphorylation mutants in *Arabidopsis thaliana*, we have demonstrated a role for PSII core phosphorylation in monomerization of the PSII homodimer. We have further demonstrated that oxidative damage is sufficient to induce the disassembly of the PSII monomer into smaller subcomplexes. Together, these results suggest at least two PSII disassembly pathways in plants, a more controlled phosphorylation-dependent pathway, and passive disassembly due to oxidative photodamage.

P83 - A Perspective of Mitochondrial Functions in Sperm Cells and Double Fertilization (UG)

Keila Jellings and Leonor Boavida
Purdue University

In angiosperms, double fertilization involves the delivery of two sperm cells to the embryo sac, where each sperm cell is destined to fuse with the egg cell or the central cell giving rise to a zygote and the nourishing endosperm. However, a long-standing question remains: are sperm cells inherently predetermined to fuse with a specific gamete? Mitochondria play a central role in the metabolism of all cells, generating ATP as the primary energy source, and as a byproduct they produce Reactive Oxygen Species (ROS). They also contribute to maintaining Ca^{2+} cytoplasmic homeostasis. Both Ca^{2+} and ROS are crucial for cellular signaling. Our previous work revealed differences in mitochondrial content between twin sperm cells, leading to our hypothesis that such cellular differentiation may have implications in sperm cellular metabolism, signaling, as well as its fusion fate or potential to activate the egg cell. To test this hypothesis, we used a sperm-specific mitochondrial fluorescent marker line in combination with genetic mutants affecting mitochondrial function or sperm cell function or fate. Along with observations of the fusion destination and mitochondria dynamics, these results are expected to contribute to our understanding of sperm cell differentiation and its role in fusion fate during double fertilization.

P84 - Deletion of Regions 2 and 3 of the Light-Response BTB Protein in *Arabidopsis thaliana* (UG)

Emily Seburn and Matthew Christians
Grand Valley State University

The ability to respond to varying intensities and wavelengths of light is crucial for plant development. Red light is detected by the phytochrome photoreceptors. When phytochromes absorb red light, they activate and move into the nucleus, where they regulate gene expression. They are then degraded by Light-Response BTB proteins (LRBs) through the Ubiquitin Proteasome System (UPS). Within the LRB protein sequence, three regions are functionally ambiguous and require further investigation. This experiment aims to elucidate the role of two of those regions. Regions two and three were separately deleted through mutagenesis and then transformed into *lrb1-1 lrb2-1* mutants, which contain no functional LRB proteins. We will analyze transformed plants for red light responses and changes in development. Understanding

the purpose of regions two and three of the LRB gene will help clarify the conserved domains of LRB in relation to its function in the degradation of phytochromes.

P85 - Engineering *Pseudomonas putida* for Increased Alginate Production (UG)

Virginia Akins[1], Lindsey Clark[2], and Jixun Zhan[3]
[1]University of Missouri, [2]Illinois Institute of Technology, [3]Utah State University

Alginate is an extracellular polymeric substance produced by various soil microbes including *Pseudomonas putida*. This polysaccharide is used in the agricultural industry to coat seeds to minimize abiotic and biotic stresses. While the majority of alginate is sourced from brown seaweed, the extraction process is expensive and water intensive. Engineering *P. putida* to produce more alginate could introduce other practical sources of this compound. Overexpressing the rate limiting enzyme can increase the production of alginate. This protein can be identified by overexpressing individual genes in the *alg* gene cluster and then quantifying the alginate produced using the carbazole assay. By cloning this gene and then ligating it into an expression vector, higher levels of alginate are expected. More sources of alginate can be discovered by studying additional bacteria species and quantifying any alginate they produce.

P86 - Halophytes and Heavy Metals: A Multi-Omics Approach to Investigate Phytoremediation Potential of *Cakile maritima* (UG)

Kathryn Vanden Hoek[1], Shawn K. Thomas[1], Tasha Ogoti[1], Ha Ngoc Duong[1], Ruthie Angelovici[1], Jacob D. Washburn[2], David Mendoza-Cózatl[1], J. Chris Pires[3], and Craig Schenck[1]
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Heavy metal pollution and soil salinization pose significant threats to ecosystems and agricultural productivity. Salt and heavy metal accumulators offer potential solutions through phytoremediation, a technique that uses plants to remove pollutants from the environment. In this study, we investigate the phytoremediation potential of *Cakile maritima* (henceforth referred to as Cakile), a salt and cadmium tolerant crop wild relative closely related to economically important mustard crops and the model species *Arabidopsis thaliana*. Elemental profiling following salt and cadmium treatment reveals that Cakile accumulates cadmium in its roots with little transport to the shoots and accumulates sodium in both its roots and leaves. Additionally, amino acid analysis indicates that Cakile is metabolically prepared for stress, with high levels of proline, an osmoprotectant, prior to stress treatment. Our findings suggest that Cakile is able to sequester sodium and cadmium ions and is metabolically primed to cope with abiotic stress, highlighting its potential for phytoremediation efforts in contaminated environments. Further exploration of Cakile's abiotic stress tolerance mechanisms could lead to the development of strategies for enhancing the remediation of polluted areas.

