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ABSTRACT BOOK

Chair: Shahid Mukhtar
Vice-Chair: Aruna Kilaru
Organizers: Magaly Rincón-Zachary and Julia Frugoli

2019 SS-ASPB ABSTRACTS

GENERAL SESSION

KH1. A Chemical Genetic Roadmap to Improved Tomato Flavor

Keynote Speaker**: Harry Klee,** Horticultural Sciences, University of Florida, Gainesville, FL 32611

Modern commercial tomato varieties are substantially less flavorful than heirloom varieties. To understand and ultimately correct that deficiency, we quantified flavor-associated chemicals in over 500 modern, heirloom and wild accessions. A subset of these accessions were evaluated in consumer panels, identifying the chemicals making the most important contributions to flavor and consumer liking. Modern commercial varieties contain significantly lower amounts of many of these important flavor chemicals than older varieties. Whole genome sequencing and a genome-wide association study allowed us to identify genetic loci affecting most of the target flavor chemicals, including sugars, acids and volatiles. Together, these results provide an understanding of the flavor deficiencies in modern commercial varieties and the information necessary for recovery of good flavor through molecular breeding.

Keywords: fruit quality, molecular breeding

GS 1. IBR5 Regulates Plant Auxin Response through Interactions with Multiple Proteins

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Auxin is a major plant hormone that regulates growth and development throughout the life cycle. Cellular responses to auxin involve the rapid degradation of a group of transcriptional repressors known as Aux/IAAs through ubiquitin proteasome pathway involving SCFTIR1/AFBs complex subsequently modulating the expression of auxin-related genes. Previous studies identified IBR5, which encodes a putative dual specificity phosphatase, as a gene involved in the auxin response pathway. Primary root growth of ibr5 mutants is insensitive to a range of auxinic chemicals. While Aux/IAA proteins are stabilized in many auxin insensitive mutants, interestingly, Aux/IAAs are rapidly degraded in ibr5 mutants. This research is focused on understanding the role of IBR5 in regulating the auxin response pathway. Through yeast two hybrid screen we identified several *Arabidopsis* proteins that interact with IBR5. Our research indicates that IBR5 regulates the degradation of Aux/IAA proteins apparently by controlling the abundance of subunits of the SCF complex, which is composed of ASK1, CUL1, RBX1, and the F-box proteins, TIR1/AFBs. Our results further indicate that IBR5 may involve in multiple regulatory points in auxin signaling pathway.

Keywords: auxin, ubiquitin-proteasome pathway, IBR5, growth and development

GS 2. *Chlamydomonas reinhardtii*: A "Rock Star" Plant Biology Teaching Tool for K-16Students

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Biological model systems play an important role in the advancement of inter- and intra-disciplinary science research and education. *Chlamydomonas reinhardtii* is a micro-green alga that retains many of the features of the green plant and of the common ancestor of plants and animals. *Chlamydomonas* is haploid, has a short replication time, cheap and easy to grow in a lab setting, can grow heterotrophically or can grow photo-autotrophically, has a sequenced genome, and is genetically tractable. These attributes make Chlamydomonas an elegant experimental model system to plant biologists, biomedical and bioenergy researchers. Some of the

biology topics in the K-16 curriculum that can be taught using *Chlamydomonas* are sexual reproduction, cell division, genetics, structure and function of eukaryotic flagella and eye spot, Optogenetics, eukaryotic photosynthesis, high light stress responses, generation of ROS and its detoxification via anti-oxidants in plant cells, photosynthetic pigment metabolism and, biomass and bioenergy production etc. Simple, inexpensive, hands-on-activities can be designed based on published *Chlamydomonas* research for either hands-on activities or for observation induced-enquiry based learning of K-16 students, ranging from 4th graders to college undergraduates. I will present some specific *Chlamydomonas* based-hands-on activities from my awarded ASPB-BLOOME 2018 project that can be used to demonstrate to the 21st century students the intra- and inter-disciplinary nature of Biology. Additionally, how concepts from selected given labs can be used for NGSS Biology core concept mapping will be presented.

