2023 Annual Meeting Midwestern Section American Society of Plant Biologists

April 22 – 23, 2023

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at

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Ames, IA

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Program At A Glance

Saturday, April 22	
7:00 – 8:00	Registration/Check-in (Location: outside *North Central*)
7:00 – 8:00	Poster Set-up (Location: *Cardinal and Gold*)
7:00 – 8:00	Breakfast (Location: *North Central*)
7:25 – 7:50	ASPB Get to Know You Bingo Game ((Location: *North Central*)
7:50 - 8:00	ASPB Ambassador remarks
8:00 - 10:00 Oral Session I: Genome-wide analyses and networks (T1-T6) - *North Central*	
8:00 - 8:05	Welcoming Remarks from Gustavo Machintosh (ASPB President)
8:05 - 8:35	Featured Speaker: Dr. Justin Walley (Iowa State University), *Unraveling Brassinosteroid and Target of Rapamycin Complex Signaling Pathways in Plants through Integrative Network Analysis of Multi-omic Data*
5 - 8:50	Eli Hartung, *Are Perennial Grass Ecotypes Locally Matched To Their Soil Microbes Across the Midwest Precipitation Gradient?*
8:50 - 9:05	Huan Chen, *Archaeological Bolivian maize genomes suggest diversity is associated with Inca cultural expansion and environmental variation in South America*
9:05 - 9:20	Ella So-Eun Kim, *Functional Characterization of novel small ORFs in Arabidopsis ABA response*
9:20 - 9:35	Kithmee de Silva, *Deciphering transcriptional regulators regulating photosynthesis in response to nitrogen and light availability in Arabidopsis thaliana*
9:35 - 9:50	Bruna Montes Luz, *Identification of soybean small secreted peptides involved in the regulation of symbiosis*
9:50 - 10:05	Lauren Sichterman, *Glycome profiling of cotton fiber cell wall polysaccharides to determine the molecular factors contributing to fiber quality*
10:00 – 11:00	**Poster Session I (Even Numbers)/Refreshments - *Cardinal and Gold***
11:00 – 1:00	**Oral Session II: Signaling and Development (T7-T12) - *North Central***
11:00 - 11:30	Featured Speaker Dr. Ruthie Angelovici (University of Missouri), *Uncovering the genetic and metabolic bases of seed amino acid composition using a multi-omics integration approach*
11:30 – 11:45	Dae Kwan Ko, *An IRE1-proteasome system signaling cohort controls cell fate determination in unresolved proteotoxic stress of the plant ER*
11:45 – 12:00	Damilola Olatunji, *The Class VIII myosin ATM1 is required for root apical meristem function*
12:00-12:15	Craig Cowling, *Roles of auxin transporter PILS6 in maize growth and development*
12:15 – 12:30	Brian Zebosi, *bds1 and bds2 function redundantly to regulate inflorescence and shoot architecture in maize via brassinosteroid biosynthesis*
12:30-12:45	Kaitlin Higgins, *Mdr1 demethylase and the intersection between the epigenome, genomic imprinting, and transposable elements in maize endosperm*
12:45-1:00	Bilal Ahmad, *Divergence of nuclear localization mechanism in HD-Zip IV family*
1:00 – 2:00	**Lunch - *North Central* (switch posters - *Cardinal and Gold*)**
2:00 – 3:00	**How to Cultivate a Successful Career in Plant Biology: Insights from Experts** • Margaret Woodhouse – PhD (Computational Biologist, MaizeGDB, USDA) • Christopher Zalewski – MPA, PhD (Vice President of Operations/Founder, Front Range Biosciences) • Carina Collins – PhD (Assistant Professor & Director of Undergraduate Research, Marian University) • Bruna Montes Luz – PhD candidate (University of Missouri)

3:00 – 4:30	**Oral Session III: Methods and Advances (T13-T18) - *North Central***
3:00-3:15	Feng Zhang, *Efficient protein tagging and cis-regulatory element engineering via precise and directional targeted insertion in plants*
3:15-3:30	Matrika Bhattarai, *Developing an in vitro GT array (i-GTray) platform for high-throughput enzyme activity testing of glycosyltransferases*
3:30-3:45	Stephen Deslauriers, *High-resolution imaging: a system for undergraduate research studying Arabidopsis development*
3:45-4:00	Zhongpeng Li, *Plasmodesmata-located proteins regulate the plasmodesmal function at specific cell interfaces*
4:00-4:15	Cailin Smith, *Thylakoid-Inner Envelope Membrane Contact Sites Facilitate Photosynthetic Membrane Synthesis*
4:15-4:30	Ashley Henry, *Uncovering the genetic controls of the Arabidopsis thaliana root growth zone and its response to gravity*
4:30 – 5:30	**Poster Session II (Odd Numbers)/Appetizers - *Cardinal and Gold***
5:30 – 6:30	**Keynote Speaker Dr. Diane Bassham (Iowa State University) - *North Central***
Dinner on your own	
Sunday, April 23	
7:00 – 8:00	Registration/Check-in/Breakfast
8:00 – 10:00	**Oral Session IV: Metabolism (T19-T24) - *North Central***
8:00 – 8:30	Featured Speaker: Dr. Sharon Kessler (Purdue University)
8:30-8:45	Conor Raymond, *Duplication and co-option of fatty acid biosynthesis potentiates plant chemical diversity*
8:45-9:00	Xingqi Huang, *Peroxisomal heterodimeric enzyme responsible for benzaldehyde biosynthesis in plants*
9:00-9:15	Ryan Patrick, *Dynamic histone acetylation coordinates temporal biosynthesis and emission of floral volatiles in petunia*
9:15-9:30	Liza Gautam, *Improving pennycress glucosinolate, seed size, and seed oil domestication traits.*
9:30-9:45	Chen Zhang, *Two evolutionarily duplicated domains individually and post-transcriptionally control SWEET expression for phloem transport*
9:45-10:00	William Thives Santos, *Engineering Plants Resistant to Defensive Non-Proteogenic Amino Acids*
10:00 – 10:15	**Coffee Break**
10:15 – 12:15	**Oral Session V: Stress and Defense (T25-T30) - *North Central***
10:15 - 10:45	Featured Speaker Dr. Cory Hirsch (University of Minnesota)
10:45-11:00	Yosef Fichman, *HPCA1 is required for systemic reactive oxygen species and calcium cell-to-cell signaling and plant acclimation to stress*
11:00-11:15	Ranjita Sinha, *Differential regulation of flower and pod transpiration during abiotic stress combination in an annual plant*
11:15-11:30	Heena Puri, *Temporal transcriptomic profiling elucidates sorghum defense mechanisms against sugarcane aphids*
11:30-11:45	Martin Alcantar, *Novel function of the CBL-CIPK Network in Plant Immunity*
11:45-12:00	Dandan Zhang, *Understanding the roles of soybean aphid effectors in soybean and soybean aphid interaction*
12:00-12:15	Katie Horton, *Chasing a molecular chimera; AvrRps4 effector family expanded via bioinformatics and new bacterial assays for lettuce*
12:15 – 12:30 Awards & Announcements (meeting adjourn) - *North Central*	

ASPB MW Section

The Midwestern section began as local society meetings at Purdue and the University of Minnesota (1926) and Illinois (1948). In 1955, at an invitational meeting in Urbana, the groups formally joined forces and became the Midwestern section and the local university sections disbanded. Today the Midwestern section boasts the largest membership of any of the sections and continues to hold annual meetings to discuss current research within the section.

States Included: U.S. – Iowa, Illinois, Indiana, Kansas, Kentucky, Michigan, Minnesota, Missouri, North Dakota, Nebraska, Ohio, Oklahoma, South Dakota, West Virginia, Wisconsin; Canada – Manitoba, Ontario

Executive Committee (2022-23)

Sen Subramanian (South Dakota State University), Vice-Chair (Senthil.Subramanian@sdstate.edu @SubramanianLab)

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Jonathan Fresnedo Ramirez (Ohio State University), Secretary/Treasurer

Bruna Montes Luz (University of Missouri), Early Career Representative (bmontesluz@mail.missouri.edu)

Dior Kelley (Iowa State University), 2023 Meeting Organizer, (dkelley@iastate.edu @KelleyDior)

Kathrin Schrick (Kansas State University), ASPB Section Representative to the ASPB Council (kschrick@ksu.edu)

Jennifer Robison (Manchester University, Indiana), Publications Manager (jdrobison@manchester.edu @OshnGirl)

Please volunteer to serve on the committee for AY2023/24

List of Posters

P001:Pei Jia Ng, Engineering arsenic tolerance by manipulating a sulfate transporter gene in Arabidopsis

P002:Ajay Gupta, High-efficiency multiplexed prime editing enables new strategies for broad-spectrum resistance to rice blast

P003:Atinder Singh, Discovering Transcriptional Regulators of Photosynthesis in Energy Sorghum to Improve Productivity

P004:Jon Cody, Viral delivery of recombinases for heritable genetic switches in plants

P005:Tanner Cook, Establishing a System for High-Throughput Screening of Factors Affecting Meiosis

P006:Evin Magner, Post-secretory synthesis of a natural analog of iron-gall ink in the black nectar of Melianthus spp.

P007:Matthew Zimmerman, A Jaltomata bohsiana nectar protein inhibits the growth of both bacteria and fungi

P008: Rylee Sokoloski, Cloning and Analyzing of Native and Engineered GLDP1 Promoters

P009:Jihee Lee, Investigation of the contribution of vesicle trafficking to the emission of volatile organic compounds in petunia flowers

P010:Mohsin Ali Nasir, Predicting protein-protein interactions between glycosyltransferases for plant cell wall polysaccharides synthesis

P011:Lilia Ernestina Montanez Hernandez, Comparative genome analysis of Bradyrhizobium strains with different nitrogen fixing capacities

P012:ATHIRA SETHU MADHAVAN, Understanding Rhizobial competition for nodulation using split root assays

P013:Anna Childers, Post-translational Modification Sites in AtTCP8 IDR 2 Influence Localization and Interaction Behavior

P014:Demi White, How do drought stress and stem parasitic plant, Cuscuta campestris, affect tomato development?

P015:Aline Rodrigues de Queiroz, Effects of exogenous antioxidants on photoprotection mechanisms in Arabidopsis thaliana

P016:Brianna Griffin, Roles of REL2 Mediated Transcriptional Co-repression in Maize Immunity

P017:shannon stirling, Karrikin-like signaling pathway is involved in the perception of volatile terpenoids in petunia

P018:Bianca Serda, The Protective Role of Isoprene Against Ozone Stress by Abating Reactive Oxygen Species Production

P019:Brett Fredericksen, Linking Hyperspectral Reflectance Data to the Genome of Panicum virgatum

P020:Maya Sealander, Phytochrome B mediated regulation of ROS production in response to high light stress

P021:Jared Haupt, Insights into Mechanisms of Chilling Tolerant Photosynthesis in the C4 Grass Miscanthus

P022:Jackson Marshall, Exploring the Phenotypic Effects of PILS2 and PILS6 Proteins in Zea Mays and Arabidopsis

P023:Fan Huang, Variations in Fatty Acid Elongation Generate Novel Hydroxy and Keto Fatty Acids

P024:Ning Zhang, The Arabidopsis xylosyltransferases, XXT3, XXT4, and XXT5 are essential to complete the fully xylosylated glucan backbone XXXG-type structure of xyloglucans

P025:Bimala Acharya, Identifying the trans-duplicated genic regions – A first step in finding Helitrons in maize genome

P026:Ang-Yu Liu, Roles of vacuolar phosphatases in nucleotide salvage pathway of Arabidopsis

P027:Hazem Khalaf Mohammed, Determining host plant responses towards competition between Bradyrhizobium elkanii strains

P028:Jordan Julian, Activity and Specificity of Xyloglucan Galactosyltransferase MUR3

P029:Armaan Sandhu, Bradyrhizobium Phenotypic plasticity: A mechanism by which soybean root exudates influence legume-rhizobia interactions

P030:Sanket Shinde, Effect of fall armyworm herbivory on transcriptional responses of sorghum

P031:Jithesh Vijayan, Exogenous application of an archaeal antioxidant enhances plant growth by attenuating biotic stress response

P032:Susan Bush, Arabidopsis root growth is enhanced by the artificial food dye Red No. 40

P033:Manoj Kumar Reddy Allam, Characterization of Glycine rich proteins in root nodule development of Medicago truncatula

P034:Aniket Singh, Cell-level auxin and cytokinin responses during root lateral organ development in Soybean

P035:Akshayaa Venkataraghavan, Elucidating the glucuronidation mechanism of heteroxylan in grasses

P036:Abhijit Sukul, Identifying small RNAs associated with Secondary Cell Wall Development in Arabidopsis thaliana.

P037:Allan Kenneth Regunton, Identifying small RNAs associated with Secondary Cell Wall Development in Arabidopsis thaliana.

P038:Ronald Myers, Extracellular ATP plays an important role in systemic wound response activation

P039:Carissa Bersche, Delineating the genetic interaction of vesicular trafficking components in plant growth and development

P040:Kristen Barwick, Antibody Production Against the EPSIN1 ENTH Domain from Arabidopsis thaliana

P041:Kelly Mason, Role of Clathrin-Coated Vesicle Components in Plant Immunity

P042:Linkan Dash, The role of GAUT10 in Arabidopsis root development

P043:Cali Gunderson, Effect of pollinators and visitors of the mint Blue Sage (Salvia azurea) in Nebraska

P044:Zawar Hussain, Characterization of a tomato extensin peroxidase in vivo

P045:Zachery Shomo, Time and Temperature: The Keys to Understanding Triacylglycerol Accumulation in Cold and Freezing

P046:Sophia Goushchina, Identifying an unknown mutation in pils2 pils6 double mutant

P047:Ilayda Korkmaz, Heterologous production of proteins involved in chloroplast lipid metabolism to determine phosphatidic acid phosphatase activity

P048:David Payne, Rice small RNA expression levels are associated with grain chalkiness under high night temperature

P049:Kenia Segura Abá, Deciphering the genetic basis of fitness of yeast in different environments

P050:Megan DeTemple, A Novel Study on Priming the Defense of Arabidopsis Plants Against Its Pathogens by Expressing Fungal Cell Wall-Digesting Enzymes

P051:Huishan Liu, Lysin-motif receptor-like kinases mediate β-1,3-glucans perception in Medicago truncatula

P052:Lahiru Ranaweera, Predicting putative cis-regulatory elements regulating transcriptional response at the single cell level in Arabidopsis roots

P053:Makenna Tressler, Analysis of Flavor Compounds in Table, Crab and Cider Apples Used for the Production of Hard Cider

P054:Anna Rowzee, Phenotypic variation and plasticity in yield traits for a biparental population grown under variable nitrogen availability

P055:Talles de Oliveira Santos, The role of PSI3 gene in Arabidopsis thaliana in the dynamic response of stomata conductance to light

P056:Supral Adhikari, Overexpression of a mobile Cuscuta gene decreases lignin composition in Arabidopsis

P057:Asha Kaluwella Mudalige, FACTORS AFFECTING Agrobacterium-MEDIATED TRANSFORMATION OF Cuscuta campestris

P058:Leon Van Eck, Identifying and characterizing resistance to the bird cherry–oat aphid in three accessions of wild barley

P059:Brianna Brown, Predicting heat stress-related genes in Saccharomyces cerevisiae and Arabidopsis thaliana

P060:Andres Gutierrez, Phenotypic characterization of a GWAS population to identify the genetic basis of mutualism variation in Medicago truncatula.

P061:Yosia Mugume, Lipid Derived Signaling Involving Chloroplasts

P062:SARA ANWAR, Functional characterization of putative transcription factors of primary cell wall deposition in rice

P063:Evan LaBrant, Candidate Proteins Located at Membrane Contact Sites Between Chloroplast Inner Envelope and Thylakoid Membranes

P064:Dinakaran Elango, Insights into microbe mediated heat stress adaptation in soybean

P065:Deena Tesfaye, **Turning over a new leaf**: **Utilizing wild *Pseudomonas* to study host effector interactions**

P066:Elissa Rhuby, TRV-AmCyan: A tool for robust visualization of viral movement and Cas9 editing in Nicotiana benthamiana

P067:Taylor Smith, Testing of a metabolic engineering strategy to increase phenylalanine in plants

P068:Madeline Stadtmueller, Modified Bt toxin to control soybean aphids

P069:Rosimeire Barboza Bispo, Understanding the response mechanisms involved in phosphorus deficiency in popcorn

P070:Santiago Franco Lopez, Conservation of stomatal traits in Arabidopsis thaliana ecotypes from arid and temperate environments

P071:Dilkaran Singh, Transcriptional regulation of CO2 and Nitrogen metabolic interactions in Arabidopsis

P072:Yiling Feng, The diversity of diterpenoid biosynthetic gene clusters in rice

P073:Arefeh Avestakh, Confocal analysis of stably-expressed Agmatine Iminohydrolase and N-carbamoyl putrescine hydrolase in A. thaliana.

P074:Bryan Drew, East Asian-North American disjunctions and phylogenetic relationships within subtribe Nepetinae (Lamiaceae)

P075:Pritha Kundu, Sequence does not matter! A tale from priming in sorghum against insects of different feeding guilds

P076:Seema Sahay, Genetic control of photoprotection and photosystem II operating efficiency in plants

P077:Caitlin Gonzales, Linking GAUT10 Function to Root Cell Wall Composition and Auxin

P078:Clair Wootan, Regulation of Heat Stress through Heat Shock Factors in the Model C4 Monocot Setaria viridis

P079:Felicity Guttmann, GmCSD1 Isoforms and Gma-miRNA398 Interactions within the Soybean Drought Response

P080:Darcy Bonds, Exploring the genetic diversity of Ketoacyl-CoA Synthetase (KCS) genes involved in plant fatty acid elongation across a diverse set of maize inbred lines

P081:Parinaz Mohtasebi, Comparative Analysis of the Root System Architecture of Vitis rupestris and Vitis riparia, Important Genetic Resources for Grape Breeding

P082:Benjamin Spears, Conservation of mechanisms for post-translational regulation of the intrinsically-disordered TCP transcription factors

P083:Adelaide Hazen, Variation in freeze tolerance and circadian clock traits among diverse Arabidopsis ecotypes

P084:Jesse Krokower, Quantitative Trait Loci (QTL) Influencing Leaf Elemental Concentrations in Grapevine (Vitis sp.)

P085:Juan Diego Rojas-Gutierrez, Genetic basis of heterosis in natural populations of Arabidopsis thaliana.

P086:Megan tenBensel, Effects of fire on plant species in grazed grasslands

P087:Su-Ling Liu, Investigating the dynamic regulation of plasmodesmata during bacterial infection

P088:fnu prema mutyala, Carbon Allocation to Mutualists in Tripartite Interactions with Medicago Truncatula

P089:India Williams, Artificial host systems (AHS) for a parasitic plant, Cuscuta Campestris.

P090:Keila Jellings, An Inducible in vitro system to reprogram somatic cells into a gamete fate

P091:Lydia Phillips, Hemp's Hide-and-Seek: Cannabis sativa L. (Cannabaceae) tested as a host for a parasitic plant, Cuscuta campestris

P092:Freddie Mildenhall, Investigation of Chrono-Freeze, a Novel Circadian Rhythm Pattern Induced by Cold Stress

P093:Katerina Holan, A Machine Learning Approach to Quantitatively Phenotype Common Rust Symptoms of Maize

P094:Matthew Wendt, Key developmental windows and environmental parameters that influence cuticular wax composition on maize silks

P095:Alexander Austin, Multi-Omic Analysis of Maize Pollen During Storage

P096:Jesus Loya, Using native rhizobia to create a drought-resilient field pea production system

P097:Haris Variz, Functional characterization of STRUBBELIG-receptor family 3 in regulating plasmodesmal function and plant immunity

~~P098:Hannah Hausman, Utilizing Oxford Nanopore Technology to Sequence the Genome of the Green Alga Trentepohlia~~

P099:Keting Chen, Characterization of the gene networks underlying cuticle production in maize silks via systems' biology approaches

P100:Yuxiang Guo, Maize orphan genes and their potential association with cuticle synthesis

P101:Dirk Winkelman, A multidisciplinary approach to assess the roles of Glossy2 and Glossy2-like in maize cuticular lipid biosynthesis

P102:Madison Lane, Characterization of candidate genes related to cuticular wax deposition on maize silks

P103:Joseph Outar, Developing a tool to examine virulence functions of co-infiltrated bacterial effectors in plant cells

P104:Mojde Sedaghat, Primary cell wall regulation: ancient genes with modern roles

P105:William Clore, LTR Predictor: A tool to identify LTR retrotransposon insertions in long-read genome sequencing data

P106:Samia Nawaz, Exploring the Adaptive Functions of the Ubiquitin-26S Proteasome System in Rice

P107:Carren Burkey, Probing the molecular basis of pathogenicity by Pseudomonas fluorescens LE6_D7 on oomycetes

P108:Raegan Mozal, Genetic characterization of TCP gene family in Physcomitrium patens

~~P109:Martin Alcantar, Novel function of the CBL-CIPK Network in Plant Immunity~~

Talk abstracts

Abstract# F1: *Session 1 Genomic networks (Featured speaker)* (Sat, Apr 22, 8:05-8:35)

Unraveling Brassinosteroid and Target of Rapamycin Complex Signaling Pathways in Plants through Integrative Network Analysis of Multi-omic Data

Justin Walley, Iowa State University

Brassinosteroids (BRs) are plant steroid hormones while Target of Rapamycin Complex (TORC) is an important regulator that integrates nutrient and energy sensing. Both of these processes modulate plant growth and stress responses. I will describe our use of systems biology approaches to integrate multi-omic datasets and unravel molecular signaling events during BR response in Arabidopsis. Moreover, we investigated the connections between BR and TORC signaling pathways and identified a common set of gene products that are regulated by both pathways. Screening of candidate genes from the reconstructed network revealed genes that when mutated exhibited altered BR response and/or modulated autophagy activity. Together, these findings demonstrate the power of integrative network analysis applied to multi-omic data and provide fundamental insights into the molecular mechanisms of BR and TORC signaling in plants.

Presenting author: Justin Walley, Iowa State University, jwalley@iastate.edu

Abstract# T1: *Session 1 Genomic networks* (Sat, Apr 22, 8:35-8:50)

Are Perennial Grass Ecotypes Locally Matched To Their Soil Microbes Across the Midwest Precipitation Gradient?

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Jumpponen, Ari, Department of Biology, Kansas State University, 1717 Claflin Rd, Manhattan, KS 66506
Lee, Sonny, Department of Biology, Kansas State University, 1717 Claflin Rd, Manhattan, KS 66506
Johnson, Loretta, Department of Biology, Kansas State University, 1717 Claflin Rd, Manhattan, KS 66506

Andropogon gerardii is a dominant grass of the Great Plains that accounts for roughly 70% of the biomass of tallgrass prairies. Its distribution across a steep rainfall gradient in the Midwest has given rise to locally adapted wet and dry ecotypes. Abiotic factors, like rainfall, in the formation of ecotypes have been well-studied. A gap in our understanding is the extent to which local soil microbes interact with ecotypes. We investigated how soil microbes influence A. gerardii growth and whether plant ecotypes are locally matched to their microbes. We predicted that ecotypes would grow better when grown with native microbes than when grown with microbes adapted to other A. gerardii ecotypes. We collected seeds and soils from six native A. gerardii populations from western KS (580mm) and Illinois (1,167mm). We isolated microbes from roots and soil, cultured them in R2A agar broth, and reciprocally inoculated wet and dry microbes weekly into garden soil where plants were grown for 12 weeks. Plant form and function were measured weekly. Preliminary results showed that ecotypes are genetically distinct and that inocula harbor distinct microbial communities. Wet ecotypes produced more biomass, had greater leaf area, and were taller than dry ecotypes. For inoculation effects, physiological traits, such as chlorophyll absorbance, a proxy for photosynthesis, were enhanced when ecotypes were growing with their local microbes. Even more notable, the dry ecotype produced ~30% more biomass when it was matched with its local microbes. Results suggest ecotype-specific microbe-mediated effects on nutrient availability. These results provide insight into how A. gerardii plants interact with their native microbiomes and have implications for restoration and forage production.

Presenting author: Eli Hartung, Kansas State University, elih@ksu.edu

Abstract# T2: *Session 1 Genomic networks* (Sat, Apr 22, 8:50-9:05)

Archaeological Bolivian maize genomes suggest diversity is associated with Inca cultural expansion and environmental variation in South America

Huan Chen1,2, Amy Baetsen-Young3, Addie Thompson2,3, Brad Day1,2,3, William Lovis4,, Gabriel Wrobel4,**

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Previous archaeological and anthropological studies have demonstrated the myriad ways that cultural and political systems shape access to and preferences for food. However, few studies have carried out biocultural analysis linking specific genotypic/phenotypic traits as evidence of cultural selection in ancient contexts. Here, we address this issue by comparing genomes of maize samples from Bolivia dating to ~500 BP included as an offering with the mummified remains of a young girl to 13 archaeological maize samples spanning at least 5,000 years of evolution, and 87 modern western hemisphere maize samples. Our phylogenetic analysis showed that the archaeological Bolivian maize (aBM) has the closest genetic distance to archaeological maize from ancient Peru, which in turn was derived from central Mexican domesticated varieties. The comparative genomic analysis identified specific modifications in the aBM genome representing traits that were selected by ancient farmers, including shorter growing season, greater drought-resistant, and sweeter taste. The genome of the aBM appears to reflect gene flow from Chile coinciding with the arrival of the Inca empire in the altiplano region surrounding modern-day La Paz, as well as selection for specific traits related to growth in this marginal environment and taste preference. Our study provides insights into the complex biocultural role that Inca culture had in determining the direction of maize diversity in South America.

Presenting author: Huan Chen, Michigan State University, chenhua9@msu.edu

Abstract# T3: *Session 1 Genomic networks* (Sat, Apr 22, 9:05-9:20)

Functional Characterization of novel small ORFs in Arabidopsis ABA response

Ella So-Eun Kim, Phong Nguyen, Larry Hsin-Yen Wu, Qiaoyun Ai and Polly Yingshan Hsu
Department of Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI, USA

Small open reading frames (sORFs) are known to play important roles in plant development and signaling, but their identification remains a challenge. Using Ribo-seq, we discovered a group of novel sORFs induced by abscisic acid (ABA) in Arabidopsis. We found that these sORFs contain one or two ABA-responsive element (ABRE) motifs in their promoter regions. This implies that these ABA-induced sORFs are part of the ABA signaling network and are controlled by ABRE-binding transcription factors (ABFs) through the ABRE. Consistent with this hypothesis, available ChIP-seq data confirmed that these sORFs are bound by ABF1 in response to ABA. Furthermore, we found that the induction levels of these sORFs by ABA are significantly reduced in the abf234 triple mutant compared to the wild type. To determine the function of these sORFs, we generated CRISPR-Cas9 mutants and overexpression lines for these sORFs. Promising results have been observed in one of the sORFs; its Cas9 mutants are resistant to ABA, while the overexpression lines are sensitive to ABA compared to the wild type. Taken together, our work demonstrates that these sORFs are part of the ABA regulatory network and involved in ABA responses. As some of the sORFs are evolutionarily conserved, ultimately we hope to apply these findings in Arabidopsis to improve crop adaptation to environmental stresses.

Presenting author: Ella So-Eun Kim, Michigan State University, ellakim@msu.edu

Abstract# T4: *Session 1 Genomic networks* (Sat, Apr 22, 9:20-9:35)

Deciphering transcriptional regulators regulating photosynthesis in response to nitrogen and light availability in Arabidopsis thaliana

De Silva, Kithmee, Department of Plant Biology, University of Illinois Urbana-Champaign, Urbana, IL
Brooks, Matthew, Global Change and Photosynthesis Research Unit, USDA-ARS, Urbana IL

Nitrogen (N) and light (L) are environmental cues with known effects on plant metabolism, physiology, and development, and both are particularly linked to photosynthesis. While the individual effects of these signals are well understood, less is known about how N and L signals are integrated. This study aims at identifying potential transcription factors (TFs) regulating photosynthesis in response to N and L availability using gene regulatory network analysis. Arabidopsis was grown in a matrix of N and L treatments (N-by-L) and the effect on physiological traits (photosynthetic efficiency, non-photochemical quenching, biomass) and gene expression were measured. The expression profiles of 6,386 N- and L-responsive genes were used to construct a gene regulatory network (GRN). The N-by-L GRN was refined using experimental data to retain high-confidence gene interactions. Gene-to-phenotype relationships were determined using machine learning and correlation of gene expression and physiological traits. Subnetworks were isolated for each trait consisting of the most highly influential/correlated genes, and TFs that were highly connected to these genes were identified. From this analysis, 117 putative TFs acting as hubs, including TFs known to regulate N and L responses were selected for downstream screening. Characterization of mutant phenotypes is underway to validate the roles of candidate TFs in integrating plant responses to N-by-L signals. By uncovering potential TFs involved in the coordination of photosynthesis in response to N and L availability, this study could reveal potential strategies for enhancing photosynthetic activity and nutrient-use efficiency in plants.