P87 - Identifying and Cloning the *MED16* Gene from Soybean and Arabidopsis (UG)

Brent Kanuho, Ashlynn Malloy, and Jennifer Robison
Manchester University

The regulation of cold tolerance impacts the growing season and range of plants. Soybean (*Glycine max*) is known to have limited cold tolerance. In previous studies, it has been shown that the cold induced molecular response of soybean resembles the phenotype of the *sfr6*/*med16* knockout *Arabidopsis thaliana* lines. The long-term goal is to examine the functionality and importance of MED16 in soybean while creating the tools required. Total cDNA was isolated from soybean and *A. thaliana* leaves via reverse transcriptase Monarch Total RNA miniprep kit. Bioinformatics was used to identify the most likely candidate for a MED16 homolog in soybean (*GmMED16*) using the *A. thaliana* (*AtMED16*) gene for comparison. Currently, *GmMED16* has been amplified from the soybean cDNA, cloned into pMiniT 2.0 vector (NEB), and transformed into competent *E. coli* cells. Confirmation of the *GmMED16* insert is being confirmed via restriction enzyme digests. The amplification of the *AtMED16* gene is being amplified via PCR but the amplification was unspecific and PCR optimization is underway. The

tools generated will be used to examine the functionality of *GmMED16* to restore cold tolerance to *med16* knockout *A. thaliana* mutants via Agrobacterium transformation with *AtMED16* acting as the positive control for eventual cold assays.

P88 - Investigating the Timing of Local and Systemic Exogenous Jasmonic Acid-Induced Protein Expression in Soybean Leaves (UG)

Jisaly Romano
Manchester University

Environmental stresses are one of the most detrimental stresses affecting soybean growth and defense. Stresses can be from abiotic (light, temperature, etc.) sources or biotic (disease, herbivory, etc.) sources. Systemic signaling molecules such as phytohormones during stress response are important in the regulation of plant growth. During wounding the production of defense hormone Jasmonic acid (JA) shields the plant from attacks and apoptosis, by acting as a systemic signal in surrounding leaves and tissue. The focus of this research is to identify the timing of local and systemic exogenous jasmonic acid-induced protein expression in soybean leaves. By utilizing one of our abiotic stress-responsive transgenic soybean lines (AtRD29Aprom:GFP/GUS, Robison et al., 2019) the increase in JA-induced protein levels was measured via GUS protein activity. Unifoliate leaves were dipped in a 1mM JA solution or in a mock control solution. Samples were taken from JA-treated leaf (local) and mock-treated leaf (systemic) every 2 hours for 24 hours. There was a significant increase in GUS activity at 12 hours in the JA-treated leaves. A corresponding increase was not seen until 48 hours in the systemic leaves. The data suggests that JA induced wounding response in systemic tissues later compared to locally wounded tissue.

P89 - Investigating the Timing of Wound-Induced Protein Expression Between Systemic and Wounded Tissue (UG)

Antonae Cofield and Jennifer Robison
Manchester University

Jasmonic acid regulates the expression of many of the defenses that a plant has. To study the wound signal in local and systemic tissue, an abiotic stress responsive transgenic soybean line with the Arabidopsis RD29a promoter driving GFP/GUS was used. Two-week-old soybean seedlings were examined for local and systemic GUS activity. One unifoliate leaf was wounded with a hole punch on either side of the midrib and the other unifoliate leaf was left untouched to examine systemic effects. Samples were taken every 4 hour for 48 hours of both the local and systemic leaf. The wound signal was assessed via GUS activity level. GUS activity was significantly increased at 12 hours in the wounded leaf tissue and 40 hours in the non-wounded leaf tissue. These data suggest that JA induced wounding response in systemic tissues at a later time compared to locally wounded tissues. The next step in the research is to examine the effect of exogenous JA application to see if it is comparable to mechanical wounded.

P90 - Potential Role of AHA2 Protein in Arabidopsis Gravitropism (UG)

Victoria A. Swiler and Sarah E. Wyatt
Ohio University

A plant's survival is largely impacted by its ability to sense and respond to gravitational changes, such as the changes that occur in spaceflight. However, the gravitropic signaling pathway is not fully understood. In the BRIC-20 experiment aboard the International Space Station, the Arabidopsis proton pump AHA2 was found to be differentially phosphorylated in microgravity compared to ground controls. AHA2 is hypothesized to be involved between plant hormone movement and differential growth during gravity signaling. To determine the role of AHA2 phosphorylation in gravitropic signaling, several lines of transgenic Arabidopsis with modifications in phosphorylation sites are being developed. Each AHA2 activation site is modified to keep the protein constitutively phosphorylated or dephosphorylated. The transformants will then be phenotyped for altered gravity response, showing if the phosphorylation of the AHA2 protein is involved in a plant's gravity

response. These findings will further knowledge on the molecular mechanisms of gravitropism to develop space-tolerant plants for life support in spaceflight and for colonization of the moon, Mars, and beyond.