*Keywords: Chlamydomona*s, photosynthesis, K-16 education, eyespot, flagella

GS 3. Can These Glasses Cure Plant Blindness?

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STEM educators need practical implementations of emerging digital media that excite their students to learn about plant biology. For these educators, web-based virtual reality (webVR) and extended reality (XR) offer exciting new avenues for education in under-addressed learning domains such as affective educational storytelling and physical manipulation of 3D models. VRplants is an interdisciplinary project from the North Carolina State University Department of Plant & Microbial Biology and the NC State Libraries to develop a suite of web-based plant biology learning modules for open utilization by educators. The project also supports a series of workshops to disseminate essential XR creation tools and increase the size of the extended reality maker community.

Keywords: virtual reality, education, web-based learning

GS4. Regulation of a Geminivirus Late Gene Promoter by PRC2

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Geminiviruses are small ssDNA viruses that cause significant yield loss in many agriculturally important crops. Upon entry into the nucleus, the viral genome is converted by host enzymes into a dsDNA replicative form (RF). The RF associates with histones to form non-integrating episomes that both facilitate virus replication and transcription and serve as targets of host defense pathways. As viral chromatin is formed de novo in infected cells, geminiviruses are unique models for examining mechanisms that target and establish epigenetic modifications. Polycomb Repressive Complex 2 (PRC2) is an important repressive regulator of developmental processes via its ability to deposit histone H3 lysine 27 trimethylation (H3K27me3), but how PRC2 activity is regulated at target genes is unclear. We propose that geminivirus gene expression is a model for PRC2 control, as we have found that H3K27me3 is localized to the viral coat protein (CP) promoter, which is repressed early in infection. We have also found that the CP promoter is bound by the plant-specific transcription factor TCP24, which has been implicated in PRC2 recruitment. Importantly, TCP24 mRNA levels decrease in geminivirus-infected cells. Thus, we hypothesize that TCP24 recruits PRC2 to inhibit premature CP expression and permit viral genome amplification. Once a threshold of genomes is produced, reduced

TCP24 levels allow CP expression, leading to virion assembly. Thus, our studies offer insight into the temp control of the geminivirus infection cycle, as well as principles underlying developmental gene regulation. (Work in the Bisaro lab is supported by NSF MCB-1158262, NSF IOS-1354636, USDA/NIFA 2015-6703-22999; work in the Sunter lab is supported by NSF DBI 1565076).

Keywords: geminivirus, transcription, PRC2, repression, epigenetics

CONCURRENT SESSION A - NON-COMPETITIVE

CS A1. Insights into the Microtubule-mediated Control of Cotton Fiber Expansion

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Cotton fibers undergo extensive elongation and secondary wall thickening as they develop into our most important renewable textile material. These single cells elongate at the apex as well as elongating and expanding in diameter behind the apex. These multiple growth modes represent an interesting difference compared to classical tip-growing cells that needs to be explored further. Furthermore, the three types of fiber tips recently discovered may well not control their cellular morphogenesis in the same way. We used In vitro ovule/fiber cultures from the two major commercial species of cotton, *Gossypium hirsutum* and *G. barbadense*, to analyze the effects of a microtubule antagonist, colchicine, on early cotton fiber morphogenesis. A method for culturing the *G. barbadense* ovules was newly developed by us, enabling the comparative experiments. The results support the mixed mode of cotton fiber expansion in all three tips types and also highlight differences between them in the role of microtubules in modulating cell expansion. The significance of the results for cotton fiber properties important in the textile industry will be discussed.

Keywords: colchicine, cotton fiber, cytoskeleton, ovule culture, plant cell growth

CS A2. The Role of the MatK Maturase in Chloroplast Group IIA Intron Excision.