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Abstract# T5: *Session 1 Genomic networks* (Sat, Apr 22, 9:35-9:50)

Identification of soybean small secreted peptides involved in the regulation of symbiosis

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As one of the main sources of vegetable oil and animal protein feed, soybean is the most important and widely produced legume worldwide. Its ability to establish a symbiotic relationship with nitrogen-fixing rhizobia reduces the need for expensive and environmentally damaging nitrogen fertilizer in agriculture. However, many molecular mechanisms involved in the soybean-rhizobia interaction remain obscure. Advances in functional genomics allow for the investigation of novel factors that affect root nodule symbiosis, such as small secreted peptides (SSPs). SSPs are short chains of amino acids secreted into the apoplast, where they act in intercellular communication and play various roles in biological processes, such as symbiotic interactions. Using TRAP-seq and in silico predictions of SSP genes, we identified hundreds of SSP genes differentially expressed in the phloem and the xylem in response to rhizobial inoculation 3 and 21 days after inoculation (DAI). Clear temporal and spatial expression patterns contribute to the modulation of the soybean-rhizobium symbiosis by SSPs, as evidenced by the clear separation between DEGs in each time point and tissue. Notably, our findings suggest that vasculature-specific TRAP-seq can aid in the identification of mobile SSPs, as gene expression of peptides previously detected in the xylem sap was induced by rhizobia in the vasculature. Rhizobia-induced SSPs are being functionally characterized by overexpression, exogenous peptide application, and gene silencing. Determining the temporal and spatial expression of these genes is instrumental in identifying new functions for small secreted peptides in soybean. Understanding how soybean and rhizobia interact at the molecular level can help us improve agricultural practices sustainably.

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Abstract# T6: *Session 1 Genomic networks* (Sat, Apr 22, 9:50-10:05)

Glycome profiling of cotton fiber cell wall polysaccharides to determine the molecular factors contributing to fiber quality

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Cotton is the largest source of renewable textiles, the value of cotton relies on its superior fiber qualities of length and twist. Each fiber develops from a cell that emerges from the surface of the seed coat and rapidly undergoes changes into an elongated twisted matured fiber. The matured cotton fiber is mostly cell wall consisting of cellulose and traces of pectin/hemicellulose polysaccharides. In order to understand the impact of cell wall composition on cotton fiber quality, multi-institutional research is being carried out. The goal is to discover the relationship between the dynamics of mRNA (transcriptome), proteins-protein complexes (proteome), and cell wall composition (glycome). Glycome profiling of the cotton fiber cell wall polysaccharide components is being carried out at ISU. Glycome profiling allows compositional changes of pectin/hemicellulose polysaccharides that occur during cotton fiber development to be analyzed. It is an ELISA-based method that employs monoclonal antibodies that are specific to specific structural epitopes of pectin and hemicellulose polysaccharides. The Gossypium hirsutum cotton bolls are harvested every day starting from 6 days post anthesis (dpa) up to 25 dpa, when fiber cell walls go through the most significant modifications. From the cotton fiber, cell-wall material and the pectins/hemicelluloses are extracted. The data obtained showed that the pectin/hemicellulose polysaccharides content gradually decreases and the cellulose content increases as the cotton fiber grows and matures. Glycome profiling of pectin and hemicellulose extracts with 71 different antibodies revealed significant changes in specific polysaccharide epitopes suggesting that specific dynamic polysaccharide compositional rearrangements occur during fiber development. Currently, we analyze glycome data together with the transcriptomic and proteomic data to reveal the cellular factors that play a crucial role in cotton fiber development and its quality.

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Abstract# F2: *Session 2 Signaling/Development (Featured speaker)* (Sat, Apr 22, 11:00-11:30)

Uncovering the genetic and metabolic bases of seed amino acid composition using a multi-omics integration approach

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Most crop seeds are deficient in essential amino acids (EAAs), i.e., those that humans and livestock cannot synthesize and must obtain from their diets. This is a problem because a diet lacking sufficient EAAs contributes to malnutrition and growth deficiencies. Unfortunately, efforts to improve the EAA profile in seed crops have been consistently stymied by the seeds themselves. A seed has an innate ability to "rebalance" its amino acid levels and composition back to a "normal" state in response to alterations (natural or engineered) to its protein composition. This proteome rebalancing mechanism, which is highly conserved across plant species, is responsible for the tight regulation of amino acids in crop seeds. While this mechanism is likely a useful coping strategy for seeds, it thwarts efforts to enhance the nutritional value of seed crops via breeding or engineering. Despite its ubiquity and its negative impact on such biofortification efforts, we know little about proteome rebalancing at the molecular level. To shed light on the genetic and metabolic bases of this phenomenon, we performed proteomic and metabolomic analyses on kernel developmental time series of the opaque-2 maize mutant. The kernels of this mutants are rebalanced despite the large reduction of its main seed storage protein, the zeins. To further identify high-priority candidate genes, we also integrated TWAS that were performed on seed bound amino acids. These analyses led to the identification of several candidate genes and uncovered the potential role of ribosomal proteins in determining and/or maintaining both amino acid levels and composition in seeds. Our findings provide new insights on the molecular mechanism of proteomic rebalancing and highlight potential new strategies to enhance our protein and amino acid biofortification efforts.

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Abstract# T7: *Session 2 Signaling/Development* (Sat, Apr 22, 11:30-11:45)

An IRE1-proteasome system signaling cohort controls cell fate determination in unresolved proteotoxic stress of the plant ER

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Excessive accumulation of misfolded proteins in the endoplasmic reticulum (ER) causes ER stress, which is an underlying cause of major crop losses and devastating human conditions. ER proteostasis surveillance is mediated by the conserved master regulator of the unfolded protein response (UPR), Inositol Requiring Enzyme 1 (IRE1), which determines cell fate by controlling pro-life and pro-death outcomes through yet-largely unknown mechanisms. Here, we report that Arabidopsis IRE1 determines cell fate in ER stress by balancing the ubiquitin-proteasome system (UPS) and UPR through the plant-unique E3 ligase, PHOSPHATASE TYPE 2CA (PP2CA)-INTERACTING RING FINGER PROTEIN 1 (PIR1). Indeed, PIR1 loss leads to the suppression of pro-death UPS and the lethal phenotype of an IRE1 loss-of-function mutant in unresolved ER stress in addition to activating pro-survival UPR. Specifically, in ER stress, PIR1 loss stabilizes ABI5, a basic leucine zipper transcription factor (bZIP TF), which directly activates the expression of the critical UPR regulator gene, bZIP60, triggering transcriptional cascades enhancing pro-survival UPR. Collectively, our results identify new cell fate effectors in plant ER stress by showing that IRE1's coordination of cell death and survival hinges upon PIR1, a key pro-death component of the UPS, which controls ABI5, a pro-survival transcriptional activator of bZIP60.

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Abstract# T8: *Session 2 Signaling/Development* (Sat, Apr 22, 11:45-12:00)

The Class VIII myosin ATM1 is required for root apical meristem function

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Myosins are evolutionarily conserved motor proteins that interact with actin filaments to regulate organelle transport, cytoplasmic streaming and cell growth. Plant-specific Class XI myosin proteins direct cell division and root organogenesis. However, the roles of plant-specific Class VIII myosin proteins in plant growth and development are less understood. Here, we investigated the function of an auxin-regulated Class VIII myosin, Arabidopsis thaliana Myosin 1 (ATM1), using genetics, transcriptomics, and live cell microscopy. ATM1 is expressed in the primary root, adventitious roots and throughout lateral root development. ATM1 is a plasma membrane localized protein that is enriched in actively dividing cells in the root apical meristem (RAM). Loss of ATM1 function results in impaired primary root growth due to decreased RAM size and reduced cell proliferation in a sugar-dependent manner. In ATM1 loss-of-function roots, columella reporter gene expression is diminished, and fewer columella stem cell divisions occur. In addition, atm1-1 roots displayed reduced auxin responses and auxin marker gene expression. Complementation of atm1-1 with a tagged ATM1 driven under the native ATM1 promoter restored root growth and cell cycle progression in the root meristem. Collectively, these results provide novel evidence that ATM1 functions to influence cell proliferation and columella differentiation in primary roots in response to auxin and sugar cues.

Presenting author: Damilola Olatunji, Iowa State University, dolatunj@iastate.edu

Abstract# T9: *Session 2 Signaling/Development* (Sat, Apr 22, 12:00-12:15)

Roles of auxin transporter PILS6 in maize growth and development

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The phytohormone auxin is essential for regulating plant growth and development. Auxin accumulation in meristems is required to maintain stem cell populations and auxin transport can facilitate cellular differentiation during organogenesis. Auxin transporters are required for maize shoot development but those that underpin maize root development are not known. Within the primary root, free indole-3-acetic acid (auxin) levels are asymmetrically distributed, suggesting that this pattern is established by regulated transport and/or biosynthesis. Using reverse genetics, we have identified two putative candidates in the PIN-LIKES (PILS) auxin efflux carrier family that are required for proper auxin transport in vivo. Loss of function transposon alleles of PILS6 displays altered auxin response in seedlings and impacts multiple aspects of maize development, including shoot height and crown root architecture. Transient expression of fluorescently tagged PILS6 protein shows it is localized to the endoplasmic reticulum. Expression of PILS6 in yeast demonstrates that it is a bona fide transporter of auxin. Loss of PILS6 leads to extensive remodeling of the proteome and phosphoproteome compared to W22. A co-expression network was reconstructed using these expression data to identify potential protein partners that may act in concert with PILS6. Based on these findings I will present a working model for PILS6 in regulating root and shoot formation, which may inform strategies to generate desirable architecture traits.

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Abstract# T10: *Session 2 Signaling/Development* (Sat, Apr 22, 12:15-12:30)

bds1 and bds2 function redundantly to regulate inflorescence and shoot architecture in maize via brassinosteroid biosynthesis

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Shoot architecture is a key determinant of grain yield in maize. Brassinosteroids (BRs) are one plant regulator affecting multiple plant architecture traits. However, the genetic mechanisms by which BRs regulate plant architecture traits in maize remain poorly understood. We recently generated and characterized a recessive, EMS-induced maize mutant, brassinosteroid deficient semi-dwarf mutant1 (bds1). Mutants have a semi dwarf stature due to compressed internodes and are partially rescued by brassinolide. Mutants also have short leaf sheaths and twisted leaf blades, display feminized tassels in the Mo17 background, and reduced tassel branch numbers in B73. Using map-based cloning and whole-genome sequencing, we localized bds1 to a small genomic region containing a point-nonsense mutation in a gene involved in BR biosynthesis. Allelism tests confirmed that bds1 encodes an enzyme likely involved in BR biosynthesis. We identified that bds1 has a close homolog that we named bds2 and generated several mutant alleles by Ds remobilization. Contrary to bds1 mutants, the bds2 single mutants are indistinguishable from wild-type plants, while the bds1-R;bds2-Ds double mutants are severely dwarfed with other defects similar to those observed with BR-deficient mutants nana1 and nana2. To understand the genetic interaction between BRs and Jasmonic Acid (JA), generated double-mutants between bds1-R with tassel-seed 1 (ts1) mutant. We observed synergistic interaction between bds1-R and ts2, the bds1-R;ts2 double mutants have dramatically increased tassel feminization and reduced plant height and tassel branches. Suggesting that BR and JA biosynthesis are required for sex determination and plant architecture regulation. Metabolite profiling analysis experiments are ongoing to confirm and characterize how the bds1 and bds2 mutants disrupt brassinosteroid biosynthesis. Based on these results, we propose that bds1 and bds2 cooperatively regulate shoot architecture and BR biosynthesis.

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Abstract# T11: *Session 2 Signaling/Development* (Sat, Apr 22, 12:30-12:45)

Mdr1 demethylase and the intersection between the epigenome, genomic imprinting, and transposable elements in maize endosperm

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In 1970 Jerry Kermicle published the first evidence for a phenomenon now known as genomic imprinting. The first imprinted gene that was discovered was R1 due to a parent-of-origin dependent phenotype in the endosperm. Since then, theories about the evolutionary underpinnings, and the search for the molecular mechanisms causing imprinting have intrigued scientists. Here we present new findings that provide insights into the role of maternal de-repression of R1(Mdr1) in maize endosperm. In 2022, Mdr1 was mapped and found to be homologous to demeter in arabidopsis. In WT plants, Mdr1 demethylates the maternal allele of R1, setting up maternal expression in the endosperm. While in the mutant, R1 remains methylated and loses expression in the endosperm. To test the genome-wide effects of mdr1 on expression, we performed RNA-seq on mdr1 mutant and wild-type endosperm at 14 days after pollination. This revealed 97 genes and 89 transposable elements that are differentially expressed in the mutant. All but one gene and three TEs are down-regulated in the mutant, as was previously seen with R1. These down-regulated genes have many shared associations with other genomic datasets, including an enrichment for overlap with differentially methylated regions (DMRs) between mutant and wild type, overlap with a helitron family with several members which are differentially expressed in the mutant, and genes that are maternally expressed (imprinted) in the endosperm. To further explore the role mdr1 plays in the endosperm epigenome we assessed the histone landscape using CUT&Tag. The H3K56ac CUT&Tag data show that a fraction of the mdr1 DMRs acquire H3K56ac in endosperm, which is dependent on Mdr1. Together, these data suggest MDR1-dependent demethylation contributes to the distinct transcriptomic environment in endosperm and to epigenome changes associated with maternally expressed genes and transposable elements.

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Abstract# T12: *Session 2 Signaling/Development* (Sat, Apr 22, 12:45-1:00)

Divergence of nuclear localization mechanism in HD-Zip IV family

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The homeodomain leucine-zipper (HD-Zip IV) transcription factor GLABRA2 (GL2) in Arabidopsis plays a vital role in the differentiation of trichomes, as well as in the development of the epidermis of the root and seed. GL2 contains a putative monopartite nuclear localization sequence (NLS), partially overlapping with its homeodomain. The current study provides evidence that deletion or substitution of basic residues (KRKRK) in the NLS affects nuclear localization and results in a gl2 null mutant phenotype. Fusing the predicted 13 amino acid NLS (GTNKRKRKKYHRH) to the enhanced yellow fluorescent protein (EYFP) is sufficient to localize it to the nucleus in roots. The functional NLS is conserved in a distinct subset of HD-Zip IV members that includes PROTODERMAL FACTOR2 (PDF2). GL2 immunoisolation from plant tissues in conjunction with mass spectrometry-based proteomics revealed several importin alpha isoforms as potential interactors. NLS structural prediction and molecular docking studies with importin alpha unveiled significant interacting residues, including those targeted for alanine substitution. Confocal imaging and electrophoretic mobility shift experiments with PDF2 indicate that DNA binding and nuclear localization are separable functions. Split-ubiquitin-based cytosolic yeast two-hybrid assays demonstrated interaction between GL2 and five importin alpha isoforms from Arabidopsis. The interactions were further validated through in vitro co-immunoprecipitation. Importin alpha triple mutants (imp-α1,2,3) showed defects in EYFP:GL2 nuclear localization in trichomes but not in roots, in line with tissue-specific functions of importin alpha isoforms in Arabidopsis. Taken together, our results provide mechanistic evidence for importin-alpha dependent nuclear localization of HD-Zip IV transcription factors in plants.

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Abstract# T13: *Session 3 Methods/Advances* (Sat, Apr 22, 3:00-3:15)

Efficient protein tagging and cis-regulatory element engineering via precise and directional targeted insertion in plants.

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Efficient and precise targeted insertion holds great promise but remains challenging in plant genome editing. An efficient NHEJ-mediated targeted insertion method was recently developed by combining CRISPR-Cas9 with phosphorothioate modified double-stranded oligodeoxynucleotides (dsODNs). Yet this approach often led to imprecise insertions with no control over the insertion directions. Here we sought to improve the precision and direction control of dsODN-based targeted insertion. First, our study revealed that phosphorothioate modification at the 3´ ends of dsODNs is necessary for efficient targeted insertion through classic non-homologous end joining. Second, we demonstrated that CRISPR-Cas9 frequently induces staggered cleavages with 1-nucleotide 5´ overhangs at the targeted sites. This Cas9-induced overhang structure can be harnessed for a directional target insertion approach with improved precision by using dsODNs with 1-nt 5´ complementary overhangs. Lastly, we applied this method to endogenous gene tagging in Setaria viridis, and cis-regulatory element engineering for disease resistance in rice. Two distinct TAL effector binding elements were inserted into the promoter region of a dysfunctional rice bacterial blight resistance gene at up to 24.6% efficiency. The resulting rice lines with heritable insertions exhibited strong resistance to the infection of Xanthomonas oryzae pv. oryzae pathogen in an inducible and strain-specific manner.

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Abstract# T14: *Session 3 Methods/Advances* (Sat, Apr 22, 3:15-3:30)

Developing an in vitro GT array (i-GTray) platform for high-throughput enzyme activity testing of glycosyltransferases

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Glycosyltransferases (GTs) are responsible for the synthesis of all carbohydrate on earth, including the polysaccharide of the plant cell wall. Advances in bioinformatics have identified a large number of GT genes associated with the plant cell wall. However, the biochemical characterization of these GTs is still lagging behind due to several technical challenges. The most direct way to determine the biochemical function of a protein is to test their enzyme activity in vitro using isolated/purified protein and appropriate donors/acceptors. However, this approach has many disadvantages: i) labor-intensive; ii) undesired products resulting from background enzyme activity; and iii) cannot be adapted to large-scale donor/acceptor specificity screening of hundreds of GTs simultaneously. Therefore, there is a need for genomic tools suitable for the large-scale biochemical characterization of GTs in vitro. To address this issue, we have developed an in vitro GT-array (i-GTray) platform for high-throughput in vitro testing of activity of GTs using cell-free coupled in vitro transcription/translation (IVTT) expression to produce tagged proteins from plasmid DNA directly on a 96-well plate. The enzyme activity product is detected by desalting paper spray-mass spectrometry (DPS-MS). DPS-MS is fast, sensitive, and can detect weak activities often displayed by GTs. As a proof-of-concept, we used i-GTray platform to test rice and rice fucosyltransferases (FUTs) members of the GT37 family (CAZy database). We were able to assign biochemical functions to nine of these FUTs. Our platform provides an excellent functional genomics tool to characterize plant GTs.

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Abstract# T15: *Session 3 Methods/Advances* (Sat, Apr 22, 3:30-3:45)

High-resolution imaging: a system for undergraduate research studying Arabidopsis development.

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Undergraduate research experiences provide unique opportunities for students to expand on their education and skill set. For primarily undergraduate institutions (PUIs), these opportunities are sometimes balanced by limited infrastructure or funding, and require creative approaches. Furthermore, it's important to capitalize on scheduling time around undergraduate coursework and building a research program where projects are easily transitioned as students matriculate. The work described here used high resolution imaging as a way to introduce undergraduates to research studies in Arabidopsis development. An area of specific focus has been seedling responses to light and temperature. While these processes have been well-characterized through endpoint analyses, studies of dynamic changes in development in Arabidopsis seedlings have traditionally been challenging based on the small nature of the seedling itself. Recently, the application of high-resolution time-lapse imaging and automated computational analysis has greatly facilitated these studies. These imaging platforms are often modular in design and can easily be adapted to the study of a wide range of phenotypes. Using this approach, undergraduates at UMN Morris have explored the effects of light and temperature (alone or in combination) on seedling development, as well as the genetic contribution to changes in growth rate during photomorphogenesis. The limits to this approach revolve around environmental consistency and human error. It remains challenging to capture dynamic changes through manual measurements in programs such as ImageJ, highlighting the importance of automated computational analysis for some studies. Even without automated analysis, the imaging platform described here represents a valuable tool for studies of plant development in real time, and is easily accessible to undergraduate research.

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Abstract# T16: *Session 3 Methods/Advances* (Sat, Apr 22, 3:45-4:00)

Plasmodesmata-located proteins regulate the plasmodesmal function at specific cell interfaces

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Plasmodesmata (PD) are membrane-lined channels connecting adjoining plant cells. PD control symplasmic intercellular communication by allowing molecules to move between cells. Because PD function as conduits, the regulation of PD aperture is considered one of the major mechanisms in modulating the plasmodesmal function. It has been well established that the plant polysaccharide callose (ß-1,3-glucan) is deposited at PD, regulating the PD aperture. Callose biosynthesis is catalyzed by callose synthases (CalSs) using uridine diphosphate glucose (UDPG) as substrates, which can be produced by sucrose synthases (SUSs). PD-located proteins (PDLPs) are other important regulators in modulating callose deposition at PD through unknown mechanisms. This study discovered that PDLP5 and PDLP6 are expressed in and function at different cell types. Overexpression of the PDLPs results in over-accumulation of callose at PD at different cell interfaces and hyperaccumulation of starch in different cell types in mature leaves. Using a proximity labeling approach, we identified several putative functional partners of the PDLPs, including SUS6. We further demonstrated that SUS6 physically and genetically interacts with PDLP6. In addition, CalS7 interacts with both PDLP6 and SUS6 and is critical for PDLP6's function. As SUS6 and CalS7 are expressed specifically in sieve elements and PDLP6 is also specifically detected in phloem, we propose that PDLP6-SUS6-CalS7 forms callose synthase complex in vasculature, likely sieve elements, to regulate the plasmodesmal function. Our studies begin to uncover cell type-specific callose synthase complexes, which govern the functions of PD at specific cell interfaces.

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Abstract# T17: *Session 3 Methods/Advances* (Sat, Apr 22, 4:00-4:15)

Thylakoid-Inner Envelope Membrane Contact Sites Facilitate Photosynthetic Membrane Synthesis

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The synthesis of photosynthetic membrane lipids does not occur in the thylakoid itself. Therefore, all the lipids that make up the photosynthetic membrane must be transported from where they are synthesized in the chloroplast envelope membranes to the thylakoid. Membrane contact sites, which are known to facilitate lipid transport between membranes in other systems, have been detected between the thylakoid and inner envelope. However, the components and functions of these contact sites are unknown. Our project combines biochemical and microscopic approaches to identify contact site proteins and their functions. Using confocal microscopy, we identified seven chloroplast-localized putative contact site proteins. Currently, we are using transmission electron microscopy to investigate how loss-of-function mutants of our candidate proteins affects chloroplast ultrastructure. So far, we found that loss of the Arabidopsis homolog to Tvp38 Family Protein (TVPFP) results in increased thylakoid-inner envelope contact site length, while loss of the Arabidopsis homolog to Annexin 2 (ANX2) results in fewer stromal vesicles. These early results excite us as we continue exploring the roles of other proteins identified at thylakoid-inner envelope contact sites. The characterization of these contact sites will strengthen our understanding of thylakoid membrane synthesis and inform further research into photosynthetic membrane remodeling and repair.

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Abstract# T18: *Session 3 Methods/Advances* (Sat, Apr 22, 4:15-4:30)

Uncovering the genetic controls of the Arabidopsis thaliana root growth zone and its response to gravity

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Root growth is a key process for water and nutrient acquisition that drives plant. Roots grow when cells produced by the meristem transiently elongate, reaching peak growth rates of 60% their length per hour before abruptly slowing at the end of the elongation zone. We developed a high-throughput method for measuring this dynamic growth in Arabidopsis roots. Our goal was to identify the genetic regions responsible for the variation in the growth rates and determine their relationship to root gravitropism traits. We used a kinematic analysis framework that enables us to measure root growth traits, such as the overall growth rate, the length of the growth zone, the maximum relative elemental growth rate (REGR), and the axial position of the maximum REGR. We measured 1,575 roots representing 162 recombinant inbred lines (RILs) derived from a Cvi x Ler cross and mapped 10 significant quantitative trait loci (QTL) for these 4 kinematic traits. In three instances, the same locus was responsible for two or more growth traits. We then explored the relationship between these growth traits and root gravitropic phenotypes, as gravitropism is driven by asymmetrical cell expansion within the growth zone and forces the root to bend. When comparing the QTLs of kinematic traits to those of tip angle QTLs from the same RIL population, there were no significant overlaps. Additionally, the principal component of the tip angle data does not correlate with any one of the kinematic traits. We then extracted the max swing rate from tip angle curves to calculate the point of greatest differential between the top and bottom sides of the root during gravitropism. This also did not correlate with any of the kinematic traits. Despite gravitropism resulting from asymmetrical cell elongation within the growth zone, the kinematic traits do not determine a root's gravitropic response.

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Abstract# KN1: *Session Keynote speaker* (Sat, Apr 22, 4:30-5:30)

Post-translational regulation of autophagy in Arabidopsis

Bassham, Diane, Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA

Autophagy is a degradation pathway in which cellular components are transported to the vacuole to recycle nutrients or to clear damaged molecules and organelles. Autophagy therefore contributes to plant survival and growth during adverse environmental conditions. The process of autophagy begins with the formation of a double-membrane vesicle called an autophagosome, which encloses the cargo for degradation. The autophagosome fuses with the vacuole, followed by degradation of the cargo by vacuolar hydrolases. The resulting breakdown products are released back into the cytosol to maintain nutrient and energy homeostasis. Autophagy is active at a low level under normal conditions and is upregulated by many different environmental stresses. We have identified a number of factors that post-translationally regulate proteins involved in autophagy, and are analyzing their function in controlling stress tolerance. I will discuss how an interplay between a protein kinase cascade and the phytohormone brassinosteroid determines the tradeoff between plant growth and autophagy, and how persulfidation of a core autophagy component controls autophagy in response to stress.

Presenting author: Diane Bassham, , bassham@iastate.edu

Abstract# F3: *Session 4 Metabolism (Featured speaker)* (Sun, Apr 23, 8:00-8:30)

Turning up the volume on intercellular communication

Sharon Kessler, Sienna Ogawa, Jing Yuan, and Yan Ju;

Purdue University

During pollination, intercellular communication between the pollen tube and the synergid cells of the female gametophyte leads to subcellular events in both cell types culminating in the rupture of the tip-growing pollen tube and release of the sperm cells to achieve double fertilization. Pollen tube arrival at the synergids triggers FERONIA receptor kinase signaling and leads to the trafficking of the NORTIA (NTA) MLO protein from the Golgi to a membrane-rich region of the synergids known as the filiform apparatus. At the filiform apparatus, NTA acts as a calcium channel to increase the amplitude of calcium oscillations in the synergids in order to ensure proper communication with the arriving pollen tube. Recent progress in understanding the role of NTA's Golgi retention and selective protein trafficking in the synergids during pollen tube reception will be presented.

Presenting author: Sharon Kessler, Purdue University, kessles@purdue.edu

Abstract# T19: *Session 4 Metabolism* (Sun, Apr 23, 8:30-8:45)

Duplication and co-option of fatty acid biosynthesis potentiates plant chemical diversity

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Torne, Tanmayee, Division of Biochemistry, Interdisciplinary Plant Group, University of Missouri, Columbia, MO 65211, USA
Schenck, Craig, Division of Biochemistry, Interdisciplinary Plant Group, University of Missouri, Columbia, MO 65211, USA

Plants make structurally diverse specialized metabolites that mediate plant-environment interactions as well as serving industrial and medicinal roles. Specialized metabolic pathways also enable understanding of pathway evolution because of their lineage-restriction and rapidly evolving characteristics. Acylsugars are trichome-synthesized specialized metabolites produced across the Solanaceae family and consist of a sugar core decorated with acyl chains. Acyl chain length is a major source of acylsugar structural variation and biological activity, however the biochemical mechanism of acylsugar acyl chain elongation remains unknown. Isotopic labeling studies suggest that acyl chains are elongated by a mechanism analogous to fatty acid biosynthesis. To identify pathway genes, we took a comparative genomics approach, focusing on the first step in the pathway catalyzed by beta-ketoacyl-ACP synthase (KAS). Phylogenetic and transcriptomics analyses in tomato and other Solanaceae species showed a Solanaceae-specific KAS gene duplication with one copy being highly expressed in the trichomes. CRISPR/Cas9 was used to knockout trichome-enriched KAS in tomato and test their roles in acyl chain elongation. Two independent mutations of trichome enriched KASII were identified: a 4 base pair deletion and a 1 base pair insertion. Metabolite analyses revealed altered acylsugar profiles compared to wild-type, without affecting fatty acid biosynthesis. These data suggest that KAS enzymes were duplicated and co-opted into trichome-localized acylsugar biosynthesis. With this knowledge we hope to engineer other species for production of biologically active acylsugars to enhance crop resilience.