P91 - Role of eATP on *Cuscuta campestris* in Artificial Host Systems (UG)

India Williams and So-Yon Park
University of Missouri

Cuscuta spp. is a stem parasitic weed that grows across central North America, decreasing growth and developments of its host by establishing connections through haustoria. Haustoria development is influenced by various environmental factors, including blue/far-red lights, physical touch, temperature, volatile organic compounds (VOCs) from host plants, and moisture levels. However, the detailed mechanism of haustoria development is unclear due to its development inside of host stems. To address this challenge, a recent study developed the artificial host system (AHS), which was designed to eliminate complications arising from environmental and host-related factors. It creates a controlled and sterile environment that allows for research that is more consistent and replicable. Although the AHS was initially developed for generating transgenic Cuscuta, we have developed the AHS protocol for broad applications to understand the haustoria structure and development factors. The modified AHS contains non-sterile Cuscuta stems on 3D printed stand with a liquid medium (or water) in a magenta box for short period. Using the AHS, the haustoria development of Cuscuta has been observed under different concentrations of extracellular adenosine triphosphate (eATP). These results will provide for the potential application of AHS for studying haustoria development without a live host plant.

P92 - Role of Nonsense-Mediated mRNA Decay in *Marchantia polymorpha* Response to Heat Stress (UG)

Sophie Vermilya†, Grace Timm†, Rebekah Ong, and Lauren Woodward
Taylor University, †Co-first authors

Our research is focused on the role of Nonsense-mediated mRNA Decay (NMD) in the stress response within the organism *Marchantia polymorpha*. NMD is a translation-dependent pathway which degrades classes of mRNAs with premature termination codons (PTCs). Additionally, NMD plays a crucial role in a variety of processes such as pathogen defense, flowering, ethylene response, circadian clock, just to name a few. To initiate mRNA degradation via NMD, a multiprotein complex is recruited to the mRNA, beginning with the association of Upf1 with RNA-binding proteins found on an mRNA undergoing premature translation termination. Interestingly, our preliminary analysis suggests some mRNAs predicted to undergo NMD are upregulated in response to heat shock while genes involved in NMD machinery (e.g. Upfs and Smgs) are downregulated. Consistent with our preliminary analysis in heat shock, *Arabidopsis thaliana* Upf1 mutants also accumulate NMD-targeted mRNAs important in plant immune response to pathogenic bacteria indicating that UPF1 is critical for NMD in and biotic stress response in Arabidopsis. To determine the impact of NMD on resilience of plants in the presence of biotic stress—namely heat shock— we describe our CRIPR/Cas9-mediated conditional knock out of UPF1 in *Marchantia polymorpha*.

P93 - Role of Squamosa Promoter Binding Protein-Like 2 (SPL2) in Regulating Root Elongation in Arabidopsis (UG)

Jocelyn Hartley†, Fuka Somatomo†, Aria Berryman, and Marta Laskowski
Oberlin College, †Co-first authors

Development of the *Arabidopsis thaliana* root system varies as plants mature. For example, the rate at which the primary root elongates speeds up after germination. The extent to which this effect may be regulated by the miR156 family of juvenility factors has not been fully established. Using GUS reporter lines that were sensitive or resistant to miR156 degradation, we observed that one of the targets of miR156, Squamosa Promoter Binding Protein-like 2 (SPL2), was expressed in the root meristem in a miR156-dependent manner. As expected, expression of the sensitive SPL2-GUS was not observed in seedlings around the time of germination when miR156 levels are known to be high. However, SPL2-GUS became visible in the root meristem by the end of the first week after germination; approximately by day 5.

Elongation of *spl2-1* loss-of-function roots between day 5 and 8 was less than in wildtype, indicating that expression of *SPL2* promotes root growth. The manner by which SPL2 has its effect is currently under investigation, with some hints pointing toward strigolactone production as a possibility.