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Maturase K (MatK) is the only chloroplast-encoded group II intron maturase in land plants. MatK has long been presumed to function as a required factor for formation of the catalytic structure needed for group IIA intron excision from seven different chloroplast precursor RNA molecules (rpl2, intron 2 of rps12, trnA, trnI, trnV, trnK, and atpF). These precursor RNAs encode proteins or tRNAs required for formation of the chloroplast translation machinery and one subunit of ATP synthase implicating an essential role for MatK in chloroplast and, subsequent, green plant tissue function. No direct assays, however, have as yet shown maturase activity for this enzyme. Further, several nuclear-encoded protein factors have been determined to be required for excision of the same target introns as MatK. We devised an assay using one of the target substrates for MatK activity, the group IIA intron within rpl2, to test MatK's functional role in group IIA intron excision. We have determined that, although the group IIA intron of rpl2 is autocatalytic under in vitro conditions, the addition of the MatK maturase to precursor rpl2 substrate significantly enhanced efficiency of intron excision in low salt buffer. This increase in efficiency occurs without additional protein components. We have also determined that expression patterns for MatK and at least one of the nuclear-encoded proteins proposed to bind to the same targets as MatK overlap supporting a possible association between these two proteins.

Keywords: maturase, chloroplast, MatK, intron, excision

CS A3. Omics in Crop Wild Relatives: Apply Untapped Genetic Diversity to Meet Global Challenges

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Climate change has generated various critical challenges to agriculture sustainability and food security. These challenges may be met by the development of novel crop varieties with increased biotic or abiotic resistance that enable them to thrive in changing environments. Crop wild relatives (CWRs) harbor a much higher level of genetic diversity than cultivated crops and have the potential to meet these challenges. The wild soybean (Glycine soja), the wild ancestor of domesticated soybeans (Glycine max), is widely distributed throughout diverse habitats in East Asia. We use wild soybean as our study system to investigate its genomic diversity, population structure, climate adaptation, and biotic stress resistance, integrating diverse omics approaches. Here we will mainly discuss the project of understanding the genetic basis of broad resistance to soybean cyst nematode (SCN), the most devastating pest of soybean, integrating genome-wide association studies, RNA-seq comparisons, and metabolomic comparisons. I will also discuss applying crop wild relatives and omics strategies in understanding other ecologically and agronomically important complex traits variation.

Keywords: crop wild relatives, genetic diversity, cyst nematode resistance, biotic stress, genomes

CONCURRENT SESSION B - NON-COMPETITIVE & COMPETITIVE

CS B1. Phylogenetic Conservation of Fine Root Chemical Traits in 36 Temperate Tree Species

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Plant traits are essential for the fitness of plants in their environments and also play key roles on nutrient and carbon cycling via affecting the decomposition of plant debris. The recent progress on the research of plant traits have revealed the lack of coordination between above-ground and below-ground traits. While leaf traits tend to follow a world-wide leaf economics spectrum, root traits, especially root morphology, exhibited a strong phylogenetic conservatism. The phylogenetic effect on root chemical traits is less understood. Most root trait studies only used carbon and nitrogen concentrations to represent root chemistry. A more comprehensive knowledge on molecular-level chemical variations in fine roots and the driving factors would improve our ability to understand and predict below-ground processes such as nutrient recycling and carbon fluxes across different biomes. Here we used GC-MS, pyrolysis-GC-MS, and FT-IR to characterize chemical properties of fine roots in 34 woody angiosperms from three major phylogenetic clades and two gymnosperms as an out-group. The results from CuO reactions followed by GC-MS and pyrolysis-GC-MS both demonstrated that phylogenetic history did not impose a significant effect on the concentrations of total lignin phenols, but the ratios of different phenol classes in more recently derived groups are significantly different from basal angiosperms, indicating a strong phylogenetic signal in lignin composition. Cell-wall bound phenols exhibited the similar trend: concentrations of total bound phenols did not differ significantly among phylogenetic clades, but the composition of bound phenols in recently derived groups is divergent

from that of the more ancient angiosperms. In addition, phylogenetic history was not a significant factor explaining the variations in cellulose concentrations and lignin : cellulose ratios in fine roots across species. To our knowledge, this study is the first to provide evidence that the phenolic profiles of fine roots in angiosperm woody species are phylogenetically structured. This finding shed new light on how phylogenetic history can contribute to the understanding of below-ground processes in terrestrial ecosystems.