Presenting author: Conor Raymond, University of Missouri-Columbia, cgrnhn@umsystem.edu

Abstract# T20: *Session 4 Metabolism* (Sun, Apr 23, 8:45-9:00)

Peroxisomal heterodimeric enzyme responsible for benzaldehyde biosynthesis in plants

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Dudareva, Natalia, Department of Biochemistry, Purdue University, West Lafayette, IN, U.S.A.

Benzaldehyde, the simplest aromatic aldehyde in nature, is one of the most widespread volatiles that serves as a pollinator attractant, flavor, and antifungal compound. Long known for its smell and taste, benzaldehyde is the most important, after vanillin, contributor to the flavor industry. It is of economic value to the cosmetic and fragrance industries and is used extensively as a precursor to plastic additives and some dyes. However, the enzyme responsible for its formation in plants remains unknown. Using a combination of in vivo stable isotope labeling, classical biochemical, proteomics, and genetic approaches, we show that in petunia benzaldehyde is synthesized via the β-oxidative pathway in peroxisomes by a heterodimeric enzyme consisting of α and β subunits, which belong to the NAD(P)-binding Rossmann-fold superfamily. Neither subunit alone is catalytically active, but they form functional benzaldehyde synthase when mixed at equal molar ratio. Phylogenetic analysis of the less diverse β subunit proteins revealed that PhBSβ homologs exist in many land plants including monocotyledonous, dicotyledonous species, and Physcomitrella patens, most of which have only a single copy of BSβ in their genomes. In contrast, plant genomes contain multiple copies of gene encoding α subunit homologs. Alpha subunits can form functional heterodimers with phylogenetically distant β subunits, but not all β subunits partner with α subunits. Analysis of spatial, developmental, and rhythmic expression of genes encoding α and β subunits revealed that expression of the gene for the α subunit likely plays a key role in regulating benzaldehyde biosynthesis.

Presenting author: Xingqi Huang, Purdue University, huan1377@purdue.edu

Abstract# T21: *Session 4 Metabolism* (Sun, Apr 23, 9:00-9:15)

Dynamic histone acetylation coordinates temporal biosynthesis and emission of floral volatiles in petunia

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Effective biosynthesis of volatile organic compounds (VOCs) depends on the coordinated regulation of complex, interconnected primary and secondary metabolic networks. In petunia, VOCs are produced in the corolla tissue, predominantly using phenylalanine as a precursor; their production and release post-anthesis operates under tight spatiotemporal control. We have found that VOC and phenylpropanoid networks are developmentally regulated during anthesis by histone acetylation (H3K9ac), which is targeted to specific genes throughout primary and secondary metabolic branches and is required for their full transcriptional activation. We have further expanded this investigation to epigenetic mechanisms underlying diurnal regulation of VOC biosynthesis. In Petunia hybrida cv. Mitchell, VOC pathway gene transcription is strongly phased to peak at dusk and facilitate nocturnal emission of volatiles. Through ChIP-Seq of several histone marks, performed using morning and evening corolla tissue, we found that histone acetylation (H3K9ac/K27ac) was specifically associated with evening expression of genes in the VOC pathway as well as important SAM biosynthesis and circadian clock genes. However, the deposition patterns at gene loci as well as differential susceptibility to histone acetyltransferase inhibitor treatments indicate that the clock and metabolic pathways are regulated in parallel by distinct epigenetic machinery. Overall, our research supports an active role for chromatin modification in mediating spatiotemporal control of metabolic activity in petunia flowers at both the developmental and diurnal time scales.

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Abstract# T22: *Session 4 Metabolism* (Sun, Apr 23, 9:15-9:30)

Improving pennycress glucosinolate, seed size, and seed oil domestication traits

Liza Gautam (1), Brice Jarvis (1), Dalton Williams (1), Mary Phippen (2), Shengjun Liu (3), Marcus Griffiths (4), Chris Topp (4),Winthrop Phippen (2), and John Sedbrook (1);

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Pennycress (Thlaspi arvense L.; Field Pennycress) holds considerable potential for producing "climate-smart commodities" including low carbon-intensity biofuels and animal feed while sequestering carbon and nutrients in farm soils. For pennycress to reach its full potential as an oilseed-producing winter cash cover crop grown on hundreds of millions of acres throughout the world, domestication traits must be improved including reduced seed glucosinolate content, larger seed size, and higher seed oil content. Glucosinolates are secondary metabolites found in Brassica species including pennycress which have pungent odors and deter herbivory by producing toxic compounds upon tissue damage. Reducing seed glucosinolate content in pennycress has been particularly challenging given the high levels this plant produces, hence its nickname "stinkweed". Using CRISPR-Cas9 mutagenesis, we generated and interrogated numerous mutations and mutant combinations, succeeding in reducing seed glucosinolate levels to near the regulatory limit of 30 micromol/gram without negatively impacting plant growth and seed yields. To improve pennycress seed size and oil content, we employed CRISPR-Cas9 genome editing to target mutations in three genes (DAI, DAR1, and UPL3) shown in other species to negatively regulate seed size by targeting cell proliferation proteins for degradation. Surprisingly, we found that combinatorial knockout of DA1 and DARI substantially increased seed size and seed oil content whereas UPL3 knockout increased seed size but minimally effected seed oil content. Taken together, our work has identified and validated gene targets and domestication trait mutations which are now being introduced into commercial pennycress varieties.

Presenting author: Liza Gautam, Illinois State University, bgautam@ilstu.edu

Abstract# T23: *Session 4 Metabolism* (Sun, Apr 23, 9:30-9:45)

Two evolutionarily duplicated domains individually and post-transcriptionally control SWEET expression for phloem transport..

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The precise regulation of gene expression in specific cell types is essential for the proper functioning of multicellular organisms. In many crop plants, including maize and Arabidopsis, SWEET sugar transporters play a critical role in mediating the efflux of photoassimilates from specific cells in leaves to non-photosynthetic sink tissues via a two-step apoplastic phloem-loading strategy. In Maize as C4 plant, ZmSWEET13a, ZmSWEET13b and ZmSWEET13c are specifically expressed in abaxial bundle sheath (BS) cells of rank-2 intermediate veins, while in Arabidopsis as a C3 plant, AtSWEET11 and AtSWEET12, only specifically expressed in phloem parenchyma (PP) cells, not the BS, which aligns with the photosynthetic mechanism used for C3 and C4 each. Despite the important roles played by these transporters, little is known about the mechanisms that determine their specific expression patterns in PP and BS cells. Our study aimed to identify these mechanisms, and we discovered two evolutionarily duplicated but independent domains in the AtSWEET11 coding sequence that are critical for PP-specific expression. We used a variety of techniques, including sequence deletions, histochemical β-glucuronidase (GUS) analysis, cross-sectioning, live-cell imaging, and evolutionary analysis, to investigate these domains. Our findings suggest that post-transcriptional regulation, likely involving RNA-binding proteins, is a mechanism conserved among vascular plants but independent of transport substrate specificity. This study not only contributes to our understanding of the mechanisms underlying SWEET transporter expression but also provides a useful experimental tool for studying PP physiology and development.

Presenting author: chen zhang, University of Illinois Urbana-Champaign Plant Biology Department, chenz6@illinois.edu

Abstract# T24: *Session 4 Metabolism* (Sun, Apr 23, 9:45-10:00)

Engineering Plants Resistant to Defensive Non-Proteogenic Amino Acids.

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Schenck, Craig, Division of Biochemistry, Interdisciplinary Plant Group, University of Missouri, Columbia, MO 65211, USA

Plants cannot rapidly evade predators or harsh environmental conditions; thus, they have some of the most fascinating survival mechanisms. Plants interact with their surroundings through chemistry, for example to deter chewing insects or to entice pollinators. Another example is the production of compounds that inhibit the growth (allelopathy) of surrounding organisms. Azetidine-2-carboxylic acid (Aze), a structural analog of the amino acid L-proline (Pro), inhibits the growth of plants and other organisms. Here we use Arabidopsis as a model to explore the biochemical mechanism(s) of Aze resistance. Arabidopsis grown on 10uM Aze-containing media showed reduced root length of around 75% compared to plants grown with no Aze. When higher concentrations of Aze were used, root growth was completely abolished. Interestingly, when media was supplemented with Pro, root growth length was fully restored. The data led us to hypothesize that Aze is misincorporated as Pro during protein synthesis resulting in reduced root growth. To test this hypothesis, we performed untargeted proteomics analysis to detect Aze misincorporation in proteins. At the global proteome level, Aze was incorporated at a rate of 0.2%. To understand the nature of Aze misincorporation, we focused on Pro tRNA synthetases, which add Pro during protein elongation. We isolated homozygous T-DNA mutants in two Arabidopsis Pro tRNA synthetase genes. Surprisingly, when the mutants were grown on Aze-containing media, they showed similar root phenotype compared to wild-type. Currently we are generating double mutants and will test their ability to grow on Aze-containing media. In the future this knowledge can be used to engineer Aze tolerant plants and increase crop resilience.

Presenting author: William Thives Santos, University of Missouri, guithives@gmail.com

Abstract# F4: *Session 5 Stress/Defense (Featured speaker)* (Sun, Apr 23, 10:15-10:45)

Machine learning to advance crop stress resilience

Cory D. Hirsch, Department of Plant Pathology, University of Minnesota, Saint Paul, MN 55108

Adverse conditions often have negative impacts on yield and end uses of crops. Moving into the future, stressful environments will be more unpredictable, quickly changing, and more severe. Researchers will need to adapt experiments and research methods to utilize advances in data collection and analysis to mitigate the negative effects of plant stresses. Here I will present three examples that use machine learning to aid in our understanding of plant responses to different stresses. These examples range from controlled and field-based experiments using different types of sensors for data collection.

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Abstract# T25: *Session 5 Stress/Defense* (Sun, Apr 23, 11:45-12:00)

Understanding the roles of soybean aphid effectors in soybean and soybean aphid interaction

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The production of soybean, an economically important crop, has been threatened by both the direct and the indirect damages caused by soybean aphid, which is a specialist colonizing only soybean plants. Our current soybean aphid management strategies rely heavily on the application of insecticides. However, the emergence of aphid populations with insecticide resistance has made the development of novel aphid control strategies an urgent need. Undoubtedly, understanding the mechanisms underlying host and pest interaction, such as soybean's responses to aphid infestation and the roles of soybean aphid effectors for successful colonization, is key to provide insights for the achievement of this goal, as well as to enrich our knowledge in basic research. In this study, we first examined soybean responses triggered by aphid infestation including the activation of soybean mitogen-activated protein kinases (MAPKs) and the production of reactive oxygen species (ROS), which are two of the most conserved plant immune responses to pathogen attack. Our study found that soybean aphid infestation activated MPK4 and MPK6 in soybean dynamically at different time points post aphid infestation. The colonization by aphids also inhibited chitin-induced ROS production in soybean but failed to suppress flg22-dependent induction. Three soybean aphid effector candidates predicted by our transcriptome analyses-based pipeline were selected and tested for their potential to influence aphid-triggered MAPKs activation and ROS production. C002, one of the putative effectors, was found to be able to inhibit both chitin and flg22-induced ROS production, and another putative effector, MP10 showed the ability to enhance both chitin and flg22-induced ROS production. These findings provide a deeper understanding of how soybean plants respond to aphid infestation and the potential roles of two effectors in soybean and soybean aphid interaction.

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Abstract# T26: *Session 5 Stress/Defense* (Sun Apr 23, 12:00-12:15)

Chasing a molecular chimera; AvrRps4 effector family expanded via bioinformatics and new bacterial assays for lettuce

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The AvrRps4 effector family, present in many economically significant bacterial plant pathogens, is characterized by conserved homology at the N-terminus and variable homology at the C-terminus. Members of this family (HopK1, XopO, and AvrRps4) are secreted into the plant cell where they are processed into N- and C-terminal fragments with individual host-specific functions related to dampening a susceptible host plant's immunity or mediating effector-triggered immunity in resistant plants. Resistant lettuce cultivars are capable of recognizing the N-terminal fragment of processed AvrRps4 (AvrRps4N) with or without the C-terminus (AvrRps4C) when transiently expressed by Agrobacterium tumefaciens but are incapable of recognizing AvrRps4C. The presence of AvrRps4C dampens recognition of AvrRps4N and reduces the subsequent activation of the hypersensitive response. To investigate this relationship as it would occur naturally, we isolated and characterized disease-causing bacterial strains from leaves of wild lettuce and transformed them to deliver AvrRps4 homologues through their type three secretion systems. Simultaneously, we used bioinformatics approaches to identify new members of the family and to investigate the evolutionary relationship between these putative homologues. The combined results have allowed us to improve models for post-processing molecular functions of the N- and C-termini within the plant cell and to deepen knowledge of lettuce-pathogen interactions.

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Abstract# T27: *Session 5 Stress/Defense* (Sun, Apr 23, 11:15-11:30)

Temporal transcriptomic profiling elucidates sorghum defense mechanisms against sugarcane aphids

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The sugarcane aphid (SCA; Melanaphis sacchari) is a key pest on sorghum in the United States that feeds from the phloem tissue, and inflicts physical damage to plants. Previously, it is reported that SCA reproduction was low and high on sorghum SC265 and SC1345 plants, respectively, compared to reference line RTx430. Here, we focused on identifying the defense-related genes that confer resistance to SCA at early and late time points in sorghum plants with varied levels of SCA resistance. We used RNA-sequencing tool to identify the global transcriptomic responses to aphid infestation on RTx430, SC265, and SC1345 plants at early time points and after extended period of SCA feeding. Aphid feeding on the SCA-resistant line upregulated the expression of 3827 and 2076 genes at early and late time points, respectively, which was relatively higher compared to RTx430 and SC1345 plants. Co-expression network analysis revealed that aphid infestation modulates sorghum defenses by regulating genes corresponding to phenylpropanoid pathways, secondary metabolic process, phytohormones, and cell wall-related genes. There were 187 genes that were highly expressed during the early time of aphid infestation in resistant line, including genes encoding leucine-rich repeat proteins, ethylene response factors, pathogenesis-related proteins, and disease resistance-responsive dirigent-like proteins. At late time point, 173 genes had elevated expression levels in resistant line and were involved in sucrose metabolism, callose formation, phospholipases, and proteinase inhibitors. Our results indicate that resistant line is better adapted to activate early defense signaling mechanisms in response to SCA feeding because of the rapid activation of defense mechanisms by regulating genes involved in monolignol biosynthesis pathway, oxidoreductase activity, phytohormones, and cell wall composition. This study offers further insights to better understand sorghum defenses against aphid herbivory.

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Abstract# T28: *Session 5 Stress/Defense* (Sun, Apr 23, 11:30-11:45)

Novel function of the CBL-CIPK Network in Plant Immunity

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Recognition of bacterial pathogens by plasma membrane-localized pattern recognition receptors (PRRs) initiates signaling cascades resulting in pattern triggered immunity (PTI). PTI-initiated plant defense responses include activation of mitogen activated protein kinase (MAPKs) cascades. Our lab identified a plasma membrane (PM) E3 ubiquitin ligase that is rapidly phosphorylated during and required for PTI. We hypothesize this protein may control plasma membrane transporters responsible for extracellular transport of molecules that induce bacterial T3SS. As the phosphorylation site of the E3 ligase is not a direct MAPK target, another kinase must be involved in regulation. Calcineurin B-Like proteins (CBLs) are a class of calcium-dependent signaling molecules, that regulate CBL-Interacting Protein Kinases (CIPKs). Our lab found that a quintuple knockout (cbl-5ko) of CBL proteins eliminating all PM-CIPK activity leads to loss of PTI similar to the knockout of the E3 ligase; and the phosphorylation sites on the E3 ligase are consistent with the upstream kinase being a CIPK. These results indicate one or more CBL proteins and their associated CIPKs may be the missing piece(s) downstream of the MAPK cascade resulting in phosphorylation and activation of the E3 ligase. Recently, we have genetically narrowed the required CBLs to only a subset of CBL proteins required for proper PTI responses. Further characterization of these mutants will be discussed.

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Abstract# T29: *Session 5 Stress/Defense* (Sun, Apr 23, 10:45-11:00)

HPCA1 Is Required For Systemic Reactive Oxygen Species And Calcium Cell-To-Cell Signaling And Plant Acclimation To Stress

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Luan, Sheng, Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA;
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As multicellular organisms, plants constantly balance and coordinate many metabolic, physiological, and molecular responses between different cell types and tissues. This process is essential for plant development, growth, and response to different environmental cues. Because plants lack a nervous system, they transmit different signals over long distances via cell-to-cell signaling. Recent studies revealed that reactive oxygen species (ROS), produced by respiratory burst oxidase homologs (RBOHs) at the apoplast play a key role in cell-to-cell signaling. A state of enhanced ROS production by one cell is thereby sensed by a neighboring cell, causing it to produce ROS, creating a continuous chain of cell-to-cell ROS accumulation termed the 'ROS wave'. This process was found to mediate systemic signals throughout the plant and is required for plant acclimation to different stresses. Although RBOHs were found to produce ROS essential for this process, the identity of the receptor(s) perceiving the apoplastic ROS signal is currently unknow. Here we reveal that the leucine-rich-repeat receptor-like kinase HPCA1 (H2O2-induced Ca2+ increases 1) acts as a central ROS receptor required for the propagation of cell-to-cell ROS signals, systemic signaling in response to different biotic and abiotic stresses, and plant acclimation to stress. We further report that HPCA1 is required for systemic calcium signals, but not systemic membrane depolarization responses, and identify key calcium-dependent signal transduction proteins involved in this process. Our findings reveal that HPCA1 plays a key role in mediating and coordinating systemic cell-to-cell ROS and calcium signals that are required for plant acclimation to stress.

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Abstract# T30: *Session 5 Stress/Defense* (Sun, Apr 23, 11:00-11:15)

Differential regulation of flower and pod transpiration during abiotic stress combination in an annual plant

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Heat waves occurring during droughts, can have a devastating impact on yield, especially if they happen during the flowering and seed set stages of the crop cycle. Global warming and climate change are driving an alarming increase in the frequency and intensity of combined drought and heat stress episodes, critically threatening global food security. Because high temperature is detrimental to reproductive processes, essential for plant yield, we measured the inner temperature, transpiration, stomatal aperture, and transcriptomic response of closed soybean flowers and pods, developing on plants subjected to a combination of drought and heat stress. Here, we report that during a combination of drought and heat stress soybean plants prioritize transpiration through flowers and pods over transpiration through leaves by opening their stomata on these reproductive tissues, while keeping their leaf stomata closed. This acclimation strategy, termed ‘differential transpiration’, lowers flower and pod inner temperatures by about 2-4°C, protecting reproductive processes at the expense of vegetative tissues. We further show that this response is associated with enhanced expression of transcripts involved in abscisic acid degradation, and that preventing it by sealing stomata causes a significant increase in internal pod temperature. Transcriptomic analysis of different soybean tissues during stress combination further suggests that leaf, flowers, and pods, developing on plants subjected to WD+HS, display a unique transcriptomic response compared to leave, or each other. We propose that manipulating stomatal regulation, stomatal size and/or stomatal density of flowers

and pods could serve as a viable strategy to enhance the yield of different crops and mitigate some of the current and future impacts of global warming and climate change on agriculture.

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Poster abstracts

Abstract# P001

Engineering arsenic tolerance by manipulating a sulfate transporter gene in Arabidopsis

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Arsenic (As) toxicity poses significant problem to all living organisms including plants. As can accumulate in the plants to levels that disrupts various physiological and biochemical processes, thus inhibits growth and development, and even plants could die. At the biochemical level, plants modulate a number of pathways that keeps active arsenic levels below the threshold limits either by complexation of metalloids with sulfur-containing compounds such as glutathione (GSH) or by increasing biosynthesis of phytochelatins (PCs) which are polymers of GSH that have metal-binding peptides. In both of the above-mentioned arsenic detoxification strategies, glutathione is the central molecule, which functions not only as a major antioxidant but also in heavy metal detoxification. GSH is derived as result of sulfate assimilation. Several lines of evidence support for a strong connection between sulfate metabolism and arsenic tolerance in several organisms including plants. This led us to hypothesize that enhanced sulfate uptake and metabolism has an important role in ameliorating arsenic toxicity, potentially via sulfate-derived metabolites such as GSH and PCs. To address this possibility, we are overexpressing a sulfate transporter gene in Arabidopsis thaliana and evaluating whether or not the transgenic plants will have greater As tolerance. We are also utilizing a knockout mutant line to assess its phenotypic response under arsenic stress. Understanding the genes and mechanisms of arsenic tolerance will pave the way for engineering arsenic tolerance in plants.

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Abstract# P002

High-efficiency multiplexed prime editing enables new strategies for broad-spectrum resistance to rice blast

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Using genetic resistance against bacterial blight (BB) caused by Xanthomonas oryzae pathovar oryzae (Xoo) is a major objective in rice breeding programs. Prime editing (PE) can potentially create novel germplasm against Xoo. Here we use an improved prime editing system to implement two new strategies for rice BB resistance. Knock-in of TAL effector binding elements (EBE) derived from the BB susceptible gene SWEET14 into the promoter of a dysfunctional executor R gene xa23 at an editing efficiency of 73.5% with biallelic editing rate of 18% in T0 generation enables an inducible TALE-dependent BB resistance. Editing the transcription factor TFIIA gene TFIIAγ5 required for TAL effector-dependent BB susceptibility recapitulates the resistance of xa5 at an editing efficiency of 88.5% with a biallelic editing rate of 30% in T0 generation. The engineered loci provided resistance against multiple Xoo strains in the T1 generation. Whole genome sequencing detected no OsMLH1dn-associated random mutations and no off-target editing demonstrating the high specificity of this PE system. This is the first-ever report to use the PE system to provide resistance against biotic stress and to demonstrate knock-in of >28-nt at high efficiency.

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Abstract# P003

Discovering Transcriptional Regulators of Photosynthesis in Energy Sorghum to Improve Productivity

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Photosynthesis is an important biochemical reaction that supports all the life on earth. While leaf photosynthesis is studied extensively, little is known about the spatial regulation of canopy photosynthesis. Most plants have evolved a mechanism to shift their protein stoichiometry to optimize photosynthesis as measured through high maximum absolute quantum efficiency of CO2 assimilation (Φ_(〖CO〗_2,max)) across the canopy height. Sorghum and a subset of related C4 species are an exception to this phenomenon and undergo a maladaptive loss of photosynthetic efficiency in shaded leaves within a canopy. Previous studies performed in these C4 species have shown that this loss in Φ_(〖CO〗_2,max) in shaded leaves relative to sun-exposed leaves is attributed to light environment and not to the leaf age. This drop in photosynthetic efficiency is predicted to cause a 15-20% loss in productivity based on current yield models. Additionally, severity in the loss of Φ_(〖CO〗_2,max)across a panel of bioenergy sorghum suggests that this trait results from the differential expression of one or more genes. Because transcription factors (TFs) regulate gene expression in response to environmental cues such as changes in light intensities, we intend to reveal the molecular basis of this maladaptive trait in shaded canopy leaves by interrogating TF targets in sorghum. We will then identify key TFs by analyzing variations in gene expression and photosynthetic traits such as Φ_(〖CO〗_2,max) across light conditions and sorghum cultivars and comparing these results to our TF-target gene database. Identifying the causes of photosynthetic inefficiency in shaded canopies and engineering solutions will help in closing the yield gap of this important bioenergy crop and further support our growing demand for energy security.

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Abstract# P004

Viral delivery of recombinases for heritable genetic switches in plants

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Genome engineering technology enables researchers to make precise and efficient manipulations of plant DNA for the purpose of advancing both basic and applied research. However, conventional methods of producing engineered plants often requires regeneration of modified cells in tissue culture, a technically challenging and time-consuming bottleneck. Alternative strategies using viral vectors circumvent tissue culture requirements in plant transformation where non-integrating, exogenous sequences are delivered and move systemically though the vasculature. Previous work from the Voytas Lab utilized tobacco rattle virus (TRV) viral vectors for delivery of gene editing reagents to Nicotiana benthamiana and Arabidopsis thaliana. In these studies, single guided RNAs (sgRNA) targeting phytoene desaturase (PDS) were engineered and expressed from the pea early browning virus (PEBV) subgenomic promoter. When delivered into leaf tissue of Cas9 expressing plants, high frequencies of somatic and heritable edits are observed in the absence of tissue culture. To expand the available tools for engineering plants using TRV, we have created viral vectors expressing a collection of site-specific recombinases (Cre, FLPe, CinH and Integrase 13). Delivery of TRV-recombinase vectors to stable N. benthamiana switch lines activates a RUBY reporter via intramolecular recombination, enabling detection of somatic and heritable recombinase-mediated modifications without special instrumentation. Demonstration of TRV-recombinase functionality in tissue-culture free approaches creates avenues for both basic and applied research where recombinases could be used for further understanding viral movement or implemented in future genome engineering projects.

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Abstract# P005

Establishing a System for High-Throughput Screening of Factors Affecting Meiosis

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While recent advances in plant biology have shed light on meiotic recombination and cycle progression, knowledge regarding the transition between meiosis induction and mitosis in plants is still very limited. Research exploring this issue has involved mostly aberrant gene analysis to see effects on meiosis, but induction research is rare. By producing a tool to screen for meiosis induction at high throughput in A. thaliana, we enable the testing of thousands of suspected meiosis inducers in a controlled and efficient manner. Quantifiable meiosis induction will provide a solid foundation to explore the intricate underpinnings in the development of germ cells. By building such a tool we hope to establish fluorescent markers to successfully differentiate gametes and somatic cells in high-throughput. With this, we aim to inform methods to accelerate crop breeding cycles to increase genetic gain.

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Abstract# P006

Post-secretory synthesis of a natural analog of iron-gall ink in the black nectar of Melianthus spp.

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Floral nectar is a sugar-rich liquid generated by plants to attract pollinators. The critical components of nectar are sugars in varying concentrations and forms, yet many also include a range of phytochemicals that attract pollinators and deter predators1. Colored nectar is a distinctive and uncommon trait of nectar. While scientists have known about colored nectar since at least 1785, it has only lately gained concerted scientific investigation2. Melianthus species produce black nectar that is believed to attract avian pollinators visually; however, the chemical identity and synthesis of the black pigment are unknown2,3. Here, we demonstrate that the black nectar produced during anthesis contains a natural analog of iron-gall ink formed from an ellagic acid-Fe complex and that it likely contributes to the appeal to passerine pollinators4. High concentrations of ellagic acid and iron give the nectar its dark black color, which may be replicated using synthetic solutions containing solely ellagic acid and iron (III). The nectar also includes a peroxidase, which oxidizes gallic acid to generate ellagic acid. In vitro processes, including nectar peroxidase, gallic acid, hydrogen peroxide, and iron (III), completely replicate the black color of the nectar. Furthermore, this black color is conspicuous to avian pollinators, according to visual modeling4. While this study focused on the impact of black nectar as a visual cue to pollinators, it may also serve other non-exclusive functions. As the pH and polyphenol content of nectar vary with the flowering stage, stage-specific filtering of floral visitors may occur5. Comparing Melianthus nectar to dark-colored nectars from Schiedea, Leucosceptrum, and Aloe species may help explain how chemistry and color influence fitness5–7.

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Abstract# P007

A Jaltomata bohsiana nectar protein inhibits the growth of both bacteria and fungi

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Flowering plants attract and reward potential pollinators by producing nectar with high concentrations of sugar. However, both bacteria and fungi can also inhabit these nectar droplets thanks to pollinator vectors. To combat this, the chemistry of nectar in flowering plants has evolved to not only include simple sugars but proteins and metabolites to deter the growth of these pathogens. Many nectar proteins contain multiple cysteine residues to create incredibly stable structures that can withstand the harsh environment outside of the cell. However, many of these small proteins have yet to be characterized fully. Here I show the potent anti-microbial and anti-fungal properties of a cysteine-rich nectar protein JbNec1a, from the Jaltomata bohsiana plant, a cousin to the tomato. Through in vitro assays it was found that JbNec1a possesses anti-microbial function at concentrations as little as 25 µM. JbNec1a was also tested in vitro against common fungal strains found in nectar and has shown an inhibition of fungal growth in concentrations as little as 10 µM. These results indicate that there is an ever expanding amount of defense type proteins found in nectar which leads to a much more complex understanding of the chemistry involved in nectar. The groundwork laid by these experiments will guide future nectar chemistry research into how plants defend their nectars against microbes. An unspoiled nectar is attractive to pollinators, which is often maintained through nectar proteins like JbNec1a.