P94 - Testing of a Metabolic Engineering Strategy to Increase Phenylalanine in Plants (UG)

Taylor Smith and Joseph H. Lynch
West Virginia University

Phenylalanine, an amino acid in all living organisms, is a building block for thousands of other key molecules. Plants use these phenylalanine-derived molecules to synthesize lignin, diminish genetic damage from UV radiation, and complete several other vital processes. Some of these molecules have potential as biofuels, medicines, and other products to improve quality of life. Phenylalanine metabolism, however, is not fully understood due to it consisting of both a plastidial and cytosolic pathway. The purpose of this study is to determine if the cytosolic pathway could be engineered to manipulate the output of phenylalanine in plants. A previous attempt to increase cytosolic production led to unintentional feedback inhibition of the plastid pathway, resulting in a lower net production of phenylalanine. Here we describe a modified strategy to avoid such inhibition. Plasmids carrying expression cassettes with different gene combinations were modified in *E. coli* bacteria. Agrobacterium-mediated transformation was used to genetically modify *Arabidopsis thaliana* plants and *Petunia hybrida* flowers. The flowers were found to contain a high expression of the gene cassette along with a significant increase in phenylalanine. Despite this, there was no significant change in net volatile production.

P95 - Three-Dimensional Structure of Haustorium in Host and Parasitic Plant Interactions via Laser Ablation Tomography (LATscan) (UG)

Demi White[1], Supral Adhikari[1], Felix B. Fritschi[1], Benjamin Hall[2], Asheesh Lanba[2,3], and So-Yon Park[1]
[1]University of Missouri, [2]Lasers for Innovative Solutions, [3]University of Southern Maine

Cuscuta spp. are stem holoparasitic plants that use haustoria to draw water, photosynthates, and nutrients from host plant vascular systems. Cuscuta serve as a model for stem parasitic plants and haustoria development, however, studies of the three-dimensional (3D) internal host-parasite interface and interconnections are limited as they are slow and tedious to assess. This study investigates an alternative method, Laser Ablation Tomography or LATscan technology, which can be used to generate 3D images of plant tissues by stacking high-resolution two-dimensional (2D) images. LATscan imaging of Cuscuta invading beet (*Beta vulgaris*) stems yielded 3D rendering and detailed images of the anatomy of Cuscuta-beet tissue interactions, including Cuscuta searching hyphae penetrating host vasculature. Differentiation between beet and Cuscuta tissues in 3D rendering and 2D images is facilitated by the color generated due to laser-tissue interactions. This study reveals that LATscan technology can be a useful and efficient tool to study tissue structure and function in parasitic plant interactions.

P96 - Understanding Protein-Lipid Interactions and Movement During Long-Distance Signaling (UG)

Curtis Chen, Luci Karakas, Ahmed Kristi, and Susanne Hoffmann-Benning
Michigan State University

A major goal of the United Nations is to "...end hunger, achieve food security, improve nutrition, and promote sustainable agriculture." As the population of Earth approaches 8 billion, it is crucial to develop an understanding of plants in order to reach the United Nations' goal. Our lab has identified several lipid-binding proteins in the plant phloem and we characterized their lipid-binding properties, localization, and stress response. One of these proteins, PLAFP, responds to the plant hormone ABA and appears to confer drought tolerance in plants. Our lab seeks to characterize if PLAFP moves, the mechanism in which it moves and the interactions it has with lipids. We use PLAFP an opotogentetically controlled promoter which induces gene expression encoding the fluorescently-tagged protein in the presence of red light but is repressed in blue light. It allows for spatiotemporal control of the gene expression and subsequent movement studies. We use Flowering Locus T, a protein, which has already been proven to move through the phloem, as a positive control and the fluorescent tag (RFP) as a

negative control. In this poster, we will describe and analyze our approach to discovering the function and movement of the PLAFP protein and present first results.

P97 - Assessing Nodulation Effects on the Photosynthetic Efficiency and Yield of Soybean (UG)

Sarah Eberly and Jennifer Robison
Manchester University

Soil microbiomes play an important role in soil and crop health. Many soils consist of their own unique microbiome. A subset of the microbiome of particular interest are endophytes which have symbiotic relationship with nutrient cycling in crops. The main interest of this research is to discover the efficiency of nutrient cycling in commercial mixtures. Soybean (*Glycine max*) cv William 82 was either soaked in commercial mixtures Exceed or TrueLeaf for 5 minutes and an uninoculated set was soaked in water as a control. Seeds were planted in a mixture of potting mix, topsoil, and manure and grown in a greenhouse. The treatments were analyzed for their photosynthetic efficiency, nodule count, and yield at developmental, and medial growth stages. Contradictory to the hypothesis, the results indicated little change between the experimental groups and the control. Thus, commercial mixtures did not improve the photosynthetic efficiency, development, or the nodule count of *Glycine max* cv William 82.