Keywords: root traits, fine roots, root chemistry, phenolics, lignin, phylogenetic effect

CS B2. Regulation of Translation in Response to Reactive Oxygen by the Protein Kinase GCN2

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The translation of cytosolic mRNAs is subject to global and mRNA-specific control mechanisms. Phosphorylation of the essential translation initiation factor eIF2alpha anchors a reversible switch that represses translation globally. The stress-responsive GCN2 kinase is the only known kinase for eIF2alpha in *Arabidopsis*. Here we show that conditions that generate reactive oxygen species (ROS) in the chloroplast, such as dark-light transitions, high light, and the herbicide paraquat all rapidly activated the GCN2 kinase, as visualized by eIF2alpha phosphorylation, whereas mitochondrial stress did not. Treatment with hydrogen peroxide activated GCN2 kinase in darkness; otherwise, GCN2 activation was light dependent. It was also suppressed by inhibitors of photosynthetic electron transport as well as ROS quenchers. Gcn2 mutants were more sensitive to continuous high light as compared to wild type in their root growth and seedling development, implicating the GCN2-eIF2alpha pathway in responses to high light and associated ROS. The GCN2 kinase suppressed the ribosome loading of mRNAs for functions such as mitochondrial ATP synthesis, vesicle trafficking, and translation. However, the global polyribosome profile of the gcn2 mutant was normal under herbicide stress conditions. The transcriptome of gcn2 was hypersensitive to herbicide, specifically in functions related to abiotic stresses including oxidative stress, as well as innate immune responses. In conclusion, we provide evidence that GCN2-mediated eIF2alpha phosphorylation is a missing link in a retrograde signaling pathway whereby the status of the photosynthetic machinery feeds back to the cytosolic protein synthesis apparatus. Supported by the US National Science Foundation.

Keywords: reactive oxygen species, translation, chloroplast

CS B3. Utilization of Fungal Endophytes as Biofertilizers

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To meet the future food demands of a growing human population, a significant surge in crop production is needed. Human population is expected to reach 9.1 billion by the year 2050, which will require an increase in the current food production by 70%. For example, annual cereal production will need to rise to about 3 billion tons from 2.1 billion produced today. Therefore, adapting new sustainable technologies, in addition to the current ones, is crucial to meet that goal. The use of microbial inoculum, i.e. fungal endophytes, is one of the promising technologies to increase productivity and improve abiotic stress tolerance. Fungal endophytes are microscopic organisms that persist between plant cell tissues and cause no apparent symptoms. One objective of our lab is to discover novel endophytes from wild plants growing in stressed environments and

test their potential role in enhancing overall crop production. In 2018, we tested a collection of endophytic fungi to determine whether or not they are capable of providing growth promotion to the model system tomato. The endophytes W11 and W14 were shown to enhance tomato's biomass and production in growth chamber and greenhouse tests when compared to non-symbiotic control plants. Endophyte W11 and W14 extract solutions increased tomato seedlings dried shoot mass by 123% and 83% respectively at 7 weeks old in a growth chamber. Additionally, endophyte W11 and W14 also increased colonized tomato's fruit production by 41% and 65% under greenhouse conditions. Furthermore, similar results were found when these two endophytes were tested in corn and bamboo under field conditions with corn crop production increasing by 35% and 29%, while in bamboo the development of new shoots increased by 54% and 19%. The use of plants colonized with fungal endophytes is producing promising results and can potentially aid agricultural industries in securing food supplies.

Keywords: plant/fungal Interactions, fungal endophytes, growth promotion

CONCURRENT SESSION C – COMPETITIVE

CS C1. Characterization of Two Putative Nutrient Transporters Required for Symbiotic Nitrogen Fixation in *Medicago truncatula*