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Abstract# P008

Cloning and Analyzing of Native and Engineered GLDP1 Promoters

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The Glycine shuttle is a carbon concentrating pathway that is used to increase net CO2 incorporation by capturing, concentrating, and re-assimilating CO2 that was released through photorespiration; this process operates between the mesophyll and bundle-sheath cells. Within plants that undergo C3 photosynthesis both the mesophyll cells and the bundle-sheath cells contain the glycine decarboxylase complex (GDC) allowing the two cells to operate independently from one another as a result do not need to utilize the glycine shuttle pathway. However, in plants that are C3-C4 photosynthesis do not contain an active GDC in the mesophyll cell but is active within the bundle-sheath cells causing the generated glycine to be transported vis the glycine shuttle from the mesophyll cell into the bundle-sheath cell to be converted into serine and shuttled back to the mesophyll cell to change the serine into 3-PG to reenter CBB-cycle and repeat the process, C3-C4 photosynthesis relies on differential gene expression. Our goal is to verify that it confers bundle sheath specificity using both native and engineered GLDP1 promoters.

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Abstract# P009

Investigation of the contribution of vesicle trafficking to the emission of volatile organic compounds in petunia flowers

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Volatile organic compounds (VOCs), produced and released from plants, play essential roles in plant defense, reproduction, and plant-plant interactions. For VOCs to be emitted from their sites of biosynthesis into the atmosphere, they must move across the plasma membrane, the cell wall, and the cuticle. However, little is known about how VOCs traverse cytosol to reach the plasma membrane. As VOCs are lipophilic low-molecular-weight molecules (100-200 Da), they are likely to partition into the hydrophobic environment, such as subcellular membranes, and get to the plasma membrane via vesicle-mediated trafficking. From the RNA-seq datasets generated from Petunia hybrida flowers, which produce high levels of benzenoid and phenylpropanoid volatiles, vesicle trafficking-related genes with expression profiles matching VOC biosynthesis and emission patterns were searched. 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. To examine the role of PhSV2s in VOC emission, the expressions of PhSV2s were downregulated. A decrease in PhSV2s expressions resulted in the reduction of VOC emission. To further identify the subcellular localization of PhSV2s, PhSV2s fused to a green fluorescent protein (GFP) and the known vesicle protein syntaxin-32 (SYP32) from Arabidopsis fused to a red fluorescent protein (RFP) were transiently co-expressed in petunia petals. GFP-PhSV2s were co-localized with RFP-SYP32, indicating that PhSV2s are localized to vesicles. This study suggests that the PhSV2s are involved in the VOC emission via vesicle trafficking.

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Abstract# P010

Predicting protein-protein interactions between glycosyltransferases for plant cell wall polysaccharides synthesis

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Glycosyltransferases (GTs) are carbohydrate-active enzymes responsible for the synthesis of all carbohydrates on Earth. The most abundant carbohydrates on Earth are associated with plant cell walls (CWs). It is estimated that up to 10% of plants genome are involved in the metabolism of plant CWs. GTs catalyze the transfer of sugars from activated nucleotide sugars to a nucleophilic glycosyl acceptor molecule. In most cases, these GTs perform their physiological functions as part of multi-protein complexes, where they form homo- and hetero-meres. Currently, protein-protein interactions (PPIs) between GTs and other proteins is not well studied due to lack of structural information and limited number of isolated/characterized multi-protein complexes. In addition, predicting PPIs between GTs is challenging and no bioinformatics tools are available to facilitate the identification of GT complexes. Thus, the main goal of this project is to develop a bioinformatics tool for PPIs between GTs (called GTome) and the creation of a database to host the findings. Our strategy consists of screening CAZy database to collect publicly available information on GTs for which 3D structures have been resolved. For GTs without 3D structure available, advanced molecular modeling and simulations to generate 3D structures will be applied to these GTs. This collection of structures will be used to map shallow binding clefts that would likely represent deep pockets involved in PPIs. The information will be used to create GTome tool.

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Abstract# P011

Comparative genome analysis of Bradyrhizobium strains with different nitrogen fixing capacities

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The genus Bradyrhizobium comprises slow growing bacteria that can induce nodulation in leguminous plants such as soybean. In contrast to other nodule-forming rhizobia, members of Bradyrhizobium are characterized for containing all symbiosis and nitrogen-fixing related genes inserted into the chromosome instead of plasmids. This feature and the inability to retain broad-host plasmids have made difficult the development of molecular tagging systems for this genus, which in turn has limited the study of the mechanisms mediating their symbiosis with soybean. We previously characterized the symbiotic properties (e.g., nodule numbers, nitrogenase activity, plant biomass and chlorophyll content) of nine Bradyrhizobium strains on soybean and classified them into high, intermediate, and low nitrogen-fixing capacity groups. We then performed whole genome sequencing, assembly, and analysis to evaluate if specific genomic features or genetic elements can be attributed to differences in nitrogen fixing capacity on soybean and to determine specific insertion sites for genetic tagging. Our preliminary analysis identified distinct organization of nodulation and nitrogen fixing genes, which may explain the symbiotic traits. Overall, the comparative genome analysis can be used to identify chromosomal insertion sites or single nucleotide polymorphisms (SNPs) that will help to develop efficient molecular tagging systems for Bradyrhizobium. Results from this work will facilitate genetic tagging of different strains and enable direct competition assays and molecular analysis using the soybean-Bradyrhizobium system at different stages during root colonization and nodulation.

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Abstract# P012

Understanding Rhizobial competition for nodulation using split root assays

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Symbiotic nitrogen fixation contributes to the majority of legume crop nitrogen needs and thus reduces the need for synthetic fertilizers. A bottleneck associated with increasing nitrogen fixation in legume crops is the rhizobial competition for nodule occupancy. Nitrogen-fixing capacity varies among Rhizobium strains and often the inoculant strains must compete with the indigenous strains in soil for nodule occupancy. Thus, legume hosts might be infected by both efficient and inefficient strains, reducing the overall nodule nitrogen supply to the plant. Host plants may employ sanction mechanisms to favor nodules with high-capacity nitrogen-fixing strains, but the timelines or types of sanction mechanisms are not well understood. We evaluated rhizobial competition in soybean using combinations of high, medium, and poor nitrogen-fixing capacity Bradyrhizobium strains. A split root system where each root half was inoculated with a different Bradyrhizobium strain was employed to evaluate host-based selection. Nodule numbers were counted at three-time points to determine if soybean favored colonization by one strain over the other based on their nitrogen fixation capacity. Results from our time course analysis indicated that soybeans might impose sanctions on poor-fixing strain (USDA 126) over high-capacity nitrogen-fixing strain (USDA 110) between 7- and 14-days post-inoculation. We also observed that soybean might promote nodulation by the intermediate capacity nitrogen-fixer (USDA 140) over the poor fixer. Interestingly, no difference in nodule numbers was observed between the high versus intermediate capacity fixers. Soybean may employ host sanctioning mechanisms against poor nitrogen fixers at different levels and stages depending on the strain combinations.

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Abstract# P013

Post-translational Modification Sites in AtTCP8 IDR 2 Influence Localization and Interaction Behavior

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Protein interactions can be affected by changes in structure, which changes behavior and causes separation from other things in the cell by phase. This is often seen in proteins with intrinsically disordered regions (IDRs), or parts of a protein with no specific structure. The plant-specific Arabidopsis transcription factor AtTCP8 is important to cellular signaling and transcriptional activities and potentially determines their response to environmental stressors. Recently the movement of TCP8 into phase-separated locations was seen in response to hormones within the nuclei of Nicotiana benthamiana, as well as interactions with other growth and defense-related transcription factors. TCP8 contains three IDRs which are likely sites of post-translational modifications that determine whether the transcription factor is enhancing growth or defense, as TCP8 cannot do both at once. We suspected that these IDRs contributed to the observed phase change. To explore this, we observed dynamic movement of TCP8 into localized condensates, using a confocal microscope. In our preliminary studies, we performed a truncation analysis that allowed us to identify sites with altered localization patterns, seen through a lack of condensates, after the removal of IDR 2. To narrow down to specific regions, we identified amino acids known to be post-translationally modified and generated site directed mutants in which they were eliminated. This mutant TCP8 protein (27) exhibits altered protein-protein interaction between TCP8 and brassinosteroid regulatory proteins. Our data points toward IDR 2 and associated PTMs as regulatory elements of TCP8 governing TCP8 phase-separation and associated behaviors.

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Abstract# P014

How do drought stress and stem parasitic plant, Cuscuta campestris, affect tomato development?

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The stem parasitic plant, Cuscuta spp., is a worldwide agricultural and ecological pest without true roots and leaves (and stomata). When Cuscuta spp. parasitizes a host, it uses specialized structures called haustoria to obtain water, carbon, and nutrients. Cuscuta spp. parasitism significantly decrease crop growth and yield in major crops such as tomatoes, potatoes, alfalfa, and carrots. In addition, drought is a global issue in agriculture, also reducing crop quality and yield. Previous studies reported that root parasitic plants severely suppress host development under drought stress. In this study, we investigated how a stem parasitic plant (Cuscuta) affects the host (tomato) growth under drought stress. To analyze the host responses, four different treatment groups, ⅰ) well-watered or ⅱ) drought conditions and ⅲ) parasitized or ⅳ) non-parasitized by Cuscuta campestris, were compared. We measured fresh weight of the pot containing soil and plant with or without Cuscuta and recorded pictures of tomato roots. Our study reveals that simultaneous stress of parasitism and drought more significantly affects tomato weight and root development compared to only parasitism or only drought. In addition, parasitized tomato plant soil had a higher field capacity than non-parasitized plants under drought stress. Interestingly, root structure was hindered in parasitized tomatoes, even though Cuscuta is a stem parasitic plant and does not directly interact with host roots. The reduced root growth in parasitized plants may explain the increased field capacity under drought conditions. This correlation between stem parasitism and host root development may be of interest in future studies involving crop root growth and development.

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Abstract# P015

Effects of exogenous antioxidants on photoprotection mechanisms in Arabidopsis thaliana

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Plants need to adapt to frequent changes in light conditions, as excessive light can trigger the generation of harmful reactive oxygen species. Therefore, plants evolved sophisticated antioxidant systems to protect themselves against oxidative stress. Previous studies reported that, when applied exogenously, antioxidants can improve growth and photosynthesis under control and stress conditions in several plant species. Antioxidants can also affect photoprotection dynamics although the effect is not consistent. For instance, the same concentration of melatonin has been reported to increase and decrease non-photochemical quenching (NPQ), the most important photoprotection mechanism in plants. We aim to determine the effects of different antioxidants on NPQ dynamics in Arabidopsis thaliana. We hypothesized that functionally or chemically similar antioxidants will have similar effects on NPQ, and that the magnitude of the effects is time-dependent. Our preliminary results suggest that antioxidants in the same biochemical pathway, such as ascorbic acid and glutathione, have oppositive effects on NPQ dynamics. Also, chemically different antioxidants can affect NPQ in a similar fashion. We have also identified short-term and long-term effects. NPQ is regulated by Photosystem II subunit S (PsbS) and the xanthophyll cycle, so our future plans include understanding the effects of different antioxidants on native antioxidant pools, characterizing their effects on the xanthophyll cycle pigments, and quantifying the expression of PsbS and enzymes involved in the xanthophyll cycle.

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Abstract# P016

Roles of REL2 Mediated Transcriptional Co-repression in Maize Immunity

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Protein acetylation is a major post-translational modification that modulates many cellular processes, including plant immunity and stress responses. Cochlibolus carbonum (Northern Corn Leaf Spot) produces the effector HC-Toxin, a lysine deacetylase inhibitor required for pathogen virulence. RAMOSA1 ENHANCER LOCUS2 (REL2) is a transcriptional corepressor homologous to TOPLESS (TPL) in Arabidopsis. TPL family members are required for a range of biological processes, including development and immunity, and are critical components of hormone responses, including auxin and jasmonate signaling pathways. We identified a lysine acetylation site on REL2 using global acetylome profiling of maize treated with HC-Toxin or C. carbonum. Furthermore, we found that rel2 loss of function mutant plants are susceptible to infection, demonstrating that REL2 is directly related to plant immunity. This work aims to elucidate how hyperacetylation impacts the biological activity of REL2 and REL2's roles in plant-pathogen interactions. Specifically, I will determine REL2-associated gene expression and elucidate how REL2 acetylation state impacts maize immunity. These objectives will be completed via proteomics, high-throughput sequencing, biochemical, and genetic experiments to further gain a detailed molecular understanding of plant immunity and to reconstruct a model for how REL2 transcriptionally regulates plant pathogen response.

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Abstract# P017

Karrikin-like signaling pathway is involved in the perception of volatile terpenoids in petunia

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Recently Petunia hybrida has been shown to utilize natural fumigation of sesquiterpenes for the proper development of reproductive organs. While the enzyme responsible for producing the terpenes has been identified, the receptor and the pathway involved in the perception of the sesquiterpenes are still unknown. Karrikin insensitive 2 receptors are known to bind karrikins, but compelling evidence suggests the primary role is the recognition of a yet unknown endogenous plant ligand. Through genetic and biochemical approaches, Karrikin insensitive 2 intermediate (KAI2ia) has been identified as the potential sesquiterpene receptor. Transgenic KAI2ia plants act as deaf receptors to sesquiterpenes and the KAI2ia-mediated sesquiterpene signaling pathway diverges from the karrikin signaling pathway.

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Abstract# P018

The Protective Role of Isoprene Against Ozone Stress by Abating Reactive Oxygen Species Production

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Previous studies have shown that isoprene can protect plants against ozone (O3) damage. These studies involved the fumigation of leaves with isoprene and O3 simultaneously, which led to the conclusion that isoprene can act as an antioxidant to directly quench O3 and other reactive oxygen species. However, the mechanism by which isoprene protects plant foliage from ozone damage is unclear. Our recent data show that isoprene alters the expression of genes required to alleviate abiotic stress. We hypothesize that the protective role of isoprene against O3 is through isoprene-mediated changes in gene expression with subsequent reductions in ROS production and programmed cell death. Our preliminary data show that priming non-emitting Nicotiana tabacum leaves with isoprene leads to protection against O3 stress in the absence of isoprene. Therefore, isoprene does not need to be simultaneously present with O3 to have a protective effect. We intend to investigate the onset of changes of antioxidant enzyme activity and growth regulator biosynthesis during the isoprene pretreatments and O3 treatments. We will carry out enzyme activity assays to measure superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase in leaf tissue harvested during isoprene pretreatments and O3 treatments. The concentration of jasmonic, salicylic, and abscisic acid will be measured using mass spectrometry. We predict that this data will show us how oxidative stress responsive genes, antioxidant enzymes, and growth regulator biosynthesis are affected during priming with isoprene and provide insights on the mechanisms with which isoprene protects plants from O3 damage.

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Abstract# P019

Linking Hyperspectral Reflectance Data to the Genome of Panicum virgatum

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Genome wide association studies (GWAS) have become a powerful tool in linking phenotypic trait data with alleles in the genome. By using natural variation of single nucleotide polymorphisms (SNPs) within plant species, we can identify genes that are significantly correlated with a trait of interest (biomass, flowering time, etc.). Spectral reflectance data represents a unique form of trait data that has the potential to integrate multiple traditional phenotypic traits. We seek to associate this spectral data directly with the genome of switchgrass (Panicum virgatum) using genome wide association. Doing so will help explain the underlying variation associated with spectral data and partition that variation into genotypic and environmental components. We sampled leaf-level spectral data at three established common gardens in Texas, Missouri, and Michigan with each common garden contained clonally replicated genotypes of switchgrass. Percent reflectance data was then incorporated as trait data into a GWAS pipeline associated with the experimental design. We find that the three populations of switchgrass represented in the experiment can be separated based on their spectra using Partial Least Squares Discriminant Analysis with >70% accuracy. Additionally, we find that spectral regions differ in their SNP associations between populations. For example, the Midwest population shows SNP associations at the red-edge, while the Atlantic population has more associations in the short-wave infrared (SWIR) region. However, even within populations, SNP associations vary greatly between sites, implying strong site effects and genotype by environment interactions within the spectra.

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Abstract# P020

Phytochrome B mediated regulation of ROS production in response to high light stress

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Reactive oxygen species (ROS) and phytochrome B (phyB) both play canonical roles in plant stress acclimation. However, ROS signaling is mediated by respiratory burst oxidase homolog (RBOH) proteins in the plasma membrane and phyB is primarily localized in the nucleus. Using Arabidopsis thaliana, we found that despite this apparent localization issue, phyB and RBOHs function as part of a regulatory module to control apoplastic ROS production, transcript expression in response to stress, and acclimation to light stress. Further, phyB can regulate ROS production during light stress even if restricted to the cytosol. PhyB, RBOHD and RBOHF coregulate thousands of transcripts in response to light stress. We currently continue investigating the interaction and co-localization between phyB, RBOHD and RBOHF using different approaches. Moreover, to unveil the molecular pathway in which phyB regulates ROS signaling and acclimation, we are testing ROS accumulation and acclimation assays with structural mutants of phyB and downstream signaling mutants, such as PHYTOCHROME INTERACTING FACTORS (PIFs) and ELONGATED HYPOCOTYL (HY) genes. Overall, our findings shed light on novel functions of phyB in which together with RBOHs, they orchestrate plants' rapid response to stress that leads to protection from adverse conditions.

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Abstract# P021

Insights into Mechanisms of Chilling Tolerant Photosynthesis in the C4 Grass Miscanthus

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Miscanthus is a genus of C4 grass with economic importance for its high biomass production. Regions occupied by Miscanthus range from subtropics of Southeast Asia to the cold-temperate continental climate of northern China and eastern Russia, making this genus excellent for studying mechanisms of chilling tolerant photosynthesis in C4 grasses. Chilling reduces enzymatic reaction rates limiting the sinks for light energy and leads to the extensive formation of reactive oxygen species and subsequent photodamage. Non-photochemical quenching is the indispensable, rapid protective mechanism from photodamage that depends on speed of de-epoxidation of violaxanthin (V) to antheraxanthin (A) and zeaxanthin (Z) via an enzyme violaxanthin de-epoxidation (VDE) that uses the cofactor ascorbate. Therefore, we studied xanthophyll conversion, VDE expression and dehydroascorbate accumulation in three Miscanthus species with contrasting chilling tolerance. Our data showed that high-chilling-tolerant relatively to low-chilling-tolerant species maintains more quenching xanthophylls, A and Z, after 12 h of dark adaptation and after 15 min of high light at 10°C. Rapid Z accumulation could not be explained by upregulation of VDE expression but rather by higher activity of VDE that was suggested by high accumulation of dehydroascorbate after 10 min at high light at 10°C and very low sensitivity of VDE to reducing agents at 4°C. The dark and chilling regulation of VDE might be a significant contributor to the unique among C4 grasses chilling tolerance observed in Miscanthus species.

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Abstract# P022

Exploring the Phenotypic Effects of PILS2 and PILS6 Proteins in Zea Mays and Arabidopsis

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The hormone auxin is an essential hormone for regulating plant growth and organ development. Auxin transport both within the cell and between cells is important for its function. PIN-LIKES (PILS) proteins are auxin efflux carriers in the endoplasmic reticulum (ER) that play a role in Arabidopsis root and shoot growth. However, the roles of PILS are not known in crops such as Zea mays (maize). Maize and Arabidopsis plants share a common ancestor and are evolutionarily similar enough to have these PILS genes conserved. However, loss of these proteins leads to different phenotypes in maize and Arabidopsis. In maize, PILS are positive regulators of shoot and root growth, while in Arabidopsis, PILS2 and PILS6 are negative regulators of organ development. It is not known why that is. Using reverse genetics and molecular biology techniques, the functional conservation and/or divergence of PILS2 and PILS6 is being examined. Fluorescently tagged ZmPILS2 and ZmPILS6 were transformed into Arabidopsis pils2 and pils6 to test for complementation. These lines will be verified for transgene expression using RT-PCR and phenotyped for shoot and root traits via ImageJ/Fiji. Then using phenotyping analysis, we can investigate if loss of PILS2 and/or PILS6 retains its negative impact on organ development in the presence of the maize orthologs. Or, if the expression of the maize orthologs in Arabidopsis can create novel phenotypes associated with of positive growth regulation, as observed in maize. The creation of stable, single-insertion transgenic lines in the respective mutant backgrounds is ongoing and the next steps are to conduct phenotypic analyses of these novel Arabidopsis genotypes.

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Abstract# P023

Variations in Fatty Acid Elongation Generate Novel Hydroxy and Keto Fatty Acids

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We have previously shown the occurrence of a process known as “discontinuous” fatty acid elongation in seeds of the Brassicaceae Orychophragmus violaceus that generates fatty acid hydroxylation through the activity of a specialized FAE1 3-ketoacyl-CoA synthetase (KCS). This enzyme “intercepts” the 3-OH intermediate during ER elongation to add two carbon atoms before the completion of a fatty acid elongation cycle to generate the C24 dihydroxy fatty acids nebraskanic (7,18-OH-24:1Δ15) and wuhanic (7,18-OH-24:2Δ15,21) acids that accumulate to ~40% of the seed oil. Here, we show a likely variation in discontinuous elongation in seeds of the closely related species O. limprichtianus that not only yields nebraskanic and wuhanic acids, but also C26 and C28 dihydroxy fatty acids and previously unknown C24-C28 keto-hydroxy fatty acids.

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Abstract# P024

The Arabidopsis xylosyltransferases, XXT3, XXT4, and XXT5 are essential to complete the fully xylosylated glucan backbone XXXG-type structure of xyloglucans

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Xyloglucans (XyGs) are the major component of primary cell wall of most plants, and it has significant role in plant growth, development and resistance to both biotic and antibiotic stresses. Although most XyG biosynthesis enzymes have been identified, the molecular mechanism that defines XyG branching patterns is unclear. Four out of five XyG xylosyltransferases (XXT1, XXT2, XXT4, XXT5) are known to add the xylosyl residues from UDP-xyloses onto a glucan backbone chain; however, the function of XXT3 has yet to be demonstrated. To understand the role of XXT3 in XyG biosynthesis, single xxt3 and triple xxt3xxt4xxt5 mutant Arabidopsis (Arabidopsis thaliana) plants were generated using CRISPR-Cas9 technology. Combined biochemical, bioinformatic, and morphological data conclusively established for the first time that XXT3, together with XXT4 and XXT5, add xylosyl residue specifically at the third glucose in the glucan chain to synthesize XXXG-type XyGs. We propose that the specificity of XXT3, XXT4, and XXT5 is directed toward the prior synthesis of the acceptor substrate by the other two enzymes, XXT1 and XXT2. We also conclude that XXT5 plays a dominant role in the synthesis of XXXG-type XyGs, while XXT3 and XXT4 complementarily contribute their activities in a tissue-specific manner. The newly generated xxt3xxt4xxt5 mutant produces only XXGG-type XyGs, which further helps to understand the impact of structurally deficient polysaccharides on plant cell wall organization, growth, and development.

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Abstract# P025

Identifying the trans-duplicated genic regions – A first step in finding Helitrons in maize genome

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Transposable elements are the DNA sequences that can move from one place to another in the genome. They are found in both prokaryotes and eukaryotes. There are two classes of transposable elements: Class I Retrotransposon and Class II DNA transposon. The maize genome contains abundant LTR retrotransposons, TIR DNA transposons, and Helitron DNA transposons. Helitrons were first identified in 2001 by computational analysis of the genome of Arabidopsis, Rice, and Caenorhabditis elegans. In maize, helitrons contribute to genomic diversity by capturing fragments of genes and moving these fragments to new positions in the genome. Helitrons are unusual in that they lack the structural features of TIR transposons and LTR retrotransposons, including both direct or inverted repeats at the TE ends and target site duplications flanking insertions. This makes helitrons especially tricky to annotate, which had led to inaccuracies in annotation of large numbers of putative genes. Accurate annotation of helitrons is critical to understanding the origin of genes and pseudo-gene sequences found interspersed throughout the genome. Here, we propose a gene-centric method for identifying helitron insertions where we first identify trans duplicated gene fragments genome-wide, then implement filtering and characterization steps to identify TE ends and family structure. Most gene fragments contained within helitrons are likely to have lost their original functions due to fragmentation and epigenetic silencing. However, the presence of a vast pool of transduplicated genic DNA is likely to have impacted evolution of the host gene function. We aim to better characterize the structure and distribution of helitrons to better understand the functional impact of these enigmatic transposable elements.

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Abstract# P026

Roles of vacuolar phosphatases in nucleotide salvage pathway of Arabidopsis

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RNA is essential in all forms of life, yet the importance of bulk RNA turnover and its role in nucleotide salvage pathways has just emerged. RNS2, a vacuolar ribonuclease that catalyzes rRNA turnover, plays roles in linking bulk RNA turnover to nucleotide salvage pathways. The rns2-2 mutant has RNA accumulation found in vacuoles along with an increased autophagy, and the mutant also has the pentose phosphate pathway shifted toward ribose-5-P production, a precursor of nucleoside biosynthesis. The increased autophagy is rescued by the treatment of inosine, a precursor of purine nucleoside biosynthesis. Thus, RNS2 is critical in keeping the homeostasis of cellular nucleotides. However, how nucleosides are generated from RNS2-produced 3'NMPs remains unclear. We analyzed this step using in vitro assays and molecular genetics. In vitro degradation of polyadenylic acid showed that purified vacuoles produce 2' 3' cyclic adenosine monophosphate (2' 3' cAMP), 3' adenosine monophosphate (3' AMP), and adenosine. Ratios of these products are shifted by the absence of PAP26, a major vacuolar phosphatase, leading to a decrease of adenosine along with accumulations of 3' AMP and 2' 3' cAMP. In addition, metabolite analyses revealed that loss of PAP26 activity alters the levels of 2' 3' cUMP, 3' UMP, and uridine in purified vacuoles, revealing that PAP26 catalyzes vacuolar nucleotide metabolism. The pap26 mutant also displays an increased autophagy, a phenotype that can be rescued by inosine treatment. This indicates that PAP26 participates in nucleotide salvage pathways through catalyzing nucleoside production in vacuoles. We propose that PAP26 also have a role in regulating RNS2 kinetics through nucleotide removal, increasing the catalytic efficiency of RNA turnover in vacuoles.

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Abstract# P027

Determining host plant responses towards competition between Bradyrhizobium elkanii strains

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Nitrogen is a crucial component for regulating the growth and development of plants. The roots of leguminous plants (such as soybeans, beans, and peas) develop symbiotic associations with a group of soil bacteria called Rhizobia that can reduce inert nitrogen into assimilable ammonia to obtain nitrogen nutrition. Competition between Rhizobium strains with different nitrogen fixation capacities for nodule occupancy often leads to a reduction in overall symbiotic efficiency in host plants. Here we aim to determine how soybean plants respond to two different Bradyrhizobium strains, one with a high nitrogen-fixation capacity (B.elkanii USDA 83) and the other with a low nitrogen-fixing capacity (B.elkanii USDA 26). We used a split root system approach for this study which allows for studying plant responses by simultaneously inoculating the separate root halves with different strains. Our previous split root assays with high and poor-capacity nitrogen-fixing strains, belonging to a different species (USDA 110 and USDA 126) showed that host plants can select and favor high-capacity nitrogen-fixing strains. In this study, we aimed to address if similar host responses can be seen with Bradyrhizobium elkanii strains. Results from this study indicated that soybean plants do not exhibit any preference towards either of the strains by 21 days post-inoculation in terms of nodule formation. However, there was a difference in nodule maturation with USDA 26. Although we did not observe any selection by host plants, this study still provides evidence for potential host sanction mechanisms that might operate at the level of nodule maturation in response to colonization by Bradyrhizobium strains with varying nitrogen fixation capacities.