P98 - Azetidine-2-Carboxylic Acid: A Bioactive Non-Proteogenic Amino Acid Altering Protein Biosynthesis (UG)

William Thives Santos and Craig Schenck
University of Missouri

Plants are rooted in place and cannot rapidly escape environmental pressures. Thus, they have evolved specialized metabolites to tolerate biotic and abiotic stresses. Azetidine-2-carboxylic acid (Aze), a non-proteogenic amino acid analog of proline (Pro) is produced by divergent plant lineages and inhibits the growth of nearby organisms. Here, we use Arabidopsis as a model to explore the mode of action of Aze in plants. Arabidopsis grown in Aze-containing media demonstrated a root length reduction of ~75%. When higher concentrations of Aze were used, root growth was completely abolished. Similar root growth reductions were observed in other plant species. Interestingly, when media was supplemented with Pro, root length was fully restored, suggesting a competition between Pro and Aze. Proteomics analysis of Arabidopsis tissues grown on Aze show that 3.5% of all cytosolically translated proteins misincorporated Aze in place of Pro. No Aze misincorporation was detected in proteins translated in the mitochondria or chloroplast. Functional enrichment analysis suggests that Aze misincorporation leads to protein misfolding and triggers the unfolded protein response, which we are currently testing experimentally. In the future this knowledge can be used to engineer Aze tolerant plants for a rapidly changing climate.

P99 - Elucidating the Role of Vesicular Trafficking Components in Plant Development and Immune Responses (UG)

Carissa Bersche, Kelly Mason, and Antje Heese
University of Missouri

Correct protein localization is critical for plants to respond to environmental stress. Vesicular trafficking is the main process by which cargo proteins are transported from the trans-Golgi Network (TGN) to the vacuole or plasma membrane (PM). We and another lab have published that loss of the TGN-localized vesicular trafficking protein EPSIN1 (EPS1) correlates with impaired cargo trafficking to the PM and the vacuole, respectively. We show that reduced PM accumulation of defense proteins correlates with impaired immune responses. However, EPS1 does not function alone; it biochemically interacts with other proteins, such as VTI11, a v-SNARE required for the fusion of TGN-derived vesicles with the lytic vacuole. To explore whether EPS1 and VTI11 may show genetic interaction(s), our lab has created *eps1 vti11* double mutants. Here, I quantified *eps1* and *vti11* single and double mutants for potential defects in plant development and accumulation of proteins with plant immune responses. My data supports that in addition to their biochemical interaction, *EPS1* and *VTI11* showed genetic interactions;

but the genetic contributions of each gene differed depending on the phenotypic defect that I investigated. My current studies investigate the subcellular localization of specific cargo proteins in these mutants to understand phenotypic defects.

P100 - Fluorescent Labeling of Proteins of Interest by the Phosphopatetheinyl Transferase, Sfp (UG)

Ritvik Mishra, Feng Wang, and Craig Pikaard
Indiana University

Visualizing and tracking proteins in real-time by single-molecule confocal microscopy allows high-resolution insights into their functions, but typically requires that proteins of interest be fluorescently labeled. Sfp, a *Bacillus subtilis* phosphopantetheinyl transferase, catalyzes the transfer of a phosphopantetheinyl group from coenzyme A (CoA) to a specific serine residue in the peptidyl carrier protein or to a derived 12 amino acid peptide tag, S6, that can be fused to other proteins. Importantly, Sfp can also use as a substrate CoA that is covalently linked, via its terminal thiol group, to small molecules, including fluorophores or biotin. We overexpressed recombinant Sfp in *E. coli* and purified it to near homogeneity. In parallel, we fused the S6 peptide to T7 RNA Polymerase and RNA-DIRECTED DNA METHYLATION 1 (RDM1), allowing both proteins to be fluorescently labeled *in vitro* using recombinant Sfp and Cy5-modified CoA. Using these, and other fluorescently labeled enzymes, our goal is to visualize RNA synthesis and other steps of the RNA-directed DNA methylation pathway using single-molecule confocal microscopy approaches.

P101 - Glimpse into Transgenetic *Cuscuta*: Using Light Microscopy to Communicate Observe Haustoria-Vascular Connections of RUBY *C. campestris* (UG)

Lydia Phillips, Supral Adhikari, Asha Mudalige, and So-Yon Park
University of Missouri

Cuscuta spp. (dodder) is a parasitic plant that parasitizes a host after forming a haustoria by penetrating the host's vascular system. Reports confirm *Cuscuta* spp. exchanges micro and macro molecules with host plants. Cuscuta serves as a model organism for understanding plant-to-plant interactions and molecular trafficking. Using Agrobacterium-mediated *C. campestris* transformation protocol, we applied and produced viable *C. campestris* plants able to flower and produce seeds that demonstrated expression of RUBY- a reporter gene that creates a visible red pigment. The RUBY reporter creates a phenotypic difference, but to confirm successful vascular connection by the haustoria, imaging was necessary. We observed successful parasitization and the diffusion of the pigments into surrounding areas of the stem interior, indicating the exchange of molecules between *C. campestris* and its host. Although this is only a glimpse into our successful introduction of RUBY and *C. campestris*, the ability to visualize the haustoria's connection to the vascular system provides engaging images for our research to be communicated across the scientific community.