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The process of symbiotic nitrogen fixation (SNF) in legume root nodules requires the exchange of nutrients between host plant cells and the rhizobia within them. In mature, nitrogen-fixing nodules, the plant gives the rhizobia, the bacteria that are housed inside the nodule cells, carbon sources in exchange for the rhizobially reduced nitrogen, also called fixed nitrogen. The endocytosed rhizobia depend on their plant host cells for other nutrients as well, which are required for rhizobial metabolism. Plant nutrient transporters have been implicated in sustaining the rhizobia inside host cells (Udvardi & Poole 2013. Annu Rev Plant Biol). Using a forward genetics approach in the *Medicago truncatula* Tnt1 mutant population, we identified two mutant lines that are defective in SNF. Whole genome sequencing revealed that the two mutant lines each had Tnt1 insertions in different putative nutrient transporter genes from the same nutrient transporter family. Both genes are highly expressed in nodules. To support the hypothesis that the defective putative nutrient transporter gene was the causative mutation in the mutants, the *M. truncatula* Tnt1 population was successfully reverse screened to find other mutant alleles of the genes. We thus obtained three different mutant alleles for one of the genes and four for the other. The mutants were back-crossed to wild-type R108 and the F2 progeny were found to co-segregate with the mutant alleles, establishing that the mutated nutrient transporter genes were the cause of defective SNF. Characterization of the mutants is underway as are experiments to understand the molecular mechanisms by which these putative nutrient transporters support SNF. These will be discussed. Support from NSF 1733470 is gratefully acknowledged.

Keywords: symbiotic nitrogen fixation, *Medicago*, transporter

CS C2. Using a Multi-Omics Approach to Identify Essential Genes Involved with Nutrient Redistribution during *Arabidopsis* Innate Immune Response

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Unlike animals, plants do not possess a circulatory immune system. Instead, they are manifested with a robust innate immune system, where each cell needs a method for detecting the presence of diverse microbes. Plants employ a suite of pattern recognition receptors (PRRs) including receptor-like kinases (RLKs) to detect evolutionary conserved microbial associated molecular patterns (MAMPs). This receptor-ligand interaction leads to a series of cellular events and triggers an effective defense response called MAMPs-triggered Immunity (MTI). On the contrary, specialized phytopathogens deploy secreted molecules called effectors to suppress MTI at various levels. It has been suggested that pathogen effectors play a role in sugar and nutrient redistribution thereby promoting their growth within the plant. Here, we use a multi-omics approach to identify specific transcription factors and genes involved in nutrient assimilation during pathogen infection.

Keywords: Arabidopsis thaliana, innate immune system, ionomics, transcriptomics, *Pseudomonas syringae*

CS C3. Plant Physiological Responses to Phosphorus Stress Could Facilitate Uranium Co-Mobilization from Stable Mineral Forms

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Phosphorus (P) is a key mineral required for plant growth, and unlike other macronutrients, most P in soil is unavailable to plants due to the pH-dependent complexation with Ca, Fe, and Al. Plants have evolved specific physio-morphological strategies to address this apparent deficiency. One of these strategies involve the exudation of metabolites from roots to facilitate resource foraging. Some of these adaptations, could also influence the dissolution of radionuclides, especially from mineral forms where the radionuclides are complexed with elements that are essential for plant growth. However, the magnitude and regulators of such mobilization remain unknown. This work, through series of sand-culture and hydroponics, investigated the changes in composition and rate of exudation of metabolites from roots of Andropogon virginicus exposed to different forms of phosphorus minerals (KH_2PO^4, $FePO_4$ $Ca_3(PO_4)_2$ and No P). The mineral form of P, and hence the bioavailability of P, affected the overall composition of the root exudates. The lower bioavailable forms of phosphorus ($FePO_4$ and $Ca_3(PO_4)_2$, but not the complete absence of P, induced greater exudation of metabolites (organic acids) that have higher chelating capacity. Thus, the exudates from these treatments were more efficient in solubilizing phosphorous. In treatment with lower P-bioavailability, the physiological amino acid concentration inside of the roots increased, whereas the concentration of organic acids in the roots decreased due to the active exudation. Thus, *A. virginicus* exhibited a P-sensing and response mechanism that was triggered more in the presence of low available P but less in the complete absence of P. The root exudate matrix of plants exposed to low available forms of P induced a >60% increase in uranium dissolution from uranyl phosphate. These results highlight the potential of using active management of soil P as an effective tool to alter the plant-mediated mobilization of uranium in contaminated soil.