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Abstract# P028

Activity and Specificity of Xyloglucan Galactosyltransferase MUR3

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Xyloglucan (XyG) is a significant hemicellulose within the cell wall of most plants. The large XyG polymer is often described in subunits. These subunits vary in structure but share a β(1-4) glucan backbone that is xylosylated to form di- or tri-xylosylated subunits. These subunits are typically described as four consecutive glucan sugars, where the fourth glucose is always unsubstituted. A single-letter nomenclature has been established to easily identify the glycosylation branching of the glucose molecules, such as G for unbranched glucose and X for xylosylated glucose, forming the most commons subunits, XXXG or XXGG. Once xylosylated, these branches can be further extended by arabinose (S), galactose (L), fucose (F), and glucuronic acid (Y), which varies between species and even different tissues within the same organism. Reverse genetics has identified the 13 glycosyltransferases (GT) involved in Arabidopsis thaliana vegetative tissue. These GTs are membrane-bound to the Golgi lumen, making them more difficult to work with. Structural and kinetic characterization of two of the 13 GTs, FUT1 and XXT1, were essential in understanding substrate binding and elucidated the differences in specificity between homologous xylosyltransferases XXT1-XXT5. In Arabidopsis, galactosylation of the second and third xylosyl residues of the subunit (XLLG) is carried out by MUR3 and XLT2. MUR3 is responsible for the galactosylation of the third xylose (XXLG), while XLT2 is responsible for the second xylose (XLXG). So far, neither galactosyltransferase has been kinetically or structurally characterized. In preparation for solving the structure of MUR3, we have expressed and purified truncated (soluble) MUR3 in HEK293S GNT1- mutant cells and demonstrated activity and specificity through in vitro assays. All these novel results will be discussed in the presentation and will serve as a significant part of the following structural study currently initiated in our lab.

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Abstract# P029

Bradyrhizobium Phenotypic plasticity: A mechanism by which soybean root exudates influence legume-rhizobia interactions

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Bradyrhizobium fixes nitrogen for soybean by forming nodules after developing association with the root, which requires a series of motility and attachment steps. These steps are moderated by bacterial surface and phenotypic properties which are influenced by root exudates secreted into the soil. There is minimal information about the phenotype of Bradyrhizobium under influence of root exudate compounds (RECs) and how phenotype impacts root attachment ability. To address this void in information, we studied the impact of twelve different RECs, one commonly used nutrient, and soil extracted solubilized organic matter on attachment and related surface properties of B. diazoefficiens USDA110. We measured attachment and related properties (surface hydrophobicity biofilm formation, attachment to cellulose and soy roots), motility related properties, (swimming, swarming, chemotaxis, flagellar expression), and surface polysaccharidal properties (colony morphology, lectin binding profile, lipopolysaccharide profiling and exopolysaccharide quantification). We found that USDA 110 displays a high degree of surface phenotypic plasticity when grown on the various individual RECs. Data and correlation analysis of phenotypic traits showed that cell surface hydrophobicity is the most root attachment influencing trait of Bradyrhizobium. Soybean RECs played specific roles in modifying the motility and root attachment processes, for instance serine increased cell surface hydrophobicity and root and cellulose attachment, gluconate and lactate increased EPS production and biofilm formation, and raffinose and gentisate promoted motility and chemotaxis. This study offers phenotypic plasticity of Bradyrhizobium as possible function for the role of root exudates in influencing legume-rhizobia interactions. Hence, it is crucial to further understand the basis of bacterial phenotypic plasticity and explore root exudate effects on plant-microbe interaction in soil environments.

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Abstract# P030

Effect of fall armyworm herbivory on transcriptional responses of sorghum

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Sorghum is one of the world's most important monocot crops grown for food, feed, and/or fuel. Simultaneously, sorghum is highly susceptible to insect pests that cause dramatic decreases in yields. Even though there is extensive natural variation for resistance against insect pests in sorghum, much of it remains undiscovered and under-utilized. This extensive genetic variation offers unique opportunities to elucidate the underlying mechanisms of sorghum resistance or susceptibility to fall armyworm (FAW; Spodoptera frugiperda). Previously, we identified varied levels of resistance to FAW in founder lines of the sorghum NAM population. Compared to wild type plants, FAW growth was lower and higher on SC1345 and Ajabsido plants, respectively. In this study, we utilized RNA-seq to identify the genes and metabolic pathways that are responsible for providing sorghum resistance/susceptibility to FAW. Co-expression network analysis demonstrates that the FAW feeding modulate the genes related to metabolic process, lipid metabolic process, and oxidation-reduction in the FAW-resistant line. The underlying mechanisms of sorghum-FAW interactions, and FAW feeding-induced signaling networks in sorghum will be discussed.

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Abstract# P031

Exogenous application of an archaeal antioxidant enhances plant growth by attenuating biotic stress response

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Redox signaling plays a vital role in all living organisms as it is a potent regulator of metabolic balance under various stress conditions and developmental stages. Redox regulation is coordinated by the combined efforts of small molecules like glutathione (GSH), NAD/NADH, NADP, NADPH, ascorbate, and their associated metabolic processes. Exogenous application of a nonnative, archaeal antioxidant (ArA) enhances the biomass yield of Arabidopsis thaliana by 5-10%. This effect is phenotypically similar to GSH application, which has similar reductant potential. Using genetically encoded redox sensing GFP (roGFP2), we deciphered that ArA-treated plants maintain more reduced plastids. Through the systems-level approach of RNAseq, we identified that various biotic stress response mechanisms are downregulated in the presence of ArA, including key regulators of salicylic acid (SA), jasmonic acid (JA), and ethylene. Interestingly, while transcription of key response genes of SA and JA signaling such as PR1, and PDF1.2 are decreased, direct measurement of these hormone levels show an increase in response to ArA. These signaling systems are regulated by NONEXPRESSOR OF PATHOGENESIS-RELATED GENE1 (NPR1). We are currently characterizing the role of NPR1 signaling in ArA-mediated growth enhancements. This work indicates that redox regulation plays an interesting role in regulating biotic stress responses which may be key to enhancing plant growth while maintaining resilience.

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Abstract# P032

Arabidopsis root growth is enhanced by the artificial food dye Red No. 40

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In humans, consumption of artificial food colorants has been linked to adverse behavioral reactions in children. Previous research has used both animal and plant models to study the genotoxic and cytotoxic effects of exposure to artificial food dyes. In this research, Arabidopsis thaliana was used as a model to test the effects of three red food dyes, with a focus on FD&C Red No. 40, the most common red food dye used in the United States. Our work shows that at levels of human consumption, growth of seedlings on media containing Red No. 40 results in an increase in root growth. Biochemical in vitro studies in other labs suggest that activity of human protein tyrosine phosphatase PTP1b can be blocked by Red No. 40, as well as other common food dyes, due to the food dyes' common aromatic ring structures. To determine whether the increased growth we see in response to Red No. 40 treatment is due to inhibition of plant phosphatases, we grew Arabidopsis mutants lacking PTP1, MKP1, or MKP2. The ptp1 mutants responded to Red No. 40 in a similar way to our wildtype Col-0 plants; mkp1 and mkp2 mutants did not show the same increase in root growth, a result consistent with inhibition of MKP1 and MKP2 by Red No. 40. Altogether, this work presents a new model for studying the cellular effects of food dyes, and provides additional data to support the adverse effects food dyes can have on cellular signaling pathways.

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Abstract# P033

Characterization of Glycine rich proteins in root nodule development of Medicago truncatula

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Symbiotic nitrogen fixation is an agronomically important biological process that results in natural input of nitrogen into agricultural soils. Legumes and soil bacteria called rhizobia that convert atmospheric nitrogen into readily available ammonia enter a partnership that leads to formation of specialized organs called nodules on legume roots. Small signaling peptides (SSPs) or peptide hormones are emerging regulators of root nodule formation and are fragments of larger polypeptides, that range from five to 65 amino acids. SSPs are classified into subgroups such as post translationally modified peptides, Cystine rich and Glycine Rich Peptides (GRPs). Although there are several studies investigating the first two subgroups, not much is known about the Glycine rich peptides. In the model legume Medicago truncatula there are 57 genes which encode glycine rich proteins, of which three have been implicated in rhizobial infection previously. Glycine rich peptides in nodules range between 60-250 amino acids and are characterized by an N-terminal secretion signal followed by stretches of glycine residues. Our research is focused on the characterization of glycine rich proteins in root nodule development of M. truncatula, as they are induced during nodule development. Of 57 glycine rich protein (NodGRP) genes, MtNodGRP31 is seen to be induced in three out of four time points post inoculation with the rhizobial partner Sinorhizobium meliloti; these include 4dpi, 10dpi, 14dpi and 28dpi. Phylogenetic analysis in MEGA X showed that the genes NodGRP31 belongs to a cluster of 13 closely related, tandemly duplicated genes on chromosome five. These 13 NodGRPs do not have orthologues in non nodulating plants such as Arabidopsis thaliana or Nelumbo nucifera.

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Abstract# P034

Cell-level auxin and cytokinin responses during root lateral organ development in Soybean

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Lateral roots and nodules are post-embryonic root organs in legumes. While lateral root initiation is largely regulated by developmental cues, the formation of root nodules is triggered by legume-rhizobium symbiosis. It has been previously demonstrated that a developmental stage-specific balance between auxin and cytokinin is essential for proper lateral root and nodule development. However, discrete hormone dynamics have been observed among lateral roots and the two types of legume nodules: indeterminate with a persistent meristem and determinate that lack a persistent meristem. The goal of this research project is to determine cell-specific auxin and cytokinin responses during lateral root and nodule development in common bean and Medicago truncatula, legumes that produce determinate and indeterminate nodules respectively. Our laboratory previously used two-photon induced 3-dimensional fluorescence microscopy to image nuclear-localized fluorescence arising from transcriptional output sensors for auxin and cytokinin to determine cell level Auxin Cytokinin Relative Output ratios (ACRO) in soybean lateral root and nodule tissues. Here we extend the research to common bean and Medicago truncatula to obtain a comprehensive view of cell-level ACRO ratios during the development of determinate and indeterminate nodules and lateral roots in legumes. The findings are likely to provide important insights into the hormonal regulation of legume-rhizobium symbiosis and evolutionarily conserved pathways across both lateral organs.

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Abstract# P035

Elucidating the glucuronidation mechanism of heteroxylan in grasses

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The structure and composition of plant cell walls (CWs) are highly complex and made up of interconnecting network of polymers such as heteroxylans (HXs). They are important in maintaining the strength of CWs required for plant growth and fitness. HXs are also useful to humans in various commercial applications, such as food, feed, and renewable energy. HXs have a backbone made up of a long chain of β(1,4)-linked xylosyl (Xyl) residues that is substituted with glucuronic acid (GlcA) as well as arabinosyl (Ara) residues. Although, the genes involved in the glucuronidation of HXs in Arabidopsis (and many dicots) have been identified as GUX1, GUX2 and GUX3, no orthologs have been identified in grasses. The goal of this project is to identify genes responsible for the glucuronidation of HXs in rice using functional genomics approaches. Using phylogenetic analysis and multiple sequence alignment between Arabidopsis and rice members of the GT8 family, we identified three putative rice GUXs. Through an in-vitro enzyme assay, I observed that they indeed transfer GlcA from UDP-GlcA (donor) to xylohexaose (acceptor). To further validate the results in-vivo, rice mutant plants were generated in these putative rice GUX genes. In addition, these rice genes were introduced in Arabidopsis gux1/2/3 triple mutant plant. This triple mutant synthesizes HX backbone without GlcA side chains and would serve as an excellent system to study glucuronidation of HXs using rice genes. Understanding glucuronidation mechanism in grasses would help manipulate the structure of HXs to improve CWs digestibility for biofuel production. The results of this work will be discussed in the meeting.

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Abstract# P036

Identifying small RNAs associated with Secondary Cell Wall Development in Arabidopsis thaliana.

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Plant cells form the cell wall in two phases. Thin, flexible primary cell walls (PCWs) are first deposited as cells grow and elongate. After growth ceases, strong secondary cell walls (SCWs) are produced to build protective barriers and help rigidify cell shape and morphology. SCWs are crucial for various research and industrial applications, but the molecular mechanisms governing their production still need to be better understood. My research aims to better understand the regulatory processes involved in secondary cell wall (SCW) biosynthesis. Prior studies have suggested that small RNAs (sRNAs) derived from cellulose synthase A (CESA) genes in barley and Brachypodium might play a role in regulating this transition1, 2. To investigate the role of sRNAs in SCW formation, a small RNAseq study using an inducible system in Arabidopsis thaliana is being conducted. The inducible system contains a master transcription factor for SCW activation (VND7), which enables the investigation of differentially expressed sRNAs before and after induction3. I hypothesize that sRNA important for regulating the SCW transition will increase in abundance after induction of VND7, enabling the transcriptional activation of SCW genes. Here, I include the progress made in plant and cell culture transformations, optimizing the timing and conditions of SCW induction, and future research plans, including small RNAseq experiments.

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Abstract# P037

Identifying small RNAs associated with Secondary Cell Wall Development in Arabidopsis thaliana.

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Plant cells form the cell wall in two phases. Thin, flexible primary cell walls (PCWs) are first deposited as cells grow and elongate. After growth ceases, strong secondary cell walls (SCWs) are produced to build protective barriers and help rigidify cell shape and morphology. SCWs are crucial for various research and industrial applications, but the molecular mechanisms governing their production still need to be better understood. My research aims to better understand the regulatory processes involved in secondary cell wall (SCW) biosynthesis. Prior studies have suggested that small RNAs (sRNAs) derived from cellulose synthase A (CESA) genes in barley and Brachypodium might play a role in regulating this transition. To investigate the role of sRNAs in SCW formation, a small RNAseq study using an inducible system in Arabidopsis thaliana is being conducted. The inducible system contains a master transcription factor for SCW activation (VND7), which enables the investigation of differentially expressed sRNAs before and after induction. I hypothesize that sRNA important for regulating the SCW transition will increase in abundance after induction of VND7, enabling the transcriptional activation of SCW genes. Here, I include the progress made in plant and cell culture transformations, optimizing the timing and conditions of SCW induction, and future research plans, including small RNAseq experiments.

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Abstract# P038

Extracellular ATP plays an important role in systemic wound response activation

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Mechanical wounding occurs in plants during biotic or abiotic stresses and is associated with the activation of long distance signaling pathways that trigger wound responses in systemic tissues. Among the different systemic signals activated by wounding are electric signals, calcium, hydraulic, and reactive oxygen species (ROS) waves. The release of glutamate (Glu) from cells at the wounded tissues was recently proposed to trigger systemic signal transduction pathways via GLU-LIKE RECEPTORs (GLRs). However, the role of another important compound released from cells during wounding (extracellular ATP [eATP]) in triggering systemic responses is not clear. Here, we show in Arabidopsis (Arabidopsis thaliana) that wounding results in the accumulation of nanomolar levels of eATP and that these levels are sufficient to trigger the systemic ROS wave. We further show that the triggering of the ROS wave by eATP during wounding requires the PURINORECEPTOR 2 KINASE (P2K) receptor. Application of eATP to unwounded leaves triggered the ROS wave, and the activation of the ROS wave by wounding or eATP application was suppressed in mutants deficient in P2Ks (e.g. p2k1-3, p2k2, and p2k1-3p2k2). In addition, expression of systemic wound response (SWR) transcripts was suppressed in mutants deficient in P2Ks during wounding. Interestingly, the effect of Glu and eATP application on ROS wave activation was not additive, suggesting that these two compounds function in the same pathway to trigger the ROS wave. Our findings reveal that in addition to sensing Glu via GLRs, eATP sensed by P2Ks plays a key role in the triggering of SWRs in plants.

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Abstract# P039

Delineating the genetic interaction of vesicular trafficking components in plant growth and development

Carissa Bersche, Kelly Mason, Kristen Barwick, Tessa Jenkins, and Antje Heese

As the global population continues to climb, a pressing issue is producing enough crops on limited land to support the needs of the growing population. Thus, optimizing crop health and yield is of great importance. The Heese lab uses the model plant Arabidopsis thaliana ecotype Columbia (Col-0) to gain insight into the roles of vesicular trafficking in plant defense, growth and development. Vesicular trafficking is the process by which cargo proteins are transported in membrane-bound vesicles from one organelle to another. The trans-Golgi Network (TGN) has emerged as a critical sorting station, sending cargo material to the vacuole or the plasma membrane (PM). Our lab is specifically interested in understanding the role of EPSIN1 (EPS1), a TGN-localized clathrin adaptor protein, in plant defense and plant growth. Mutations in the EPS1 gene correlate with impaired cargo trafficking to the vacuole and the plasma membrane. However, EPS1 does not function alone as it biochemically interacts with other vesicular trafficking proteins. One such protein is VTI11, a v-SNARE that provides specificity for the fusion of TGN trafficking vesicles with the lytic vacuole. Loss of VTI11 leads to a zigzag pattern of the inflorescence. To explore whether EPS1 and VTI11 show a genetic interaction in plant growth and development, our lab has created eps1 vti11 double mutants. The purpose of my project is to confirm the eps1 zig1 double mutant by polymerase chain reaction (PCR) genotyping and quantify potential phenotypic defects in their single and double mutations on inflorescence and leaf tissue area. I provide PCR genotyping data confirming that we have isolated the eps1 vti11 double mutant line. Currently, I am quantifying leaf size, inflorescence length and zigzag appearance by comparing the double mutant to the eps1 and vti11 single mutants and Col-0 wildtype using ImageJ analyses, and these results will be discussed.

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Abstract# P040

Antibody Production Against the EPSIN1 ENTH Domain from Arabidopsis thaliana

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Because plants are a major food source for humans and life-stock, understanding how plants protect themselves against pathogenic infection is vital to protect the world's food supply. With large percentages of yearly crop yield lost to pathogens, our lab's research will provide new insights into how to engineer more resistant plants, reducing crop losses and providing food security for a rapidly growing world population. The Heese lab focuses on the cellular machinery that shuttles the critical plant immune receptor FLAGELLIN SENSING2 (FLS2) to the cell surface. FLS2 must be at the cell surface to detect extracellular bacteria and trigger defense responses. EPSIN1 (EPS1) is an adaptor protein which plays a role in the formation of vesicles trafficked to the cell surface. Our lab has published that Arabidopsis eps1 null mutants are more susceptible to infection by pathogenic bacteria due to decreased levels of FLS2 and other defense proteins at the cell surface. EPS1 has an Epsin N-terminal Homology (ENTH) domain which is highly conserved between plant orthologs; but role(s) of ENTH in physiological response is unknown for any plant EPSIN [3]. As a first step towards understanding roles of plant ENTH-domains, my project goal is to produce a polyclonal antibody against the EPS1 ENTH domain and confirm its specificity. I expressed a 6His-tagged ENTH domain into bacterial cells and purified it using nickel affinity chromatography. I then sent my purified ENTH protein to the company Eurogentec for polyclonal antibody production in rabbits. I received and tested the antibody bleeds to confirm by immunoblot analysis using Col-0 wild-type and eps1 mutants that these αENTH antibodies detect Arabidopsis EPS1 in vivo. My future work will assess whether this antibody also detects Zea mays EPS1 and Arabdiopsis EPS1 deletion forms in planta to gain insight into the physiological roles of EPS1 subdomains.

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Abstract# P041

Role of Clathrin-Coated Vesicle Components in Plant Immunity

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To feed the world's growing population, crop production will need to double within the next 30 years. Each year, a substantial percentage of crops are lost to pathogen infection resulting in decreased crop yield. Understanding the molecular mechanisms behind plant immunity can help to inspire novel approaches to engineering more resistant crop species. The plasma membrane (PM) is a crucial contact point between a plant cell and its environment. For effective immune responses, plant PM proteins perform critical functions to facilitate pathogen perception as well as initiation, amplification, and attenuation of defense responses. One strategy that plant cells utilize to control the PM protein composition is by protein cargo trafficking to/from the PM by vesicular trafficking including secretion and endocytosis. Vesicular trafficking has emerged as a key regulator of plant defense because mutations in vesicular trafficking genes result in altered immune responses. In plants, clathrin-coated vesicles (CCV) form at both the PM and the trans-Golgi Network (TGN); however, our understanding of how they control plant immunity is limited. The goal of this project is to delineate the roles of CCV components in regulating the PM composition for proper initiation and attenuation of plant immunity in Arabidopsis thaliana. Our lab has recently shown that AtEPS1, a CCV adaptor that recruits clathrin components for vesicle formation at the TGN, regulates the PM abundance of the immune receptor FLAGELLIN SENSING2 (AtFLS2) for effective defense responses. In eps1 mutants, AtFLS2 accumulates to reduced levels in the PM correlating with impaired immune signaling. We will expand the role of AtEPS1 by investigating its genetic interaction with the CCV component CLATHRIN HEAVY CHAIN (AtCHC). We have evidence that both eps1 and chc single mutants have altered immune responses. Here, we report of the isolation of chc eps1 double mutants and their genetic interaction in plant immunity.

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Abstract# P042

The role of GAUT10 in Arabidopsis root development

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Cell wall properties of the root apical meristem (RAM) are poorly understood compared to the elongation and maturation zones of the developing root. GAUT10 is a pectin biosynthesizing enzyme required for root growth in Arabidopsis in a sucrose-dependent manner. Using live-cell microscopy, I have determined that the short root phenotype of gaut10-3 is due to a reduction in both RAM cell number and epidermal cell elongation. In addition, the absence of GAUT10 leads to a reduction in the lateral root cap and epidermal cell marker line expression, indicating root cell differentiation defects in this mutant. GAUT10 is required for normal pectin and hemicellulose composition in primary Arabidopsis roots, implicating these particular cell wall states in influencing normal RAM properties. Specifically, loss of GAUT10 leads to a reduction in galacturonic acid and xylose in primary cell walls and alters the presence of rhamnogalacturonan (RG) I and homogalacturonan (HG) polymers in the root. In addition, gaut10-3 roots exhibit altered auxin pathway gene expression and abnormal auxin metabolite composition in primary roots, suggesting that cell wall composition may impact auxin metabolism. A working model of GAUT10 action concerning two growth cues, auxin, and sugar, will be presented.

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Abstract# P043

Effect of pollinators and visitors of the mint Blue Sage (Salvia azurea) in Nebraska

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The Blue Sage (Salvia azurea), a central Great Plains native mint species (Family Lamiaceae), plays a vital role in Nebraska's ecosystem through its value for native pollinators. Native bees commonly are attracted to the nectar of the abundant flowers of this species. We hypothesized that Salvia azurea is primarily pollinated by bumblebees and simply visited by other pollinators. We documented the pollination patterns of Salvia in Cottonmill Park, Buffalo County, Nebraska. In total, we collected 45 pollinators. From the 45 specimens collected, we documented that 58% of the pollinators were bumblebees, 31% were other bees, and 11% were butterflies. From the identified bumblebees, we observed that 64% were American bumblebees (Bombus pensylvanicus) and 36% were common eastern bumblebees (Bombus impatiens deayi). Given that S. azurea is primarily pollinated by native bumblebees, planting and conserving this species across the Great Plains can maintain and support bumblebee diversity.

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Abstract# P044

Characterization of a tomato extensin peroxidase in vivo

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Extensin (EXTs) comprise a diverse group of cell wall structural glycoproteins. EXTs are network forming glycoproteins that undergo self-assembly and covalent crosslinking. Covalent crosslinking is mediated by EXT peroxidases that polymerize EXT monomers to create a polymeric scaffold. Scaffold formation is critical for plant growth and development, mechanical wounding, and response to pathogen infection. Previous work identified an EXT peroxidase from tomato (TomEP)1. TomEP has been characterized in vitro, but its physiological role(s) in vivo has not been explored. This project focuses on the characterization of mutants of TomEP generated through CRISPR/Cas9. Here we identify homozygous knockout tomato mutant lines that are Cas9-free and with no apparent off-targets. To assess the impact of TomEP loss-of function, biochemical characterization of Tom EP mutant lines is underway. Additionally, the subcellular localization of TomEP is being explored. We find that TomEP is localized to the plasma membrane (PM) by live-cell imaging in tobacco leaves. Addition of either H2O2 or a synthetic EXT analog (YK8) appears to shift the subcellular distribution of TomEP from PM to apoplast. These data have interesting implications regarding the regulation of EXT scaffold formation in vivo. References 1Dong W, Kieliszewski MK, Held MA 2015. Identification of the pI 4.6 extensin peroxidase from Lycopersicon esculentum using proteomics and reverse-genomics. Phytochemistry 112: 151-9

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Abstract# P045

Time and Temperature: The Keys to Understanding Triacylglycerol Accumulation in Cold and Freezing

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As winter approaches, plants acclimate to the cold and modify their intracellular structure to withstand low temperatures. During this time, plants increase desaturation in their membranes to prevent damage and prepare for more severe cold. More intense remodeling occurs when temperatures descend into freezing, preventing rigidification. Unfavorable lipids are removed, producing diacylglycerol, which is quickly acylated into triacylglycerol (TAG). This process suggests TAG accumulation is a sink for remodeled lipids allowing for stronger membranes to form. In Arabidopsis, two enzyme families carry out this function. The diacylglycerol acyltransferases (DGATs) and phosphatidyl-diacylglycerol acyltransferases (PDATs) use different acyl chain donors to generate TAG, suggesting unique roles in stress response. While DGAT1 and PDAT1 have been linked to thermotolerance, the combined and temporal responses of the families during cold and freezing has yet to be determined. Here we show TAG accumulation during cold and freezing requires both families of acyltransferases and the isoforms uniquely respond to different severities of low-temperature stress. To test this, we used two complementary approaches with loss of function mutants in Arabidopsis, dgat1,2 3 and pdat1,2. The first assayed membrane integrity via electrolyte leakage. dgat1 and dgat2 membranes were most impaired, while pdat1 and pdat2 had the lowest TAG levels. The second quantified lipid changes over 8 days of low-temperature treatment followed by post-freezing recovery. Results showed pdat1 accumulated the least TAG after freezing and had the most impaired recovery. dgat1 had a deficit in TAG early in cold treatment but was able to recover. Lastly, we show a correlation between the fatty acid composition of TAG and monogalactolipid after cold treatment in dgat1. Collectively, this work demonstrates the importance of measuring multiple time and stress scales when defining gene relevance and assessing function.

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Abstract# P046

Identifying an unknown mutation in pils2 pils6 double mutant

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Auxin is an essential hormone for plant growth and development. Auxin transport occurs via diverse protein families including the PIN-LIKES (PILS) which are localized to the ER and regulate nuclear auxin concentrations. PILS2 and PILS6 are paralogs that are conserved in angiosperms. Previous work on Arabidopsis PILS2 and PILS6 demonstrated that these proteins are negative regulators of organ size. Phenotyping of primary root and lateral root traits in published mutant alleles of PILS2 and PILS6 indicates that these genes have non-redundant functions. To examine their potential overlap in organ size control, a novel pils2-2 pils6-1 double mutant was created. Surprisingly, pils2 pils6 double mutants exhibited two classes of rosette size phenotypes among an F2 population: normal and dwarf. The new dwarf rosette phenotype emerged among homozygous pils2 pils6 individuals that co-segregate with PCR markers for the pils2-2 and pils6-1 genotypes, suggesting the presence of a third mutation. Proposed genetic experiments to investigate the nature of this background synthetic lethal mutation will be presented and a CRISPR allele of PILS2 will be described. These data further our understanding of genotype-phenotype relationships among paralogous auxin efflux carriers in Arabidopsis.

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Abstract# P047

Heterologous production of proteins involved in chloroplast lipid metabolism to determine phosphatidic acid phosphatase activity

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Monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) make up around 80% of the lipids in the thylakoid membranes where photosynthesis takes place. Diacylglycerol (DAG) is the precursor of MGDG and DGDG; it is produced through the dephosphorylation of phosphatidic acid (PA). The enzyme that dephosphorylates PA in the plastid is unknown; therefore, the PA phosphatase (PAP) activity of various A. thaliana candidate proteins are being investigated through production in S. cerevisiae and E. coli, and PAP activity assays. The candidate proteins include predicted chloroplast lipid phosphate phosphatases LPPγ, LPPε1, and LPPε2. Because a knockdown mutant of known acyltransferase ATS1 may have lower PAP activity, and a knockout mutant of predicted rhomboid protease RBL10 is deficient in the conversion of plastid PA to MGDG, these proteins are also being investigated as possible PAPs. Gene expression and protein extraction are being optimized for the aforementioned proteins in order to carry out PAP activity assays that will show which ones will catalyze PA dephosphorylation and produce DAG.