P102 - Investigating the Host/Parasite Relationship Using *Cuscuta campestris* and Tomato Under Drought (UG)

Felicity Guttmann, Demi White, India Williams, Kate Kottman, Ava Oelrichs, Charles Krueger, Felix Fritschi, and So-Yon Park
University of Missouri

Cuscuta campestris is a stem parasitic plant that takes water and nutrients from the host, leading to a significant reduction in crop yield, particularly in crops like tomatoes. The negative impact of parasitism can become even more pronounced when the host also experiences abiotic stress. In this study, we examined tomatoes exposed to four varied growth conditions to analyze the physiological responses of tomato hosts facing parasitism and drought conditions. These conditions included well-watered without Cuscuta (WW-C), well-watered with Cuscuta (WW+C), drought without Cuscuta (D-C), and drought with Cuscuta (D+C). Physiological data, including host leaf water potential (LWP), fresh weights, and dry weights, were collected to gauge the degree of stress in each treatment group. The most notable difference between treatments was LWP. LWPs for WW-C and WW+C treatment groups indicated high water availability. Drought-treated plants had LWP values indicating low water availability. Interestingly, the D-C LWPs exceeded the D+C LWPs, suggesting higher stress levels in a drought condition

without Cuscuta than a drought condition with Cuscuta. D+C hosts were expected to experience greater stress, but physiological results seem to contradict this assumption. This raises questions about water retention under parasitism, as well as mobile genes between Cuscuta and hosts.

P103 - Quantifying Allelopathy Resistance in Soybeans to Pennycress Glucosinolates (UG)

Devon C Jonaus[1], Lucas M Roberts[2], and Aaron J Lorenz[2]
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During the winter, farmers in the Midwest often end up with empty fields between planting corn and soybeans. To change this, the USDA NIFA CAP project IPREFER was created with the goal of making pennycress, a common weed, into an oilseed crop used on formerly fallow fields during winter months. One issue with this is the presence of glucosinolates: pungent compounds found in Brassicas that have the potential to inhibit germination and negatively affect plant growth. When herbivores damage plant tissue, glucosinolates and myrosinase are able to interact and form highly damaging hydrolysis products. This deters further consumption and harms surrounding plants, thus minimizing resource competition. In pennycress, the glucosinolate sinigrin hydrolyzes into allyl isothiocyanate (AITC), best known for being the pungent compound in horseradish. In the Upper Midwest, soybeans must be planted several weeks before pennycress is harvested to give them enough time to grow. This relay cropping system means that soybeans are most exposed to these compounds during early development. My research looks at how these two compounds affect juvenile soybean growth in order to effectively breed more tolerant soybeans.

P104 - Regenerative and Antimicrobial Activities of *Veronicastrum virginicum* Extracts (UG)

Isabelle R. Arnold and Rachel M. McCoy
St. Norbert College

Veronicastrum virginicum is a plant native to the eastern United States historically known for being used by Native American tribes for medicinal purposes including wound healing, treating rheumatism and other human diseases. Other members of its family have also been used medicinally to treat a variety of conditions including treatments for coughs, fevers, aliments, and to assist with childbirth. We hypothesize that *V. virginicum* extracts include medicinally relevant metabolites such as catalpol and/or iridoids. To test our hypothesis, we evaluated extracts of *V. virginicum* for regenerative and antimicrobial activities. We extracted metabolites from leaves, roots, and stems for further testing and used disc diffusion assays on these extracts against various ESKAPE pathogens. Preliminary results suggested that the antimicrobial properties are present as a zone of inhibition around the leaf extract with the *Staphylococcus epidermidis* bacteria. To test regeneration activities, *Dugesia japonica* were cut and placed into specified plant extract environments. Preliminary results suggest that the roots of the plant promote increased regeneration rate compared to the other extracts. Further work will be able to identify and characterize the bioactive compounds within the extracts and further characterize their medicinal activities.

P105 - Does the NHP6A Protein Affect *mPing* Transposition in Yeast? (UG)

Kaili Renken and C. Nathan Hancock
University of South Carolina Aiken

DNA transposable elements are found in virtually all eukaryotic organisms and can jump around the genome and insert/excise themselves from the DNA. *mPing* is a *Tourist*-like miniature inverted repeat transposable element derived from the autonomous element, *Ping*. Currently, *mPing* is being developed into a targeting insertion tool for genome editing in plants. It has been shown that *mPing*, mobilized by transposase proteins, can be inserted into Cas9-cleaved sites. Our goal is to identify proteins that promote transposition and thus increase the efficiency of *mPing*-based gene discovery and genome editing. A study found that HMGB1 protein increased the mobility of a *Mariner*-type transposon, *Sleeping Beauty*, in mammalian cells by bending the transposase binding site to facilitate transposase binding. This project is testing if NHP6A, the yeast homolog of HMGB1, affects *mPing* transposition. An overexpression *NHP6A* construct was made amplifying *NHP6A* and

Gateway cloning it into the pAG426 GPD plasmid. *NHP6A* null mutant yeast will also be transformed with a *hp_mPing:URA3* construct, designed to integrate into the genomic copy of *ADE2*. Using an *mPing* transposition assay that measures excision from *ADE2*, we will be able to determine the *hp_mPing* transposition frequencies with various levels of NHP6A. We anticipate increased transposition when *NHP6A* is overexpressed and decreased transposition in the *NHP6A* knockout. Moving forward, our next steps would be to test if expression of a *NHP6A* homolog in *Arabidopsis* will also increase transposition in plants.