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Abstract# P048

Rice small RNA expression levels are associated with grain chalkiness under high night temperature

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Increasing global temperatures will cause rice to be exposed to a higher frequency, duration, and intensity of heat stress (HS). The increasing incidence of High Night Temperature (HNT) is particularly harmful as it not only reduces the yield of rice, but also results in the production of low-quality “chalky” grains due to reduced deposition of starch and protein in the caryopsis. However, certain rice cultivars have a higher tendency than others to maintain high grain quality under HNT. Several investigations have been performed on how rice cultivars of differing quality regulate genes under HS, and how these differing regulation patterns affect the chalkiness phenotype. However, the role of small RNAs (sRNAs) is largely unknown. Therefore, we performed a differential expression analysis of sRNAs using two pairs of cultivars that are genetically related, but have contrasting grain quality under HNT. We found that under HNT, the caryopsis tissue of the chalky cultivars had higher expression of osa-miR1867, which inhibits a starch synthase, than the good quality cultivars. Additionally, the low-quality caryopsis tissue had a higher abundance of antisense siRNAs to HAP5 genes, one of which is NF-YC10, an essential regulator promoting grain development. In HNT-stressed flag leaf tissue, chalky cultivars had higher expression of osa-miR530-5p, which inhibits grain yield. It is therefore evident that sRNA expression levels play an important role in determining grain quality under HNT and could be useful in engineering HS-resistant cultivars.

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Abstract# P049

Deciphering the genetic basis of fitness of yeast in different environments

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For thousands of years, the budding yeast Saccharomyces cerevisiae has widely been used for domestic and industrial processes. During these processes yeast undergo different types of stress, affecting productivity and tolerance. Therefore, mitigating these stress responses requires understanding the mechanisms underlying changes in fitness in different stressful environments. In this study, we utilize statistical and machine learning approaches to predict fitness of a collection of S. cerevisiae isolates grown in 35 stress environments using genomic data, including single nucleotide polymorphisms, open reading frame presence/absence, and copy number variation. We aim to understand how the environment impacts the fitness effects of genes and to unravel the gene-by-environment (GxE) relationship underlying fitness variation. Preliminary results reveal that different genomic data types are useful for predicting fitness in different environments beyond population structure. To further understand why fitness in certain environments is better able to be predicted, we investigate several factors (e.g. narrow-sense heritability of fitness, stress severity, and correlations of fitness profiles across environments) potentially contributing to fitness predictions in each environment. We also aim to quantify the contribution of each gene to fitness for each isolate and identify a set of genes that are informative of stress response mechanisms impacting fitness in each environment. This work will expand our understanding of the genotype-to-phenotype map of fitness and how GxE interactions modulate the impact of genes on fitness in different environments.

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Abstract# P050

A Novel Study on Priming the Defense of Arabidopsis Plants Against Its Pathogens by Expressing Fungal Cell Wall-Digesting Enzymes

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The cell wall is the plant's first line of defense against biotic and abiotic stresses. Microbial pathogens produce a notable quantity and variety of cell wall digesting enzymes to get past the cell wall and invade the plant cell. When the cell wall is modified by microbial enzymes, the plant perceives this change and activates cascades of signaling pathways, triggering host immune system pathways to fight against the invading pathogens. We can take advantage of the plant's defense mechanisms by introducing fungal cell wall digesting enzyme genes individually or in combination into the plant genome that modify the cell wall to prime the plant immunity. In this approach, only a minor change in the cell wall occurs without compromising the normal growth and development of the plant. When these modifications occur in the cell wall, the plant will sense the changes in its cell wall and upregulate its defense genes and confer higher plant resistance to infection. A few genes have previously been confirmed to confer resistance, but an additional 17 more uncharacterized genes will now be evaluated in Arabidopsis thaliana against the funal pathogen Botrytis cinerea. Later this novel technology will be transferred to Maize and other crops to help alleviate costs associated with crop diseases. The results of the initial broad scale screen of the 17 transgenic lines will be presented in this poster.

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Abstract# P051

Lysin-motif receptor-like kinases mediate β-1,3-glucans perception in Medicago truncatula

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β-glucans are the major component of the outer cell wall layer of many fungi and oomycetes, which are mainly connected via β-1,3-linkages with regularly occurring β-1,6-branch and function as the microbial-associated molecular patterns (MAMPs) to trigger innate immunity in plants. Compared to the well-studied β-glucan signaling in mammalian system, it is still largely unknown how plants recognize β-glucans from bacteria and fungi and which receptors are responsible for β-glucans perception. Through genetic screening, we identified two LysM receptor-like kinases MtLYK9 and MtLYR4 that are required for defense responses induced by linear β-1,3-glucan laminaripentose (Lam5). These two receptors, however, were not involved in laminarin-activated immune signaling, suggesting that plants have specific receptors for perception of different β-1,3-glucans. We will be further exploring the molecular mechanism on how different receptor complexes to initiate the immune pathway and symbiotic pathway respectively.

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Abstract# P052

Predicting putative cis-regulatory elements regulating transcriptional response at the single cell level in Arabidopsis roots

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Transcriptional regulatory elements interact with transcriptional regulatory proteins (transcription factors,TF) to precisely regulate gene expression in spatiotemporal and conditional manner. The discovery of transcriptional regulatory elements allows us to understand the functionality of a gene in terms of the condition or cell type where it is expressed. These regulatory elements make connections with TFs to function in complex regulatory networks to perform cellular functions such as development. Although experimental based approaches are commonly used to uncover regulatory elements that function in specific contexts, to uncover all the regulatory elements in the regulone of a context specific expression scenario can be time consuming. Recently, data-driven approaches such as machine learning (ML) have been successfully implemented in combination with different omics data to uncover cis-regulatory elements (CREs) and the larger regulatory networks they participate in at a global scale. Although these data-driven approaches have been able to identify CREs that modulate expression at the tissue level, discovery of transcriptional regulators at the single cell level haven't been implemented. In this study, we will use a published Arabidopsis root expression atlas obtained at single-cell resolution to predict gene expression in specific cell types using different ML approaches. Specifically, small DNA sequences (k-mers) that are highly predictive of expression in different cell types will be considered putative CREs (pCREs). To understand the transcriptional regulatory networks at the single cell level in which these pCREs may be associated with, genome-wide transcription factor binding site information from Arabidopsis will be used to identify TFs that bind to the pCREs. The resulting transcriptional regulatory networks at a single cell resolution will be used to understand the different transcriptional regulators modulating root development.

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Abstract# P053

Analysis of Flavor Compounds in Table, Crab and Cider Apples Used for the Production of Hard Cider

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Cider-making is historically relevant, and cider has become a common home treat. Hard cider requires a different flavor profile of apple, allowing it to ferment well and become more tasteful. Understanding the blend of flavor compounds and where in the apple fruit they originate is crucial in the production of hard cider. In this study, fruits from two commercial table apple varieties, Honeycrisp and Gala, as well as a number of cider and crab apple varieties grown on the WVU Animal Research Farm orchards were analyzed for their flavor profile. All apple varieties were tested as a whole fruit. In addition, selected varieties were separated in three isolated fruit parts - skin, pericarp, and core - and analyzed for their flavor profile. For each apple variety, fruit samples were extracted with an organic solvent, methyl tert-butyl ether (MTBE), and subsequently the solvent extract containing flavor compounds was up-concentrated. These concentrated samples were then analyzed by combined gas chromatography/mass spectroscopy (GC/MS) to characterize the profile of flavor compounds for each variety and tissue. The retention time and mass spectrum determined for each compound allows for their tentative identification, and comparison with an internal standard to determine relative abundance. We observed that crab apples accumulate significant amounts of benzaldehyde (almond oil) in the fruit pericarp, while in table apples this desirable flavor compound is only found in the core. We are now studying the gene expression level of the heterodimeric benzaldehyde synthase in different apple varieties and tissues by RT-PCR analysis.

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Abstract# P054

Phenotypic variation and plasticity in yield traits for a biparental population grown under variable nitrogen availability

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Large-volume applications of nitrogen (N) during maize production can be economically and ecologically costly. The ability to produce maize with less N would be beneficial for both farmers and the environment. Past research identified root growth angle as a key trait for N acquisition efficiency where a steep-angled root system is better able to acquire N more available at depth. Using a set of recombinant inbred lines (RILs) whose parents exhibit contrasting plasticity responses to low N conditions in root growth angle, we are working to identify relationships between root phenotypes and yield traits. We grew the B73 x Oh7B RIL family in the field under high and low N conditions and quantified root growth angle phenotypes, total yield per plant, and 100-seed weight. Though our work is still underway, we are already detecting widespread variation in all traits measured. Interestingly, we observed variation in grain shape and size, which we will pursue as another trait of interest moving forward. Phenotyping efforts for this RIL family should conclude soon, but we plan to repeat this experiment during the upcoming field season. These data will be included as part of a larger project that will use linkage mapping to identify genomic regions associated with root phenotypes and high performance in low N conditions.

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Abstract# P055

The role of PSI3 gene in Arabidopsis thaliana in the dynamic response of stomata conductance to light

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The PSI3-1 gene family was first characterized as playing a crucial role in plant growth and development. We hypothesize that this gene may also play an important role in stomata conductance (gs). Thus, this study aimed to characterize two A. thaliana mutant lines (psi3-1 – SALK_086423 and psi3-2 – SALK_114872) and their respective wild types (WT). The plants were grown in a growth chamber (10 h photoperiod, 200 μmol m-2 s-1 light intensity, 22°C day/night and RH at 65-70%) for 6 weeks. The gs versus time curve of the plants was then obtained using an Infra-Red Gas Analyzer (LICOR) with 45 set points spaced at one-minute intervals. Stomata area (SA), density (DS), length (SL), and width (SW) and epidermal cell density (ECD) were obtained with Inverted Light Microscopy by photographing fully developed leaf prints. Our findings show that the mutants did not differ from WT in terms of DS, but had significantly smaller stomata (SA, SL, and SW; $P< 0.01$). Only the psi3-1 showed higher ECD ($P< 0.05$) and, therefore, lower stomata index ($P< 0.001$). The reduced stomata size in both mutant likely drove the reduction in stomata opening in response to time of light exposure. A clear difference in the shape of this response was observed, particularly between psi3-1 and WT. The asymptotes of time response of gs (gsmax) were 3.2 and 2.3 times higher in the psi3-1 and psi3-2, respectively. WT showed a faster stoma opening response, however, since at time-point zero, its values were 2.8 and 7.3-fold higher than psi3-1 and psi3-2. Although statistical difference was not detected, possibly due to a low number of biological replicates, this study strongly indicates that overexpression of the PSI3 proteins could serve as a novel approach to increase the speed of stomata reaction to light.

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Abstract# P056

Overexpression of a mobile Cuscuta gene decreases lignin composition in Arabidopsis

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Cuscuta spp. (dodder) is a holoparasitic plant that has a specialized structure for parasitism called a haustorium. The haustorium invades the host's vascular tissues and translocates water, nutrients, mRNAs, and proteins (Wu et al., 2022 and Jhu & Sinha, 2022). To identify mobile proteins between Cuscuta campestris and Arabidopsis, we explored using liquid chromatography coupled with tandem mass spectrometry; 97 mobile Cuscuta proteins in Arabidopsis and 447 mobile Arabidopsis proteins in Cuscuta were discovered through this analysis. Among 97 mobile Cuscuta proteins in Arabidopsis stems, 23 KDa Jasmonate Induced Protein (CcJIP23) was the most abundant Cuscuta protein. Thus, we attempted to discover the function of the mobile CcJIP23 gene. Using the anti-CcJIP23, the movement of the CcJIP23 protein was confirmed. Arabidopsis plants overexpressing CcJIP23 (35S:CcJIP23) were generated and analyzed to understand the gene function. Interestingly, we found that the lignin deposition of 35S:CcJIP23 Arabidopsis plants was reduced by 50% compared to control plants. Lignin is a complex aromatic biopolymer that stiffens the middle lamella and secondary cell walls of plants to resist biotic and abiotic stresses. Lignin biosynthetic enzymes help to assemble various defense compounds against pathogens. Thus, we hypothesize that Cuscuta may deliver the CcJIP23 proteins into Arabidopsis host plants to inhibit the lignin deposition for successful parasitism.

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Abstract# P057

FACTORS AFFECTING Agrobacterium-MEDIATED TRANSFORMATION OF Cuscuta campestris

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Cuscuta spp. (dodder) are holo-parasitic plants that depend on a host plant for all their life cycles. As a weed, it causes severe damages on major crops (such as tomato and legumes) in the world. Recently, Cuscuta has been studied as a model plant for understanding plant-plant interactions because mobile molecules (DNAs, RNAs, and proteins) are exchanged between Cuscuta and host plants through a unique organ called haustoria. Although Cuscuta genome sequences and multiple RNA-seq studies have been reported, molecular mechanisms of Cuscuta parasitism or host-parasite interactions have been poorly understood due to the lack of a stable genetic transformation system. In this study, we investigated Agrobacterium-mediated transformation of Cuscuta campestris to develop a novel protocol. We used two reporter genes (YFP and GFP) to track the transformation efficiency and transformation stability. Cuscuta seedlings were inoculated by two different Agrobacterium species, Agrobacterium rhizogenes and Agrobacterium tumefaciens, to compare the susceptibility. At the same time, different factors; explant type, culture media with different plant growth regulators, and co-cultivation conditions have been tested to optimize the protocol. Taken together, we found some Cuscuta explants and callus showing strong YFP or GFP activities as results of transient and stable transformation. Under different regeneration media, some transformed calli even showed regeneration initiation, suggesting the possibility of stably transformed Cuscuta explants. This transformation method will accelerate functional characterization studies of Cuscuta.
Keywords: Cuscuta campestris, Agrobacterium, Yellow Fluorescence Protein, Green Fluorescence Protein

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Abstract# P058

Identifying and characterizing resistance to the bird cherry–oat aphid in three accessions of wild barley

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Phloem-feeding insects increasingly threaten the world's cereal crops, whether through direct plant damage or by acting as viral vectors. Developing aphid-resistant small grains is therefore an important objective of crop resilience breeding. Although none of the currently deployed barley cultivars are resistant to aphid attack, exploration of the secondary and tertiary barley gene pools is underway to identify and characterize resistance genes with potential for plant improvement. We calculated intrinsic rate of increase for bird cherry–oat aphid (Rhopalosiphum padi) foundresses in caged feeding studies on barley (Hordeum vulgare) and its wild relative (H. vulgare ssp. spontaneum). Compared to the cultivar 'Morex', our results indicate decreased aphid fecundity when feeding on three accessions of wild barley, WBDC053, WBDC117, and WBDC336. The behavior of R. padi on wild barley as assessed by electropenetography (EPG) suggests that aphids feeding on WBDC053 spend about a quarter less time in E2 salivation, which corresponds to phloem sap ingestion. Quantitative PCR-based gene expression analysis of the barley stress-related genes LOX2:3 and SOD1, combined with histological staining using 3,'3'-diaminobenzidine, implicate the involvement of oxidative defense in the responses of these wild barley accessions to aphid feeding. Our studies support the growing interest in crop wild relatives as reservoirs of genetic diversity potentially useful in crop improvement for food security.

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Abstract# P059

Predicting heat stress-related genes in Saccharomyces cerevisiae and Arabidopsis thaliana

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Organisms today experience different levels of heat stress and have specific mechanisms in place to increase thermotolerance. In the near future, global warming will lead to increased levels of heat stress in organisms. Thus to address this issue, it will become even more crucial to know what genes are related to heat stress and to what degrees they are essential. One way organisms respond to increasing heat stress is by changing expression of specific genes, such as heat shock proteins, protecting protein structure. However, changes in gene expression during heat exposure is not always indicative that the gene is important for thermotolerance. Definitively labeling a gene as heat stress-related usually involves creating knockout mutants of that gene and recording the phenotype under heat stress conditions. Although experiments are an excellent way to determine a heat stress-related gene, these experiments become increasingly expensive and time consuming as the number of genes in an organism increase. Machine learning (ML) approaches can partially alleviate costs incurred through experimental study by utilizing previously produced data to identify genes that have not yet been recognized as heat stress-related. S. cerevisiae and A. thaliana are good model species to create predictive ML models that can identify potential heat stress-related genes using well-annotated genomes and abundant publicly available multi-omics data. This study aims to use ML approaches to determine potential heat stress-related genes by integrating multi-omics data from both S. cerevisiae and A. thaliana. Furthermore, these models create a framework for identifying candidate heat stress-related genes and further identify functional relationships between heat stress-related genes.

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Abstract# P060

Phenotypic characterization of a GWAS population to identify the genetic basis of mutualism variation in Medicago truncatula.

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The legume-rhizobia symbiosis is considered one of the most important plant-microbe interactions in the biosphere. The relevance of this symbiosis lies in the possibility of replenishing the soil with fixed-nitrogen, action that can contribute to the development of sustainable agriculture and reduce the use of fertilizers. The understanding of the molecular events underlying the symbiosis is key to enhance the nitrogen-fixation efficiency. Medicago truncatula has been used as model plant for studying the plant-microbe interaction in legumes. The availability of genome sequence, gene expression data and mutant collections makes M. truncatula an easy choice when carrying out molecular studies in legumes. The advent of new resources in molecular biology, as next generation sequencing, has made possible to obtain new data to elucidate the underlying molecular mechanisms in different biological processes. Genome-wide association studies (GWAS) has been an important tool that combines newer resources available in molecular biology, to produce genome-wide single nucleotide polymorphisms SNPs, and complex statistical analysis to search for the genetic architecture of complex traits. Currently, the lab is conducting GWAS analysis to identify the genetic basis of the mutualism variation in M. truncatula – Ensifer spp. complex. Based on Batstone, et al. 2021, two Ensifer spp. strains were selected, and they were used in a subset of 149 accessions of the M. truncatula hapmap population to identify genes associated with the partner choice selection of M. truncatula for a specific Ensifer spp. strain. This preliminary analysis is showing the phenotypic data obtained after the inoculation of both strains under similar controlled conditions, the data shows accessions with different strain affinity. With the molecular analysis, we await the identification of genes related to this phenomenon in the Medicago genome.

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Abstract# P061

Lipid Derived Signaling Involving Chloroplasts

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Chloroplast membranes are characterized by an extensive photosynthetic membrane uniquely composed of galactolipids which are absent from membranes of other cellular organelles. Three recently characterized lipases, PLIP1, 2, 3, cleave 18:3 fatty acid from Arabidopsis chloroplast membrane lipids. The released polyunsaturated fatty acids can be subsequently oxygenated via enzymatic reactions or chemical oxidation to yield oxylipins such as Jasmonic acid (JA). Overexpression of PLIP1, 2, 3 resulted in stunted plant growth with altered leaf morphology and accumulation of JA and other oxylipin metabolites, redirecting the metabolism from growth to defense. Moreover, the expression of PLIP3 is responsive to abiotic stressors and ABA, and loss of function plip1, 2,3 triple mutants show sensitivity to abscisic acid (ABA), suggesting a possible PLIP3-based mechanism connecting JA and ABA signal transduction pathways in Arabidopsis. To gain a deeper understanding of how chloroplast membrane lipid-derived signals are involved in coordinating biotic or abiotic stress responses, we have conducted a genetic suppressor screen in the PLIP3-OX (PLIP3 overexpression) background to query the information chain from the origin of the lipid-based signal to its perception, transduction, and modification by other signaling pathways. Plants exhibiting (partially) restored growth or reversion of morphological phenotypes such as leaf shape and pigment accumulation were selected. Mutated loci will be identified by bulk sequencing analysis of segregating suppressor mutant populations and the affected proteins will be characterized. In addition, we designed a Course-based Undergraduate Research Experience and provided over 300 students the opportunity to work on an authentic research project in the classroom and for some in the research lab, creating a science-education community engaging both scientists and undergraduate students.

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Abstract# P062

Functional characterization of putative transcription factors of primary cell wall deposition in rice

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The structure of plant cell walls is highly dynamic and complex. Plant cell walls are typically classified into two types which are: primary cell wall and secondary cell wall. The primary cell wall (PCW) is mainly composed of cellulose (the most abundant polymer on earth), hemicellulose, and pectin. Secondary cell wall (SCW) comprised of hemicellulose (xylan and glucomannan), cellulose and lignin. Plant cell walls are also used as a source of renewable biomass to produce biofuels and bioproducts. To understand how cell walls biosynthesis contributes to plant development and biomass production, it is important to unravel the molecular mechanisms that regulate their deposition. While many transcription factors that regulate the SCW deposition are known, but very little is known about the transcription factors (TF) regulating the PCW. Currently, one study identified ERF035, a member of the AP2/ERF transcription factor family in Arabidopsis, as the first master switch for PCW biosynthesis. No such TFs is known in grasses. To overcome this issue, we used GAN analysis to identify TFs that are co-expressed with genes associated with PCW synthesis ana we identified five rice TFs (Os10g850400, Os09g0501600, Os02g0182800, Os08g0524800, Os03g0123500) that belong to Myb and KNOX family TFs. I hypothesize that these TFs participate in regulating the biosynthesis of PCW in rice. Thus, the goal of my project is to characterize these five putative TFs in rice by generating loss-off function mutants by using CRISPR-Cas9 technology and over-expressing lines. Then, I will do characterization of these mutants by biochemical and phenotypic analysis. Results of this work are highly significant, and it would be beneficial for both the agriculture and biofuels application.

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Abstract# P063

Candidate Proteins Located at Membrane Contact Sites Between Chloroplast Inner Envelope and Thylakoid Membranes

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Chloroplasts perform a variety of functions necessary for plant viability, particularly the capture and conversion of light energy by thylakoid membranes. Thylakoid membranes lack de novo glycerolipid synthesis, relying on lipid trafficking from the inner envelope membrane (IEM). However, mechanisms by which thylakoid lipids are exchanged with the IEM are largely unknown. Research into thylakoid/IEM lipid trafficking and the proteins facilitating the movement is largely hampered by a lack of robust function/targeting prediction and a paucity of bait proteins available for probing the physical gap between thylakoids and IEM. To address this, we compiled a set of candidate proteins that may participate in chloroplast lipid trafficking using three metrics. First, we interrogated public datasets for kingdom-wide functional homologs predicted to localize in chloroplasts. We supplemented this literature-based approach by using proteomic detection in double-fractionated chloroplast membranes, as well as enrichment in protein populations residing at MCS using a split-biotin ligase system. Candidate coding sequences were then cloned and fluorescently tagged for confocal laser scanning microscopy using transient expression in Nicotiana benthamiana by agroinfiltration. Of the 30 candidates screened thus far, six localized to chloroplasts, while several others localized to compartments near the chloroplast. These include proteins with domains predicted to confer lipid binding and/or MCS tethering functions, as well as proteins lacking known functions. Candidates are currently being assessed by co-localization with fluorescent protein MCS markers, co-fractionation with known chloroplast membrane proteins, and effects of knock-out/over-expression on chloroplast membrane morphology during low temperature de-etiolation. By experimentally determining constituents of lipid trafficking within the chloroplast, we aim to gain a better understanding of thylakoid membrane lipid dynamics.

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Abstract# P064

Insights into microbe mediated heat stress adaptation in soybean

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Heat stress is one of the major limiting factors for soybean productivity worldwide. Recent research has highlighted microbes crucial role in helping plants adapt to heat stress. Microbes modulate hormone levels, enhance nutrient uptake, induce stress-responsive genes (induced systemic resistance), improve soil health, and ultimately, protect the plants from enemies by forming biofilm in and around the rhizosphere. Therefore, our objective was to understand the role of native soil microbes in heat tolerance adaptation through 16S and ITS sequencing and develop an understanding of key genes, metabolites, and root anatomical traits in soybean. The experiment was conducted with four contrasting soybean genotypes (Williams 82, IAS 19C3, PI 639693, and PI 89008) for heat stress tolerance with (non-autoclaved) and without (autoclaved) microbes under high (day: 38o C for 16 hrs and dark: 28o C for 8 hrs) and optimum (day: 28o C for 16 hrs and dark: 21o C for 8 hrs) temperatures with 60% relative humidity and current ambient CO2 levels (400 ppm). Autoclaving did not change the physical properties of the soil, but the chemical property was affected. We have observed significant respiration differences between autoclaved and non-autoclaved soils; non-autoclaved soils showed a fourfold cumulative CO2 flux difference compared to autoclaved soils. The high-quality rhizospheric soil DNA was used for 16S (bacteria) and ITS (fungi) library preparation and MiSeq sequencing. Alpha and beta diversity revealed significant differences between the treatments. Higher bacterial abundance was observed at optimum temperature with microbes treatment. Pseudomonadaceae species were abundant across the treatments. Genotype-specific bacterial abundance was also observed across the treatments. Our study will help holistically understand the heat and microbial interactions to breed soybean cultivars for wider adaptation to variable future climatic conditions.

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Abstract# P065

Turning over a new leaf: Utilizing wild *Pseudomonas* to study host effector interactions

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The current system for studying effector triggered immunity in (lettuce) via transient expression with isn't sufficient to study the natural interaction between the host plant's immune system and phytopathogen derived effector proteins. This is because -mediated transient expression leads to unnaturally high levels of effector in the plant cell for an extended time, while most bacterial plant pathogens deliver a precise amount of effectors at fixed points during the colonization process via the type III secretion system (T3SS). The T3SS is evolutionarily diverse and effectors have secretion signals within the first 20-50 amino acids that direct the effector into the correct T3SS. Therefore, a naturally pathogenic bacterium of the same genus as the original isolate is required to investigate the delivery of type III-secreted effectors. To study the natural interaction between AvrRps4, a bipartite effector originally isolated from . , and the lettuce immune response we first collected diseased wild lettuce leaves from Columbia, Missouri. Bacteria were isolated from the leaves and we used DNA barcoding to identify strain KHDT2201. We infiltrated KHDT2201 into lettuce cultivars 'Ninja' and 'Kordaat' and found it to cause disease in both. Once transformed with AvrRps4, we expect to see recognition characterized by the easily-observed hypersensitive response in 'Kordaat' and not 'Ninja' based on previous results with Agrobacterium. AvrRps4 is found in a diverse range of crop pathogens and understanding its recognition by lettuce may contribute to the development of improvements for crop plants.

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Abstract# P066

TRV-AmCyan: A tool for robust visualization of viral movement and Cas9 editing in Nicotiana benthamiana

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Tobacco rattle virus (TRV) vectors are used for delivery of gene editing reagents to plants in the complete absence of tissue culture. Upon infection of transgenic Nicotiana benthamiana plants expressing Cas9, engineered TRV vectors moves systemically through the plant vasculature, expressing single guide RNAs (sgRNAs) that target endogenous sequences, such as phytoene desaturase (PDS). The lack of robust tools to measure infection frequency and track viral movement has left researchers to use labor intensive methods of RNA extraction and RT-PCR on random leaf samples, which can result in false negatives. Recent unpublished work in the Voytas Lab has shown that including the AmCyan fluorescent reporter on TRV is a reliable method to visualize viral movement in real-time. To build on the utility of the TRV-AmCyan visualization strategy, this work has focused on correlating AmCyan signal with Cas9 editing and assessing heritability of edits. If AmCyan signal can be correlated with editing in somatic cells, this strategy could be used to quickly determine tissues that contain potential edits for screening, reducing laborious sampling methods. The results obtained from this study will be an important foundation for efficient translation of TRV-mediated editing in different plant species.

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Abstract# P067

Testing of a metabolic engineering strategy to increase phenylalanine in plants

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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 would be useful in creating biofuels, medicines, and other products to improve quality of life. The phenylalanine metabolic pathway, however, is not fully understood due to its dual nature. In phenylalanine synthesis, there are two separate pathways that exist: the plastidial and cytosolic pathways. The purpose of this study is to determine if the cytosolic pathway could be engineered in order to manipulate the output of phenylalanine in the cytosol. 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 petunia plants. It is expected that in the petunia, the metabolic changes will increase the net production of phenylalanine and phenylalanine-derived aromatics. These observable changes would support the conclusion that the cytosolic pathway is a viable target for manipulating phenylalanine production.

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Abstract# P068

Modified Bt toxin to control soybean aphids

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The soybean aphid is a significant pest of soybean in the US Midwest. When left untreated, aphid infestations can lead to yield loses of up to 40%. Common management strategies involved insecticide applications that increase production costs and have negative environmental impacts. Thus, novel strategies of soybean aphid control are needed. A well-established strategy to control insect in other crops is the use of transgenic plants expressing Bacillus thuringiensis (Bt) toxins. However, Bt toxins do not have effect on aphids. In previous work, the Bt toxin Cyt2Aa protein was altered by the introduction of peptides that increase binding the pea aphid, which resulted in increased toxicity to that aphid species in in vitro feeding assays. To study the potential of a similar strategy against the soybean aphid, a Bt toxin gene was modified by inserting sequences encoding gut-binding peptide sequences in different parts of the protein sequence and transgenic plants were generated by Agrobacterium transformation. The inserted DNA fragment also included glufosinate herbicide resistant gene as a selection marker. Multiple lines of soybean seeds were produced. We screened the progeny of these lines and identified plants that have herbicide resistance and are thus transgenic. Transgene segregation was observed in several lines. Phenotypic analysis suggests that the modified Bt toxin does not have deleterious effects on plant germination or vegetative growth. RT-PCR analyses to confirm to confirm expression of the transgene and western blots to assess accumulation of the Bt protein are underway. Plants that accumulate significant levels of Bt toxin will be evaluated using aphid bioassays to determine protection against soybean aphids.