P106 - Identifying the Causative Mutations Underlying a Dwarf Soybean Phenotype (UG)

Samantha Marie Burns and C. Nathan Hancock
University of South Carolina Aiken

Glycine max, commonly known as soybean, is an important crop that make up 90% of all United States oilseed production. The U. S. is currently #1 in the world for soybean production and #2 in the world for soybean export. Identifying the genes that control soybean growth is critical to the genetic improvement of this crop. The Hancock laboratory identified a G. max plant line with decreased height, smaller leaves, and significantly lower yields in a mutant population. To identify the mutations present in the dwarf mutant, we Illumina sequenced the entire genome and assembled it to the Williams 82 reference soybean genome using Burrows-Wheeler Aligner. Picard was used to mark duplicates and convert it to a BAM file. Genome Analysis Toolkit (GATK) was then used to identify the variants present in the genome. Candidate mutations that disrupt genes will be identified using SNPEff. Once candidate genes are identified, the team will use PCR analysis of an F2 segregating population to determine which mutation is linked to the phenotype. Once the Hancock laboratory team can identify what gene is causing the mutation, further research into the function of the gene can be conducted.

P107 - Expansins: Prospective Candidates for Leaf Angle Improvement in *Sorghum bicolor (UG)*

Jack R. Erickson, Aeson S. Akins, and Martha Ibore Natukunda
Augustana University

S. bicolor, or sorghum, is the fifth most important cereal crop in the world and the third most important in the US. Sorghum is highly drought-tolerant, resulting in an increasing reliance on the crop in areas of the world with low rainfall and areas with harsher droughts due to the effects of climate change. By increasing sorghum biomass, the photosynthetic efficiency of sorghum can be improved; therefore, sorghum must be engineered towards a "smart canopy," in which the angles of leaves are large near its base and get progressively smaller up the plant. Currently, sorghum's architecture is inverse of the proposed smart canopy model, and manipulating the lamina joint (or collar cells) of S. bicolor may lead to improved leaf inclination, light absorbance, and carbon allocation. We seek to improve sorghum leaf angle by studying expansin genes whose protein products are involved in plant cell elongation and expansion through cell wall microfibril acidification. Using expansin genes — identified previously through RNA sequencing — as prospective candidates for leaf angle improvement, we aim to isolate and genetically characterize expansin genes that differ in expression between canopy layers.

P108 - Stratification of Potassium in Tillage vs. No-Till Systems and Uptake in Corn (*Zea mays*) in Western Kentucky (UG)

Ava Isaacs, Kyle Krieger, Christopher Powell, and Dr. Megan Taylor
Murray State University

The demand for potassium is high in agricultural systems including corn. Potassium increases tolerance to water stress and disease resistance. Potassium is often not readily available for plant uptake in the soil and is largely lost during harvest, it is also an important component of plant cell walls and other metabolic functions, which could affect yield potential and soil fertility. This study investigated different tillage systems and the movement of potassium both in and out of the soil. Potassium can be a limiting factor for successful corn production. This study was conducted at two locations in Murray, Kentucky, both with the primary soil complex Grenada Silt Loam. One field has historically been under conventional tillage and had a tillage event in 2022. The other field has been in no-till for over 20 years. Soil and

tissue samples were taken during various stages of the growing season. Grain samples were also collected to determine the total uptake and usage of potassium. This study aimed to describe the possible stratification of potassium under different tillage systems and how that affects uptake into the corn plant as well as describing crop physiological differences as well as effects on grain quality in both systems.

P109 - The Role of KFB in Gravitropism (UG)

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A plant's perception and response to gravity is necessary for normal growth and survival, but the pathways involved are not fully understood. The Wyatt Lab studies the biochemical components of a plant's gravity response to better understand these pathways. In the BRIC-20 spaceflight experiment, we identified 968 genes that were differentially regulated in spaceflight, compared to ground control replicates. A group of proteins that functions in the ubiquitin pathway, E3 ligases, were differentially expressed. These proteins are involved in the pathway that labels molecules for degradation in the cell. Two of these E3 ligases were later identified to be gravity mutants through reorientation experiments conducted on Earth: KFB and PP2-A13. My current research has been characterizing the role of KFB, a kelch domain-containing F-box protein, (AT1G23390, kfb) in the gravitropic pathway through knockout and overexpression mutants. Once mutant lines are identified, mutant phenotypes are analyzed through root curvature, shoot curvature, gravity persistent signaling, and clinostat disorientation experiments. These data are then compared to the wild-type response to identify the effect of KFB on gravitropism. These findings will further the knowledge on gravitropism and aim to improve our ability to grow plants in space to fuel long-term spaceflight missions.