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Abstract# P069

Understanding the response mechanisms involved in phosphorus deficiency in popcorn

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Agriculture expansion combined with the need for sustainable farming activities is a major drive for breeders to introduce plant cultivars better adapted to abiotic stress conditions such as nutrient deficiency. Phosphorus (P) is an essential plant nutrient for photosynthesis and therefore plant growth, however phosphorus is mainly found in soil in forms that are not directly available by the plant making it a major limiting factor for growth and development of crops. Here we investigated the response to low and high P availability in two hybrid lines of popcorn (Zea mays L. var. everta); L80 (P-inefficient) and P7 (P-efficient), selected from previous work for their different ability to use P based on their grain yield. In addition to physiological data to measure the P efficiency and plant growth, a proteomics approach was used to determine which metabolic pathways are responsible for the improved phosphorus use in P7. Significant differences in protein abundance among P supply conditions and between the P7 and L80 lines were observed. A total of 421 differentially accumulated proteins (DAPs) were identified in the P-inefficient line and 436 in the P-efficient line. Among the identified DAPs, 67 and 52 proteins were unique to high P, and 121 and 151 proteins were unique to low P in L80 and P7, respectively. These proteins were involved in photosynthesis, but also in secondary metabolites and protein biosynthesis. Our results also allow us to understand the major differences in the P-deficiency response mechanisms between P7 and L80 and provide new insights into the mechanisms needing investigation to improve use of P in popcorn.

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Abstract# P070

Conservation of stomatal traits in Arabidopsis thaliana ecotypes from arid and temperate environments

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Plasticity is a critical dynamic response to environmental changes. In response to water scarcity, stomatal plasticity is especially important due to its function as a regulator of water loss from the leaf. Stomatal plasticity can occur anatomically via reduction in number (stomatal density, SD; stomatal index, SI), area (stomatal area, SA), and total pore coverage on the leaf, measured as stomatal pore index (SPI). To interrogate both adaptative and anatomical characteristics of stomatal plasticity in response to water scarcity (WS) conditions, we assessed stomatal trait plasticity in response to WS of Arabidopsis ecotypes from diverse environments. Interestingly, ecotypes from low and high aridity environments exhibited relatively lower SPI across both WW and WS treatments driven mainly by a decreased SA on the adaxial side of the leaf and SD on the abaxial side of the leaf. SPI response was positively correlated with the theoretical maximum gas exchange (gsmax). These ecotypes also showed relatively lower SI across both treatments. However, no ecotype exhibited a significant change in SI or SPI between WW and WS conditions. These results suggest that the reduction SPI and SI is conserved across different ecologies. Further, our data also support a trend of evolutionary selection for Arabidopsis in arid ecology to reduce both SI and SPI to mitigate water loss as suggested by decreased gsmax. As our experiment directly assesses anatomical leaf characteristics but not additional variables (e.g., transpiration as a function of stomatal closure) future research is merited and will focus on assessment of physiological responses to WS and the effect of other environmental variables.

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Abstract# P071

Transcriptional regulation of CO2 and Nitrogen metabolic interactions in Arabidopsis

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Plant Carbon (C) and Nitrogen (N) metabolism are intricately connected. Rising atmospheric CO2 levels directly affect the C and N metabolism and their interactions. Elevated CO2 grown C3 plants has altered allocation of N causing reduced investment in Rubisco and has reduced carboxylation rate (Vc,max). These changes contribute to the negative acclimation of photosynthetic capacity. A decline in plant N concentration is also observed under elevated CO2 leading to decreased nutritional quality of C3 plants. Altering genetic factors controlling CxN metabolic crosstalks could provide genotypes better suited for high CO2 conditions, i.e., has little to no acclimation of photosynthetic capacity and maintain N concentration similar to ambient CO2-grown C3 plants. We constructed a gene regulatory network of Arabidopsis plants grown under CO2 and N treatments. We found bZIP1 as one of the regulators of CxN responsive genes. Next, we analyzed the overexpression of bZIP1 TF experimentally. bZIP1-overexpression (bZIP1-OX) line accumulated more plant biomass under elevated CO2 conditions than wildtype plants, while N and protein concentration remained similar between the two genotypes. RNA seq analysis of bZIP1-OX plants revealed CxN responsive genes differentially regulated by bZIP1-OX are enriched in Jasmonic Acid (JA) signaling and synthesis pathways. Further, co-expression network analysis was utilized to identify the gene modules correlated with above-ground biomass. Modules having a significant positive correlation with biomass constitute genes related to JA-related processes. In conclusion, our study shows bZIP1 as a potential target for biomass increase under elevated CO2. RNA seq analysis shows in bZIP1-OX plants, biomass increase, and JA-related genes' expression are correlated. This indicates that JA plays a role in C & N crosstalks in Arabidopsis and may play a role in biomass regulation under elevated CO2 conditions.

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Abstract# P072

The diversity of diterpenoid biosynthetic gene clusters in rice

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Rice (Oryzae sativa) is a staple food crop and a model cereal plant. Instead of central metabolites, plants also produce a large number of secondary metabolites, which reflect the current ecological interactions with environment. One kind of important secondary metabolite in rice is labdane-related diterpenoids (LRD), serving as phytoalexins and playing an important role against rice pathogens. However, the rice diterpenoid metabolic network is complex and not well-known. Until now, there are three biosynthetic gene clusters discovered in rice which are related to diterpenoid biosynthesis, including chromosome 2 BGC (c2BGC), chromosome 4 BGC (c4BGC) and chromosome 7 BGC (c7BGC). All these three clusters contain terpene synthases which form the initial backbone defining the types of diterpenoids, and downstream tailoring/decorating enzymes which add oxygen to enhance polarity and hydrogen-bonding capacity. Different from bacteria functionally cluster genes co-transcribed as a single mRNA, each rice cluster gene is transcribed as a single mRNA. However, they still show strong collinear and syntenic relationship on chromosome. Notably, in contract to widely conserved c2BGC and c4BGC, c7BGC is subspecies (ssp.) specific, being prevalent in ssp. Japonica and rarely in ssp. Indica. Another not conserved gene is OsKSL8j discovered in ssp. Japonica cv Nipponbare firstly, which is required for the Oryzalexin S biosynthesis. But it is being replaced by its allele OsKSL8i in ssp. Japonica, with concurrent disappearance of Oryzalexin S. These results indicate that these biosynthetic genes are dynamic and changeable, resulting in birth and loss of natural products in rice.

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Abstract# P073

Confocal analysis of stably-expressed Agmatine Iminohydrolase and N-carbamoyl putrescine hydrolase in A. thaliana.

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Putrescine is the smallest of the polyamines, a group of metabolites essential for plant growth and development. In A. thaliana, one of the biosynthetic pathways for putrescine occurs via Arginine decarboxylase 1, which was localized to the ER (You et al. 2019), and the subsequent metabolism of agmatine to putrescine by the enzymes Agmatine Iminohydrolase AIH and N-carbamoyl putrescine amidohydrolase respectively (NLP1). This research describes the confocal analysis of stably transformed F1 plants expressing AIH.1-GFP and NLP1.1-GFP in young A. thaliana seedlings. Both proteins are localized to a membrane bound organelle within the cytoplasm of cells consistent with that of the ER. AIH.1 was localized to the membrane abutting the developing chloroplasts in etiolated hypocotyls. In the same tissues, NLP1.1 was localized to subcellular regions distant from chloroplasts. The differential localization pattern of these genes was mirrored in the transient expression of these isoforms in N. benthamiana. These results provide additional support for an emerging model indicating that the ER is a major hub for the synthesis and export of putrescine.

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Abstract# P074

East Asian-North American disjunctions and phylogenetic relationships within subtribe Nepetinae (Lamiaceae)

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Biogeographic disjunctions are found across plant lineages and have been of major interest to biologists for centuries. Research on this subject has been reinvigorated by recent advances in molecular dating and associated comparative methods. One of the “classic” disjunction patterns is that between Eastern Asia and Eastern/Western North America. It has been speculated that this pattern is the result of vicariance following the sundering of a widespread Acrto-Teritary flora. The subtribe Nepetinae in the Lamiaceae (mint family) is noteworthy because it contains three genera with this intercontinental disjunction pattern: Agastache, Dracocephalum, and Meehania. These disjunctions are ostensibly the result of three separate events, allowing for concurrent testing of the tempo, origin, and type of each biogeographic event. We use phylogenies based on chloroplast and nuclear data to estimated divergence times and analyze the historical biogeography of Nepetinae. We show that the three disjunctions are “pseudo-congruent”, with unidirectional movement from East Asia to North America occurring at slightly staggered times during the late Miocene and early Pliocene, about 3.5-10 million years ago. With the possible exception of Meehania, we find that vicariance is likely the underlying driver of the disjunctions.

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Abstract# P075

Sequence does not matter! A tale from priming in sorghum against insects of different feeding guilds

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Sorghum, one of the world's most important cereal crops, suffers severe yield losses due to attack by insects of different feeding guilds. In most instances, emergence of these pests are not secluded incidents and are followed by another or can also co-occur. Sugarcane aphid (Melanaphis sacchari) (SCA) and fall armyworm (Spodoptera frugiperda) (FAW) are the two most important and destructive pests of sorghum, which belongs to sap-sucking and chewing feeding guilds, respectively. Defense-priming is an induced physiological state where the plants are trained to hyperactivate their defenses upon environmental challenges. In this study, sequential feeding on the sorghum RTx430 genotype by either FAW primed-SCA or SCA primed-FAW were monitored to unravel the mechanisms underlying defense priming, and its mode of action. Regardless of the order of herbivore arrival on sorghum RTx430 plants, a significantly induced defense phenotype was observed in the primed state compared to the non-primed condition, irrespective of their feeding guild. Additionally, gene expression and secondary metabolite analysis revealed differential modulation of the phenylpropanoid pathway upon insect attack by different feeding guilds. Our findings suggest that priming in sorghum plants upon sequential herbivory induce defense by the accumulation of the total flavonoids/ Jasmonic acid and lignin/ Salicylic acid in FAW primed-SCA and SCA primed-FAW interaction, respectively.

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Abstract# P076

Genetic control of photoprotection and photosystem II operating efficiency in plants

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Photoprotection against excess light via nonphotochemical quenching (NPQ) is indispensable for plant survival. However, slow NPQ relaxation under low light conditions can decrease yield of field-grown crops up to 40%. Using a semi-high-throughput assay, we quantified the kinetics NPQ and photosystem II operating efficiency (ΦPSII) in a replicated field trial of more than 700 maize (Zea mays) genotypes across two years. Parametrized kinetics data were used to conduct genome-wide association studies. We identified numerous candidate genes involved in NPQ and ΦPSII kinetics in maize. For six top candidates, we verified the orthologs in arabidopsis (Arabidopsis thaliana): two thioredoxin genes, and genes encoding a transporter in the chloroplast envelope, an initiator of chloroplast movement, a putative regulator of cell elongation and stomatal patterning, and a protein involved in plant energy homeostasis. Since maize and arabidopsis are distantly related, we propose that genes involved in photoprotection and PSII function are conserved across vascular plants. The genes and naturally occurring functional alleles identified here considerably expand the toolbox to achieving a sustainable increase in crop productivity.

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Abstract# P077

Linking GAUT10 Function to Root Cell Wall Composition and Auxin

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Decades of genetic and biochemical experiments have led to a deep understanding of the molecular components that influence Arabidopsis primary root growth. An outstanding question in the field is how cell wall composition within the root apical meristem (RAM) is modulated to impact cellular growth and differentiation. The Kelley laboratory has recently identified an auxin regulated cell wall modifying enzyme, GAUT10, to be required for RAM maintenance. In this project I will use genetic and biochemical approaches to delineate the molecular mechanisms associated with GAUT10 activity within the RAM and further our understanding of Arabidopsis root development. For this project I will use the plant model Arabidopsis thaliana (Arabidopsis) and existing genetic resources available in the Kelley lab. I will be responsible for genotyping F2 populations generated from F1 individuals which were created by Dr. Kelley by crossing gaut10 mutants with three different loss of function mutants: tob1and dao2. These mutants have been previously characterized and were selected because these genes are all elevated in gaut10 roots compared to wild-type. I will test the hypothesis that loss of these genes can suppress the gaut10 short root phenotype. For each F2 population I will grow out and genotype around 60-70 plants, with the expectation that 1/16 will be double mutants. First I will perform DNA extractions and then genotype each F2 population for three alleles (GAUT10/gaut10-3, TOB1/tob1-2, and/or DAO2/dao2-1) using polymerase chain reactions (PCRs). Genotyping the F2 populations allows me to identify the desired double mutants. I will collect seeds from F2 individuals with the genotype(s) of interest and then plant F3 individuals for phenotypic analyses. I will measure root lengths using the segmented line tool in Fiji/ImageJ and perform a ANOVA analysis to determine if the double mutants are the same or different compared to the single parental mutants.

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Abstract# P078

Regulation of Heat Stress through Heat Shock Factors in the Model C4 Monocot Setaria viridis

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Cereal grains are vital for feeding and supplying energy for a continually growing population; however, climate change poses an increasing risk to key crops. As stressful abiotic conditions become more common, it is vital to understand how cereals respond to heat stress. Thermotolerance is the process by which plants respond and remember heat stress events and is linked to decreased growth and yield. Heat Shock Factor (HSF) is a major transcription factor (TF) family in eukaryotes and is essential for heat tolerance. This family is conserved in all eukaryotes and is highly expanded and diversified in plants. The current model of genetic and phenotypic responses to thermal stress events come from eudicots such as Arabidopsis and tomato; however, a deeper understanding of the responses of C4 monocots could bring better crop yields through improved heat tolerance. To address this gap, we aimed to evaluate the existing eudicot-centric model in Setaria viridis, a model C4 monocot, to evaluate key functional drivers of thermotolerance and construct a putative regulatory pathway. The key HSFs, HSFA1, HSFA2, and HSFA6, will be knocked out through CRISPR/Cas9 genome editing. We will assess the phenotypes of mutant plants, as well as diverse wildtype ecotypes, to investigate the regulatory hierarchy of genes during heat stress.

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Abstract# P079

GmCSD1 Isoforms and Gma-miRNA398 Interactions within the Soybean Drought Response

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Drought is a major cause of low yields in soybean production. The drought response pathway in soybean plants is regulated by microRNAs, small RNA sequences that silence genes by binding to specific complementary mRNA sequences, post-transcriptionally. Multiple mRNA isoforms can be transcribed from the same DNA strand through the process of alternative splicing. In this study, we decided to use two different soybean lines to investigate this pathway: drought resistant (PI416997) and drought susceptible (PI398965), with each line in both drought and well-watered conditions. After extracting total RNAs, we measured transcription levels of miRNAs and their target genes with gene-specific primers. Among three candidate miRNAs, miRNA398 was highly expressed under the well-watered condition and not expressed as much under the drought condition. Interestingly, the transcription levels of miR398 had more dramatically decreased in the drought resistant line. This result led us to investigate the transcriptional analysis of target genes. GmCSD1 is a well-known target gene of miR398, but one isoform, GmCSD1b, does not have the MBS. The GmCSD1 gene minimizes the harmful effects of drought by eliminating reactive oxygen species (ROS), a harmful substance in the cell that can lead to reduced metabolic activity and potentially cell death. qRT-PCRs revealed that GmCSD1a was more highly expressed in the drought condition than in the well-watered condition. GmCSD1b expression showed no significant difference between the different lines and conditions. This relationship is important for understanding how soybean plants respond to drought and how microRNA affects alternatively spliced mRNA regulation in the plant.

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Abstract# P080

Exploring the genetic diversity of Ketoacyl-CoA Synthetase (KCS) genes involved in plant fatty acid elongation across a diverse set of maize inbred lines

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The cuticle produced by the plant epidermis performs many essential functions, including acting as a water barrier to seal in moisture, facilitating removal of debris, protecting against pests and pathogens, and shielding photosystems from excess UV radiation. The cuticle is comprised of a cutin matrix that is infused with and laid atop by cuticular waxes, which can include very long chain fatty acids, fatty aldehydes, alcohols, hydrocarbons, ketones and/or wax esters. The precursors of cuticular waxes are very long chain fatty (VLCF) acyl-CoAs. VLCF-acyl-CoAs are products of the Fatty Acid Elongation (FAE) pathway that operates in the endoplasmic reticulum of the plant cell. The first step in the FAE pathway is the condensation of a VLCF-acyl-CoA with malonyl-CoA, which is catalyzed by a 3-ketoacyl-CoA Synthetase (KCS) to form a 3-ketoacyl-CoA. There is a great deal of genetic redundancy for this step in the pathway, with 26 unique KCS genes present in the reference maize genome (inbred B73). In this study, we examine the genetic diversity of KCS genes across 26 genetically diverse inbred maize lines that are the parental founders of the Maize Community's Nested Association Mapping (NAM) as well as three additional inbred lines of agronomic value (B104, Mo17, and W22). Importantly these maize inbred lines have been sequenced and exhibit structural variations when comparing across genomes, including the presence/absence of sequences, including genes. Using a suite of sequence comparison and phylogenetic analysis tools, we are assessing whether the KCS gene family is dynamic in gene number across inbred lines and the breadth of genetic diversity that exists among these genes. This work will shed light on the genetic diversity of KCS genes and provide the foundation for studying potential diversity in function across the gene family.

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Abstract# P081

Comparative Analysis of the Root System Architecture of Vitis rupestris and Vitis riparia, Important Genetic Resources for Grape Breeding

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The North American grapes species Vitis rupestris Scheele and Vitis riparia Michx have been the pillars of rootstock breeding for the past 150 years. Though a large body of viticultural knowledge has been accumulated on their impact of grafted scions, the architecture of their root systems has received limited scientific attention. In this study, we generated and comparatively analyzed adventitious root systems from dormant cuttings of 22 V. riparia and 22 V. rupestris accessions obtained from the USDA grape Germplasm collection in Geneva, NY. We photographed the roots and then extracted 35 phenotype elements (phenes) of the root system architecture (RSA) from two-dimensional images using the software RhizoVision Explorer. Principal component analysis (PCA) of seven uncorrelated phenes showed that PC1 explained 56.2% of the variation and arranged the two species into partially overlapping but clearly separate clusters. Phenes that differed most significantly were width ($p = 0.000009$), number of roots ($p = 0.00002$), convex area ($p = 0.0005$), total root length ($p = 0.001$), depth ($p = 0.002$), lower root area ($p = 0.002$), network area ($p = 0.001$), and perimeter ($p = 0.001$), all of which were greater in V. riparia. V. rupestris, however, had a higher frequency of steep root angles ($p = 0.004$), greater root diameter ($p = 0.004$), and root system solidity ($p = 0.02$), indicating that the two species differ in the overall structure of the RSA, in addition to the size of the root system. We also collected RSA data from a V. rupestris X V. riparia F1 hybrid progeny. Using a GBS marker-based integrated linkage map of the parents, we mapped two QTL on the V. riparia genome which explained 13.1 % and 12.6% of the variance in the number of roots and the depth of the root system, respectively, and two QTL on the V. rupestris genome which influenced a range of phenes, including width, depth, perimeter, the lower root area, and the number of roots.

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Abstract# P082

Conservation of mechanisms for post-translational regulation of the intrinsically-disordered TCP transcription factors

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Plants respond to changing environmental conditions through rapid, reversible, and dramatic changes to their gene expression profiles- this frequently come at significant metabolic cost to the plant, leading to 'stress-response tradeoffs', as seen in the inhibition of growth through activation of immune signaling. Current models of plant transcription describe the modulation of transcription factors (TFs) along a spectrum of activity caused by changes in the accumulation, aggregation, and interactions of diverse sets of TFs within the cell. Transient alterations to net gene expression can then occur in an efficient and reversible manner. A plant transitioning from benign to stressful growth conditions, may therefore respond appropriately by altering the subcellular location and composition of transcriptional regulatory protein complexes governing associated genes. Proteins that exhibit this complexing behavior often lack a strictly defined structure, or rather are enriched in 'intrinsically disordered regions' (IDRs) or Prion-Like Domains (PLDs) that allow flexibility to adopt many structures- and with them new sets of interacting partners, locations, and gene expression profiles. Similar patterns of disorder between groups of proteins may reflect adoption of similar regulatory mechanisms perceived environmental signals. One notable family of highly conserved, plant specific TFs exhibiting these structural motifs are the TEOSINTE BRANCHED1/CYCLOIDEA/PCF proteins or TCPs. Here, we present data supporting a mechanism for post-translational control of TCP transcriptional activities and the conservation of this mechanism between vascular (A. thaliana) and nonvascular (P. patens) plant systems.

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Abstract# P083

Variation in freeze tolerance and circadian clock traits among diverse Arabidopsis ecotypes

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The circadian clock, a 24-hour oscillator present in all kingdoms of life, coordinates physiological changes with respect to the time of day and surrounding environmental signals, such as light and temperature. Defined by a set of transcriptional-translational feedback loops that generate time of day gene expression, the circadian clock regulates genes involved in abiotic stress response such as freeze stress. To harness and apply the benefits of the circadian clock, the genes key to time of day stress response must first be identified. We evaluated the circadian rhythm and freeze stress tolerance of ten geographically diverse Arabidopsis thaliana ecotypes. Freeze response phenotypes were determined by measuring Fv'/Fm', size, damage level, ROS, and time to flower after a freeze stress event. The results showed differential levels of freeze tolerance between ecotypes. To phenotype their circadian clocks, we performed a leaf movement experiment under cold and control conditions. This demonstrated variation in the circadian period between ecotypes which may contribute to their differential stress tolerances. Differences in temporal gene expression between a cold-tolerant and cold-sensitive ecotype was determined through time course RNA sequencing. Tissue for RNA sequencing was harvested every four hours for one day during a cold acclimation period of 10º C day/4º C night, and once again after a -7º C drop. Results indicate differences in expression of circadian genes as well as cold responsive genes between the two genotypes. This dataset will allow us to identify temporal regulators of cold response and candidate targets for crop improvement.

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Abstract# P084

Quantitative Trait Loci (QTL) Influencing Leaf Elemental Concentrations in Grapevine (Vitis sp.)

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In grapevine, the influence of elemental concentrations (the ionome profile) on fruit and wine quality is well established, yet the genetic basis of the ionome profile received limited scientific attention. In this study, we analyzed the leaf ionome of 131 interspecific F1 hybrid progeny from a cross between a Vitis rupestris Scheele (♀) and a Vitis riparia Michx (♂) plant. This F1 progeny was replicated in Western New York (NY), South Dakota (SD), Central Missouri (CMO), and Southwest Missouri (SWMO). We sampled leaves in all four experimental vineyards at three different times during the growing and had the concentration of 19 elements measured using ICP-MS. Principal component analysis (PCA) performed on the concentration of all 19 elements demonstrated that PC1 and PC2 accounted for 10.1-27.3% and 7.2-15.7% of variance, respectively. Using a GBS-based linkage map and the concentration of individual elements as phenotype, significant recurring QTL were mapped to chromosomes 2, 4, and 7. A V. riparia QTL was identified at p=0.010 in the region between 83.1 and 86.4 cM on chromosome 4 which explained 39.7-41.9 % of variation in Zn concentration in both SWMO and CMO. In V. rupestris, two QTL were found: one mapped to the 7.9 to 12.-cM region on chromosome 2 and explained 22.7-38.7 % of variation in Ni concentration ($p < 0.014$); the other mapped to the 65.8 to 67.1-cM region on chromosome 7 and explained 31.2-47.9% of variation in Rb accumulation (p=0.014). Both V. rupestris QTL were detected in both the CMO and the NY vineyards. Interestingly, when using the PC1-derived score as a phenotype to represent the complete ionome, a significant QTL was mapped to exactly the same locus on V. rupestris chromosome 7 as the QTL for Rb concentration. The PC1 score QTL was detected in CMO and NY (p=0.010, and p=0.012) where it explained 10.05 and 12.14% of the variation, respectively.

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Abstract# P085

Genetic basis of heterosis in natural populations of Arabidopsis thaliana.

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Most new mutations are on average partially deleterious and partially recessive. In natural populations with small effective population size, the probability of fixation of some deleterious partly recessive alleles increases due to a reduction in the effectiveness of natural selection because of random drift. Crosses between individuals of different populations are expected to be heterozygous for fixed partially recessive alleles and could exhibit heterosis, defined here as the increase in fitness of F1 cross relative to the mean of the parental lines. I study the genetic basis of heterosis in two natural populations of A. thaliana from Italy and Sweden using a genome-wide panel of homozygous Near-Isogenic Lines (NILs). Each NIL was backcrossed to the background parental ecotype to generate a panel of heterozygous NILs. The two parental lines were also crossed to generate heterozygous F1 lines in each region where the parental ecotypes differed. The heterozygous NILs will allow me to partition the overall heterosis of the F1 crosses between the parental ecotypes into the effects of individual genomic regions. I used field data from multiple years to simulate in a chamber the Italy ecotype environmental conditions to measure heterosis for fitness components (fecundity and survival). Overall heterosis for the F1 cross between the parental ecotype was 51%. Only one heterozygous NIL showed heterosis respect the parental ecotype. However, six heterozygous NILs showed a lower fitness with respect the parental ecotype. Results suggests that somewhat heterosis is evince as a possible consequence of additive effects and dominance complementation. However, at the same time the reduction in fitness for some of the crosses is possibly due to negative epistatic interactions.

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Abstract# P086

Effects of fire on plant species in grazed grasslands

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In late April of 2022 the Road 702 Wildfire, a stand-replacing event, ravaged an estimated 41,155 acres in Furnas County, Nebraska. The most recent fire of this magnitude occurred an estimated 136 years ago. The intention of this study is to discover how plant species diversity varies among pastures that have historically been grazed to different extents. Specifically, this study compares three different pastures: One pasture has been impacted by both the fire and overgrazing due to cattle, the second was impacted by the fire but was not grazed, and the third pasture, a control, consists of a piece of land that was not impacted by the fire, and has not been grazed for two decades. The variety of sites used in this study allows for a wide diversity of plant species. Using a random sampling collection quadrat method to measure density and diversity within the different pastures, we took a base sample of plant species during the late fall of 2022. Monitoring precipitation levels throughout the summer of 2023, and monitoring plant population fluctuations in these grasslands plots are essential for the practicality of this study. This study will continue for a minimum of four years in order to collect the data necessary to assemble conclusive results regarding the effects of native and invasive plant species in grazed settings that result after a prescribed burn or wildfire. This study will help inform fire prescription management techniques by elucidating plant diversity patterns under the influence of fire and grazing.

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Abstract# P087

Investigating the dynamic regulation of plasmodesmata during bacterial infection

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Plasmodesmata (PD) provide physical connections between adjoining plant cells, serving as communication channels. Given their fundamental roles, PD are important for plant survival and defense. It has been reported that PD-located proteins (PDLPs) play important roles in plant immunity. The expression of PDLP5 is upregulated by bacterial infection, whereas Pseudomonas syringae (Pst) effector HopO1-1 targets Arabidopsis PDLP5 and PDLP7. We recently implemented enzyme-catalyzed proximity labeling (PL) to identify functional partners of PDLP5. We further utilized the PL assay to capture the dynamic changes in PDLP5-containing protein complexes upon bacterial infection. We identified over 200 proteins that might function with PDLP5 during bacterial infection. GO enrichment analysis shows that proteins involved in response to stress (GO:000695), vesical-mediated transport (GO:0016192), and localization (GO:0051179) are significantly enriched by bacterial infection. We first selected over 20 candidates to examine their PD association. We detected 9 proteins to be associated with PD. We then focus the functional characterization of cysteine-rich receptor-like kinases (CRKs). CRK2 has been reported to function at PD and phosphorylates NADPH/respiratory burst oxidase protein D (RBOHD). Using protein complex modeling program AlphaFold-Multimer (AF-M), we predicted CRKs-PDLP5, CRKs-RBOHD, and CRKs-RBOHF heterocomplexes. Our preliminary data suggest the role of the CRKs as negative regulators of callose accumulation at PD and plant immunity against bacterial infection. We hypothesize that CRKs regulate reactive oxygen species production during bacterial infection to regulate the PD function and plant immunity. Our study demonstrates the power of the PL assay and AF-M in studying the dynamic regulation of PD during plant immunity.