P110 - Identification and Characterization of Suppressor Mutant in a Jasmonic Acid Accumulating Arabidopsis Line (UG) (Also T4)

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Jasmonic Acid (JA) and its derivatives are important plant hormones involved in plant growth and stress responses. Plastid Lipase 3 (PLIP3) cleaves 18:3 (number of carbons: number of double bonds) acyl groups from chloroplast membrane lipids which are then metabolized to oxylipins including JA. The *PLIP3* overexpression line (*PLIP3*-OX) showed increased levels of JA and its derivatives, altered leaf morphology, and stunted plant growth. We are interested in discovering novel components of JA synthesis and signaling processes. Toward this goal, we conducted a suppressor mutant screen in the *PLIP3*-OX line. One candidate, suppressor mutant 97, carries a recessive mutation leading in the homozygous state to a partial reversal of the *PLIP3*-OX phenotype in addition to yellow leaves and a lipid phenotype similar to *PLIP3*-OX, but with a reduced level of 18:3 acyl groups. Through next-generation DNA sequencing of bulk DNA from an F2 mapping population consisting of 93 homozygous suppressor mutant individuals, a set of mutated candidate genes likely including the one casually responsible for the observed phenotypes was identified. To ultimately determine the causal mutation, we are currently crossing independent T-DNA mutants disrupted in the candidate genes with *PLIP3*-OX and suppressor line 97.

P111 - Advancing equity in plant science: NSF RCN:LEAPS: ROOT & SHOOT

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The American Society of Plant Biologists

ROOT & SHOOT (Rooting Out Oppression Together and Sharing Our Outcomes Transparently) is a five-year, multi-organizational NSF-funded LEAPS (LEAding cultural change through Professional Societies) Research Coordination Network (RCN) that draws together the efforts of seven plant science organizations (APS, ASPB, ASPT, BSA, IS-MPMI, MGC, NAASC) and other partners to facilitate cultural changes in the plant sciences to advance diversity, equity, inclusion, and access. Specifically, the RCN aims to use "*evidence-based practices to remove barriers for individuals historically excluded from science based on gender, gender identity, disability status, sexual orientation, ethnicity, or*

race." A key activity has been carried out by the Inclusive Conferences Working Group (ICWG). This team, comprising individuals from across career stages and organizational affiliations, has developed a comprehensive set of documents to promote awareness of and adherence to inclusive practices for conference attendees and organizers that will embed inclusivity and reduce harms. Another major activity has been to educate those with decision-making power within each organization about the complex issues that affect individuals' sense of belonging and desire / ability to persist in plant science through a pan-organizational year-long training program, which culminated in organization-specific pilot programs that address systemic inequities. The RCN also provides funding for student members of SACNAS and MANRRS to attend and network with plant scientists at plant science conferences; curates internship, grant, and award opportunities from across the plant sciences; and compiles a reading list of articles addressing equity in STEM. Our latest initiative is the formation of a Working Group that is developing a culturally-aware mentoring training program. Please join our mailing list (https://rootandshoot.org/) and follow us on Twitter @RootandShootRCN to be informed as these and other opportunities arise.

P112 – Investigating Potential Transgenic Silencing in Three Transgenic Soybean Lines (UG)

Rachel Fedor, Jennifer Robison
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Genetic modification has played a key factor in our ability to select for, eliminate, or change specific traits in a plant. One factor that poses risk to genetic modification is transgene silencing. Genetic silencing is a natural self-defense mechanism in plants which can result in the silencing of transgenes. The chance of silencing increases with every generation after the introduction of the transgene. It is crucial to check transgene expression for silencing. This research investigates the potential transgene silencing in three RD29A prom::GFP/GUS transgenic soybean lines. This study utilized the GUS assay to observe GUS activity in the soybean plants as a measure of transgenic expression. The study consisted of four soybean lines. One of the soybean lines was wild-type Williams82, which was used as the control, while three transgenic soybean lines were examined. A statistical analysis indicated that transgene expression levels are significantly above background compared to Williams82. Although this was discovered, when compared to previous research in 2019, it was found that slight transgene silencing has occurred in transgenic lines 22-23, and 28-5 and significant gene silencing in transgenic line 17-9 from generation three to current generation six. These findings highlight that gene expression is slowly being inhibited in these transgenic lines and it would be prudent to continue checking the lines as generations progress.

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