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Abstract# P088

Carbon Allocation to Mutualists in Tripartite Interactions with Medicago Truncatula

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Mutualism is prevalent in plants, yet different mutualists affecting the same host are rarely studied. Here, we study the leguminous plant, Medicago's tripartite interaction with two symbionts: rhizobia and arbuscular mycorrhizal fungi that benefits plants by fixing atmospheric nitrogen and enhancing the phosphate and nitrogen uptake from the soil respectively. As a result, plants provide them with carbon. With our previous experiments, we found that arbuscular mycorrhizal fungi can successfully compete with rhizobia for the host carbon when they have access to an exogenous nitrogen source. Now our goal is to identify the time of shift of carbon allocation by the host to arbuscular mycorrhizal fungi when provided with ammonium or the other symbiont, rhizobia is functionally defective in nitrogen fixation.

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Abstract# P089

Artificial host systems (AHS) for a parasitic plant, Cuscuta Campestris.

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Cuscuta Campestris is a destructive parasitic plant that grows throughout most of central North America, mainly inhabiting anthropogenic croplands. Haustoria, an organ within C. Campestris, connects the parasite to its host plant, which exudes from the parasite's shoot and infiltrates the host's phloem to receive nutrients necessary to complete its life cycle. The development of these parasitic organs depends on environmental factors such as the presence of far-red light, physical touch, temperature, and moisture content (Furuhashi et al., 2021). C. Campestris has been shown to complete haustorium development in these artificial host systems (AHS) (Bernal-Galeano et al., 2022). These systems can help remove environmental and host-related factors that complicate research involving plant hosts and create a contained and sterile environment that allows for easier repetition of research. In this study, we have developed the AHS protocol for future applications. In several preliminary tests, C. Campestris shoots were placed under agarose gels, as well as attempting to inoculate C. Campestris onto wooden dowels wrapped in KimTech wipes. All of these projects yielded minimal haustoria development. After many designs, we modified the previously reported AHS with Magenta Tissue Culture Boxes (Bernal-Galeano et. al 2022). In this final design, each AHS consists of a fibrous stick with capillary ability on which C. Campestris is inoculated, placed in a liquid solution, and held up by a 3D-printed stand within tissue culture boxes. These 3D printed pieces, which were designed by Max Sproull and made by the MU 3D printing lab, are made of polylactic acid (PLA), cost $0.50 each and took 1 hour 37 minutes to make. With this system, we will investigate the physiological and molecular biological analysis of haustoria development.

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Abstract# P090

An Inducible in vitro system to reprogram somatic cells into a gamete fate

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Fertilization is a critical biological process for species survival and genetic diversity. In plants, seeds are the direct products of double fertilization and are the basis of nearly all our food. Despite its importance in agriculture, little is known about the processes that lead to fertilization success and the communication between plant male and female gametes. Because fertilization occurs deep into the flower tissues and involves just a few cells in the plant, studying sperm-egg interactions is technically challenging. To overcome these difficulties, we established an estradiol-inducible system in Arabidopsis thaliana to test the potential to reprogram somatic leaf cells into gametes. Inducible UBQ10p:RKD2-GFP and UBQ10p:DUO1-mCherry, master regulators of sperm-egg cell identity, were introduced into the background of several transgenic lines that express egg and sperm cell-specific markers. Protoplasts isolated from four to six-week-old rosette leaves were monitored using fluorescence microscopy for nuclear expression of RKD2-mCherry and DUO1-GFP following induction by β-estradiol. In addition, we verified the expression of plasma membrane gamete-specific markers to validate a shift from somatic to sperm or egg-like state. We will report our preliminary findings on the ability of this system to reprogram somatic cells into sperm and egg cell fate. If successful, this novel in vitro "artificial gamete" platform can facilitate the study of protein-protein interactions occurring at the cell surface of gametes and ultimately lead to a better understanding of how fertilization and seed development are regulated in plants.

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Abstract# P091

Hemp's Hide-and-Seek: Cannabis sativa L. (Cannabaceae) tested as a host for a parasitic plant, Cuscuta campestris

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Cuscuta spp. (commonly known as dodder) is a parasitic plant that latches onto a host with the formation of haustorium, penetrating the host's vascular system to collect life sustaining nutrients, water, and metabolites. There are around 200 species of Cuscuta, each with its own favorite host plants. Cuscuta campestris was the first Cuscuta species to report full genome sequences, launching its wide used for host-parasitic plants interaction. In research of C. campestris, tomatoes, beets, and Arabidopsis are commonly used host plants for understanding host-parasites interaction. Here, we explored the host preference of C. campestris with industrial hemp (Cannabis sativa L., Cannabaceae) which has not been reported as a host of Cuscuta. To investigate industrial hemp and Cuscuta interaction, we observed Cuscuta parasitism on industrial hemp. Seven days after Cuscuta inoculation, we found successful parasitism were Cuscuta tightly coiled on industrial hemp and developed haustoria. After the successful Cuscuta haustoria development stage, Cuscuta stopped growing on industrial hemp, notably never dying. Sectioning and Tolidine blue O (TBO) staining assays revealed that Cuscuta fully penetrated the host stem. However, due to the positioning of industrial hemp's vascular system, Cuscuta searching hyphae were unable to reach host vascular tissue. Future research can explore inoculation of newer hemp tissue with underdeveloped cortex- the barrier between haustoria and the xylem and through staining hopefully reveal how the Cuscuta was able to survive past the haustoria development stage. Alternatively, further research can explore other varieties of Cuscuta, such as C. australis or C. reflexa, that have shown to have more aggressive haustoria that could possibly penetrate hemp's central vascular system.

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Abstract# P092

Investigation of Chrono-Freeze, a Novel Circadian Rhythm Pattern Induced by Cold Stress

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Abstract: Previous studies have identified a novel phenomenon, called chrono-freeze, in Solanum lycopersicum, where circadian clock genes lose their oscillation pattern and maintain a static expression level under cold stress. This study aims to investigate the prevalence and molecular mechanisms of this phenomenon in angiosperm species. The primary hypothesis of this study is that the phenomenon of chrono-freeze observed in tomato is present in all angiosperms but specifically lost in cold-adapted species, suggesting that the loss of chrono-freeze is connected to the evolution of cold stress tolerance. Our results show that when Solanum lycopersicum plants are subjected to a three-hour cold stress treatment (4°C) in the morning, essential circadian clock genes lose their oscillation pattern and maintain a static expression level. Additionally, when subjected to varying durations of cold stress treatment (4°C) for 3, 6, and 12 hours, clock genes lose their oscillation pattern for the duration of the cold stress. Upon returning to ambient temperature (24°C), cold-treated plants act as if they are seeing light for the first time in the morning and show peak expression in CCA1 and PRR7 clock genes after the same amount of time as the no-cold control after it saw the light. Our results suggest that the input signal from cold suppresses the light signal that tomato plants receive during cold treatment. In conclusion, our findings provide insights into the prevalence and molecular mechanisms of the chrono-freeze phenomenon in angiosperms, which may have significant implications for understanding plant adaptation to changing environmental conditions.

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Abstract# P093

A Machine Learning Approach to Quantitatively Phenotype Common Rust Symptoms of Maize

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Computer-vision based approaches to plant disease phenotyping are becoming more common, which can lead to faster and more reliable phenotyping. However, pipeline development can be a lengthy process, due to the large amount of training data required and the time it takes to annotate that training data. Our research investigates the applicability of U-Net convolutional neural network models to common rust of maize, caused by the fungal pathogen Puccinia sorghi, in which the fungus develops small and numerous pustules on leaf surfaces. In particular, we assessed the overall performance of U-Net models as well as their ability to replicate ground truth results from a fungicide efficacy experiment and a plant resistance gene differential experiment. U-Net models were trained with an increasing number of leaf images and their respective annotations, from six leaves to 352 leaves, with training leaves chosen either randomly or from a subset of phenotypically diverse timecourse images. Overall, we found that increasing the number of training images greatly improves model performance, particularly for models with fewer leaves, but the rate of improvement decreases. The likelihood that an individual model will be able to corroborate ground truth findings steadily increases as training dataset size increases as well.

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Abstract# P094

Key developmental windows and environmental parameters that influence cuticular wax composition on maize silks

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Desiccation of maize silks hinders fertilization; thus, silk drought tolerance is a potential breeding goal to enhance maize productivity in increasingly dry environments. Maize silks, and other aerial organs, are coated with a hydrophobic cuticle that limits water loss and protects against other abiotic and biotic stresses. Prior work has found silk cuticular wax accumulation increases dramatically in growing seasons that experience low precipitation. Moreover, cuticular wax composition can vary among genotypes and accumulate to higher concentrations in portions of the silks that are emerged from encasing husk leaves into the external environment. While genotype-by-environment effects have been implicated in cuticular wax accumulation, a true multivariate analysis of cuticular wax phenotypes is still needed. To parse the effects of genotype and environment on cuticular waxes, 468 Wisconsin Diversity Panel inbreds were grown in both Iowa and Minnesota in 2016 to 2017, providing silk cuticular wax data for 45 metabolites across three environments, with high resolution weather data at each site. A similar experiment was performed in 2020 wherein 11 strategically selected inbreds from this panel were grown across 6 planting dates in both Iowa and Minnesota. Linear modeling was used to

find putative associations between silk cuticular waxes and weather throughout plant development. Low precipitation and high solar radiation immediately prior to silking were correlated with increased cuticular wax accumulation, though intensity of solar radiation weeks prior to silking may also influence the silk cuticle. This analysis provides a foundation for controlled environment studies that assess the impact of specific weather parameters at specific stages in plant development on the silk cuticle. Ultimately, this research will lead to a better understanding of abiotic factors that impact cuticle composition and whether these modified cuticles protect against specific stresses.

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Abstract# P095

Multi-Omic Analysis of Maize Pollen During Storage

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Pollen is a defining feature of flowering plants and contributes to the reproductive success of many key agricultural crops. Decades of genetic and molecular research have contributed to our understanding of pollen morphology and development. While orthodox (i.e., trees) pollen types may survive in a desiccated state for months, most recalcitrant (i.e., grass) pollen types are known to exhibit short timeframes of viability. PowerPollen has recently developed novel proprietary technology to facilitate removing grass pollens from the natural pollination system and keep pollen viable under storage until pollination is needed. An outstanding question in the field is what molecular changes occur within pollen after maturation and before death. To determine if altered gene expression profiles are associated with maize pollen viability we performed global proteomics, phosphoproteomics, and transcriptomics of fresh maize pollen at maturity and under storage conditions known to prolong viability. To assess phenotypic differences in gene expression patterns associated with maize inbreds we also profiled two unrelated inbred genotypes with three technical replicates. In total, 43,573 transcripts, 7,946 protein groups, 11,502 phosphorylation sites, and 3,253 phosphoproteins were identified between both inbred genotypes and across storage treatments. Differential expression of these analyses revealed a functional skew toward protein synthesis/degradation, carbohydrate/energy metabolism, cell wall metabolism, and cytoskeleton dynamics. This study enhances our understanding of pollen biology and will advance our understanding of molecular factors that contribute to reproductive success.

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Abstract# P096

Using native rhizobia to create a drought-resilient field pea production system

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Increasing abiotic stressors, such as drought and salinity, lead to difficulties in agriculture production. Man-made fertilizers are a continual cost for successful yields, but only a small amount is absorbed in the soil, leading to chemical run-off. Plant growth promoting bacteria can be used as field inoculants to supplement plant nutritional needs. Symbiotic rhizobia colonize legume plants and form organ-like nodules in the roots where they convert atmospheric nitrogen into a usable form in a process known as biological nitrogen fixation. Rhizobia also promotes plant growth in ways such as nutrient acquisition, hormone production, and the modification of plant stress responses. Companies adopt a “one-size fits all” approach in developing microbial inoculants for use in a variety of regions. This leads to volatile results when inoculating in fields where the microbes are not adapted to regional stress and soil conditions. By isolating native rhizobia from regional soils in stressful environments, we attempted to develop microbial inoculant that performed comparably, or better, than commercial controls in field testing for field pea. In this work Midwestern soils were sampled from areas near vetch legume plants. This soil was used to “trap” compatible rhizobia when grown with field pea to produce nodules. The rhizobia were identified using 16SrRNA sequencing, and tested for salinity stress tolerance, phosphate solubilization, and indole-3-acetic acid production. In combination with greenhouse testing, the rhizobia isolates were used in field testing to achieve results comparable to commercial inoculant controls. Further work is still needed to test additional isolates for yield testing, as well as to expand the scope of tested plant-growth promoting phenotypes.

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Abstract# P097

Functional characterization of STRUBBELIG-receptor family 3 in regulating plasmodesmal function and plant immunity

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Plants rely on effective cell-to-cell communication to grow and respond to external stimuli such as pathogen infection. Plasmodesmata (PD) provide the plasma membrane, endoplasmic reticulum, and cytoplasmic continuity between adjoining plant cells, allowing direct cell-to-cell communication. Given their fundamental roles, PD are essential for plant survival. Recent studies highlighted the important role of PD-located proteins (PDLPs) in regulating PD function through an unknown mechanism. Using enzyme-catalyzed proximity labeling, we identified STRUBBELIG-receptor family 3 (SRF3), which is a leucine-rich repeat receptor-like kinase, as a functional partner of PDLP5. SRF3 was previously reported to be associated with PD and involved in iron homeostasis, root growth, and sensing bacterial flagellin (flg22). We confirmed the PD association of SRF3-sfGFP in Nicotiana benthamiana. Using co- immunoprecipitation, we demonstrated the physical interaction between PDLP5 and SRF3. We showed that srf3 mutants are compromised in the flg22-induced activation of mitogen-activated protein kinases. In addition, srf3 mutants are compromised in flg22- induced resistance to Pseudomonas syringae pv. tomato DC3000. In addition to PD association, SRF3-sfGFP is also detected in membrane-bound vesicles formed at PD. The formation of PD- associated vesicles can be induced by bacterial infection. The vesicles accumulate reactive oxygen species (ROS). The findings suggest the role of SRF3-containing vesicles in maintaining ROS homeostasis during bacterial infection. In line with the hypothesis, genes involved in ROS responses are misregulated in srf3 mutants. Together, our findings suggest that SRF3 might function at PD to regulate ROS homeostasis during bacterial infection.

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~~Abstract# P098~~

withdrawn

Abstract# P099

Characterization of the gene networks underlying cuticle production in maize silks via systems' biology approaches

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The plant cuticle is comprised of a cutin polymer matrix infused with and coated by cuticular waxes that together form a protective layer. The silk cuticular waxes are comprised of very-long-chain fatty acids (VLCFAs), and VLCFA-derivatives including aldehydes and hydrocarbons. These metabolites are linked via enzymatic reactions as presumed precursors, intermediates, and end-products for cuticular wax biosynthesis. Herein, we collected the silks from inbreds B73 and Mo17, and queried the cuticular waxes and transcriptomes along the length of silks that captures the developmental progression and the environmental transition as silks emerge from the husks. We noted that cuticular wax biosynthesis is sensitive to silk environmental transition and the genotype, reflected by varying wax compositions between the husk-encased and emerged silks, and between B73 and Mo17. Joint statistical metabolome-transcriptome analysis identified ~300 genes associated with the cuticular wax variation between B73 and Mo17, and along the silk length. These cuticular wax-associated genes include those confirmed to participate in cuticular wax biosynthesis and the ones from pathways that directly or indirectly interact with cuticular wax biosynthesis, including cell wall biogenesis, proteasome-mediated protein degradation, and vesicle trafficking. In addition, 42 IBMRILs that demonstrate broad variation in silk cuticular wax compositions were selected for expression-QTL mapping. The genome-wide eQTL distribution and the eQTL distribution for the cuticular wax-associated genes were thus identified, allowing for further exploration into the potential regulatory relationships within the gene networks identified by the multi-omics integration pipeline. In conclusion, this systems' biology approach identifies the gene network underlying silk cuticular waxes, demonstrating the complexity of metabolic context that can determine cuticle deposition.

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Abstract# P100

Maize orphan genes and their potential association with cuticle synthesis

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Orphan genes are genes encoding species-specific proteins that do not share orthology to any other annotated genes in the biosphere. Recent studies on the functionality of orphan genes demonstrate their impact on the development and metabolism of an organism. In plants, for example, the QQS (Qua-Quine Starch) orphan unique to Arabidopsis modulates carbon and nitrogen allocation (Li et al., 2015). In maize, approximately 39,000 putative orphan transcripts have been identified from publicly available maize transcriptome data, however their potential functions are unknown. The goal of the work is to identify putative orphan genes that are associated with plant cuticle development, particularly in the reproductive silks. The hydrophobic cuticle covers the plant epidermis and protects the plants against stressful environments. It is composed of a cutin polyester matrix and cuticular waxes that are infused within or laid on top of the cutin matrix. In this study, we identified putative silk-specific orphan genes via a phylostratigraphy approach, examined differential expression of the putative orphan genes along the silk length and between B73 and Mo17 inbred lines, and queried potential associations between these orphans and the cuticular waxes. We discovered significant expression differences of silk-related orphan genes between husk-encased and emerged portions of the silks, and have identified interesting correlations between orphan gene expression and cuticle composition. These discoveries will pave the way toward the broader understanding of the functions of orphan genes and the genetic networks that underlie important metabolic pathways.

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Abstract# P101

A multidisciplinary approach to assess the roles of Glossy2 and Glossy2-like in maize cuticular lipid biosynthesis

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The cuticle is a hydrophobic barrier that covers all surfaces of the aerial organs of land plants. It provides the first line of defense from biotic and abiotic stresses that are detrimental to plant health. The cuticle is composed of a network of lipids that are both intercalated within and laid atop an insoluble cutin polyester matrix. The solvent extractable cuticular wax mixture, depending on organ and stage of development, is comprised of combinations of different lipid classes, such as very long chain fatty acids (VLCFAs) and their derivatives, including hydrocarbons, alcohols, aldehydes, ketones, and wax esters. Classical genetic strategies have identified approximately 30 glossy genes required for normal cuticle deposition in maize, and molecular characterization of these genes is providing new insights on cuticle formation. This study focuses on the maize Glossy2 (Gl2) gene, which encodes a protein that is archetypal for the BAHD class of acyltransferases. Although Gl2's biochemical function remains unclear, homozygous gl2 mutant seedlings exhibit cuticular waxes of shorter chain lengths, presumably due to an alteration of the maize fatty acid elongase complex (FAE). Recently, GLOSSY2-LIKE (encoded by Gl2-like), which shares 63% amino acid similarity to GL2, was also shown to play a role in VLCFA elongation when heterologously expressed in Arabidopsis. To assess the in planta physiological function of Gl2-like, six unique maize mutant alleles have been generated via CRISPR-Cas9 genome editing. These gl2-like mutants, in combination with gl2 mutants, will enable the characterization of the functional relationship between GL2 and GL2-LIKE. In parallel, the roles of GL2 and GL2-LIKE proteins are being investigated using a heterologous expression system, in which the entire maize FAE pathway is being re-constructed in a yeast host. This combination of multidisciplinary strategies is unraveling the roles that Gl2 and Gl2-like serve in maize cuticle biosynthesis.

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Abstract# P102

Characterization of candidate genes related to cuticular wax deposition on maize silks

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The hydrophobic cuticle, which covers aerial portions of plants, is the first line of defense against environmental stresses, including drought, UV radiation, temperature, and insects and pathogens. This cuticle is comprised of a cutin polyester matrix that is infused with and laid atop by cuticular waxes, comprised of differing combinations of very long chain fatty acids (VLCFAs), hydrocarbons, aldehydes, alcohols, esters, and ketones. The cuticle on maize silks is rich in hydrocarbons, with minor amounts of VLCFAs and trace aldehydes and alcohols that together provide important protection for this tissue during the pollination period. Cuticle biosynthesis within the epidermal cells is tightly regulated at both the transcriptional and post-transcriptional levels. Many of the transcription factors known to regulate cuticle biosynthesis and deposition in Arabidopsis and in maize are not expressed in silks, suggesting different transcriptional regulation in this important organ. We have identified two transcription factors through multi-omics approaches that are putatively related to cuticle composition in either silks or seedlings (i.e. FDL1 and a bZIP TF). In addition, we have identified genes involved in the fatty acid elongation pathway (e.g. a Ketoacyl-CoA Synthetase) and in lipid transport (e.g. lipid transfer proteins that may facilitate wax transport through the cell wall) that are putatively associated with cuticular wax composition on maize silks. The potential functions of these candidate genes in regulation of cuticle biosynthesis and deposition on silks have been assessed by profiling cuticular waxes on silks from UniformMu mutants of these candidate genes. Characterization of these genes will allow a better understanding of how the plant cuticle is formed and deposited, and lays the foundation for future applied breeding approaches to generate “designer” protective cuticles.

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Abstract# P103

Developing a tool to examine virulence functions of co-infiltrated bacterial effectors in plant cells

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Bacteria rely on many tools to infect plant tissues, chief among them are effector proteins responsible for the manipulation of cellular processes once in the cell. AvrRps4 is a bipartite bacterial effector originally isolated from Pseudomonas syringae pv pisi, that is processed into two functional pieces within the plant cell. Both the N terminus and C terminus of AvrRps4 act as individual effectors, but the exact target of the N terminus is not known. We hypothesize that it can conceal the presence of other effectors by interfering with EDS1, a necessary part of immune signaling. To test this hypothesis, we will co-infiltrate lettuce and tobacco leaves with AvrRps4 and a second effector called XopQ. XopQ is produced by the bacterium Xanthomonas campestris pv. vesicatoria (Xcv) and is recognized in tobacco via the same EDS1 signaling pathway that we hypothesize AvrRps4 interrupts. XopQ has been cloned into the expression vector pHM1 and will be transformed into an Agrobacterium strain. As XopQ has been shown to be recognized in tobacco, both plants will be used in this study to confirm the interaction of AvrRps4 and EDS1. We expect to see a reduction in XopQ recognition by tobacco when AvrRps4 is present to inhibit the recognition. This research will allow us to have a clearer understanding of how bacterial pathogen-derived effectors are recognized by plants when multiple effectors are present and the effect that could have on crop improvement.

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Abstract# P104

Primary cell wall regulation: ancient genes with modern roles

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During plant development, cell wall (CW) determines the morphology and mechanical features of plant cells. Plant CW is a complex network made up of polysaccharides, proteins, and polyphenolics, that can be both tough and flexible. Two types of CWs are found in higher plants depending on their thickness and flexibility: Primary and secondary CW. The deposition of these two types of CWs is dictated by a complex transcriptional regulation network. While we have extensive information about the transcriptional regulation of secondary CW, especially in dicots, regulation of primary CW deposition is still largely shrouded in mystery in both monocots and dicots. Unraveling this mystery can help scientists engineer plants with better characteristics suitable for society needs, including sustainable source of biofuel. Using global weighted gene associated network (GAN), we identified four putative transcription factors (TF)s in rice (Oryza sativa L.) that are co- expressed with glycosyltransferases associated with primary CW deposition. These TFs are good candidates for the first layer of master switches for primary CW deposition in grasses. To examine this hypothesis, I applied CRISPR/Cas9 technology to knock out the genes encoding these potential TFs in rice and determine their impact on plant growth and endosperm development. Morphological and biochemical characterization of these mutants confirmed their role as a Primary CW TFs in monocots.

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Abstract# P105

LTR Predictor: A tool to identify LTR retrotransposon insertions in long-read genome sequencing data

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Long Terminal Repeat (LTR) retrotransposons are the most abundant type of transposable element (TE) found in the maize genome, characterized by having long terminal repeats on either side of a coding sequence or deleted coding sequence. LTR retrotransposons make up the majority of the maize genome, but only a handful of new insertions have ever been identified in controlled experiments. With recent advances in long-read sequencing technologies, we are now able to fully sequence single molecules that include new insertions of long TEs along with enough surrounding DNA to map the insertion with high confidence. However, existing tools used to annotate LTR retrotransposons based on structural features could not be applied in this context. Here we describe a new tool, LTR Predictor, which can be used to identify LTR retrotransposon candidates from inserted sequences defined through long-read sequencing. LTR Predictor searches sequences for long terminal repeats and primer binding sites, filters out insertions that are too short or composed entirely of simple repeats, then creates output files in table and graphical formats for high-quality candidates. Since this tool relies on structural features rather than homology, prior knowledge of LTR retrotransposons already present in the genome is not required. This tool will aid in discovery of novel LTR retrotransposon insertions in plant genomes, paving the way for downstream studies on the consequences of TE insertions on genome function.

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Abstract# P106

Exploring the Adaptive Functions of the Ubiquitin-26S Proteasome System in Rice

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The ubiquitin-26S proteasome system (UPS) utilizes a small 76-amino-acid peptide, called ubiquitin (Ub), to modify a myriad group of intracellular proteins to regulate their activities, turnover and localization. Three enzymes assist Ub to attach its substrates: E1 (Ub activating enzyme), E2 (Ub conjugating enzyme), and E3 (Ub ligase). Among E3 ligases, SKP1- Cullin1-F-box complexes (SCF) have been recognized as the largest group to recognize protein substrates for ubiquitylation. Within an SCF complex, the F-box protein determines the specificity of a substrate, while Skp1 is an adaptor bridging the F-box into the complex. After proteins are recognized and ubiquitylated by the SCF complexes, they are often targeted into the 26S proteasome for degradation. The 26S proteasome is a huge protein complex that is composed of 19S regulatory particle and a 20S core protease complex. This study is aiming to apply CRISPR/Cas9 mutagenesis to create mutations in Oryza sativa Skp1 Like (Osk) 1/20 and Oryza Sativa PAG1 genes that encode Skp1 and the CP subunit PAG1, respectively, in rice. The resulting mutants will be complemented with HA-Osk1 and PAG1-FLAG, allowing us to study the adaptive changes of Osk1-mediated ubiquitylation proteome and proteasome composition, respectively.

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Abstract# P107

Probing the molecular basis of pathogenicity by Pseudomonas fluorescens LE6_D7 on oomycetes

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Fresh market production of vegetables like spinach, lettuce, tomatoes and arugula is shifting to hydroponic greenhouse operations. In hydroponics systems, Pythium species is a problematic plant pathogen and can be introduced by airborne dust particles from neighboring farm fields and cause root rots that results in stunting or yellowing of leaves. To identify bacterial antagonists of oomycetes, we surveyed a collection of pseudomonads from a Lake Erie diatom bloom, known to also contain oomycetes. Using a high throughput competitive plate assay, I have identified and sequenced three strains of Pseudomonas fluorescens that exhibit contact dependent killing of Pythium dissotocum P. oopapillum P. ultimum, one strain of Saprolegnia parasitica (a fish pathogen), and Pythium strains from New Jersey, California and Indiana at a lowest concentration of 400 cells/ml. Bioinformatic analysis of the P. fluorescens isolate that we have tested most completely (LE6_D7), indicates that it has no ability to degrade cellulose, pectin/starch, and contains no recognizable animal effector proteins. To address the utility of this isolate as a biopesticide in a complex mixture, we added 50 ml of bacterial culture to a 6 L tub of contaminated water from our experimental hydroponic system filtered aliquots and filters grown overnight on antibiotic V8 plates. To identify the molecular basis of this inhibition, we have implemented a targeted gene knockout strategy, generating a first set of candidate gene markers that resulted in loss of virulence.

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Abstract# P108

GeneticcharacterizationofTCPgenefamilyinPhyscomitriumpatens

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TCP-family transcription factors have been well-studies as diverse regulators of plant physiology, but largely in vascular plants. Nonvascular plants like Physcomitrium patens represent a simpler physiological system to work in with fewer gene redundancies to complicate phenotypic studies. The roles of PpTCPs are unknown, however, their gene structure is remarkably similar too that oof Arabidopsis thaliana, and it is possible that their respective proteins behave similarly. The work described in this poster aims to use molecular cloning to generate several tools for characterizing the PpTCP transcription factory gene family. One tool utilizes a CRISPR:Cas9 system for generating mutant plant lines to observe phenotypic changes. While another set of constructs will be made to visualize and characterize the behavior of PpTCP proteins in a tobacco expression system. This project focuses on building toward a larger comparative genomic study of the PpTCP gene family with a future goal of phenotypic characterization.

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~~Abstract# P109~~

Talk abstract duplicated erroneosuly