Keywords: phosphorus stress, root exudates, *Andropogon virginicus*, radionuclide mobilization

CS C4. *Arabidopsis* Bax Inhibitor 1 (ATBI-1) Interacts with ATIRE1A to Execute its Pro-survival Function

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Abiotic and biotic stresses can severely perturb endoplasmic reticulum (ER) function. The unfolded protein response (UPR) is a three-pronged signaling axis dedicated to preserving ER homeostasis. If these mechanisms of adaptation and survival are insufficient to recover the ER homeostasis, cells will initiate apoptosis. One of the branches of UPR is controlled by IRE1 (Inositol-requiring enzyme 1). Upon the accumulation of misfolded or unfolded proteins, IRE1 directly cleaves AtbZIP60 (basic leucine zipper 60) mRNA in response to various environmental stresses, leading to the production of an active transcription factor that promotes the expression of multiple ER stress-responsive genes. However, the AtIRE1-dependent mechanisms that regulate bacterial-pathogen-triggered cell fate decision remain unclear. Here, we demonstrated that AtIRE1a interacts with a highly conserved cell death suppressor AtBI-1(Bax-Inhibitor 1). The interaction of AtIRE1a and AtBI-1 appears to be tightly regulated by the phosphorylation status of two amino acids in IRE1a. The interaction of AtBI-1 with AtIRE1a directly promotes the AtIRE1a-dependent AtbZIP60 splicing in vitro. Our data also position caspase-like activity as an essential player regulated by AtIRE1-AtbZIP60 and AtBI-1 in cell fate decisions upon avirulent bacterial pathogen attack.

Keywords: Arabidopsis thaliana, inositol requiring enzyme 1 (IRE1), Bax Inhibitor 1 (BI-1), ER stress, UPR, cell death, caspase

CS C5. Nutrient Availability Drives Kin Recognition in the Model Angiosperm *A. thaliana*

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Plants alter their root system architecture (RSA), exudate production, and nutrient acquisition profiles, based on the genetic relatedness of their neighbor. Kin recognition (KR), the ability to discriminate the genetic relatedness of neighboring allows plants to modify their growth in response to the neighbor. Such interactions with associated variation in functional traits play a crucial role in maintaining ecosystems and provide valuable insight into strategies for balancing growth and nutrient acquisition within communities. Yet, the chemical and molecular signals and mechanisms driving KR are poorly understood, due to a lack of model systems for this purpose. The discovery that the model plant *Arabidopsis thaliana* is capable of KR provides an incredible resource for determining the molecular mechanisms, signals and phenotypic responses associated with this phenomenon. In the present study, we have investigated the extent to which genetic distance and nutrient scarcity is involved in KR responses. Our findings impact our broader understanding of the KR phenomenon as well as how best to study it in this model angiosperm.

Keywords: Arabidopsis thaliana, Kin Recognition, nutrient availability

CS C6. Elucidating the Factors that Regulate Specificity of Crop-AMF Association in Resource-Limited Soils.

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The symbiosis between plants and arbuscular mycorrhizal fungi (AMF) is the most ancient and widespread plant-mutualistic association. Plants benefit from this symbiosis by obtaining a majority of their phosphorus (P) requirement and greater tolerance to environmental stress. As AMF form a symbiotic association with 70-80% of plant species, there is less specificity in this association at the level of fungal colonization. However, when different AMF genotypes associate with the same plant genotype, the response/outcome of this symbiotic association is different. The reasons for the high specificity in the response/outcome of this symbiosis is remarkably less explored. An unprecedented understanding of the factors regulating the differential response/outcome of plant-AMF symbiosis would help to better utilize this symbiotic association in crop production systems. We studied the effect of different species of AMF on the uptake of phosphorus

from iron phosphate (sparingly available) and potassium phosphate (readily available) using Sorghum bicolor as a model species. In this experiment, we utilized different sorghum genotypes that differed in their defense chemistry and different AMF species (Rhizophagus intraradices and Gigaspora gigantea) that differed in their functional traits such as competitiveness and tolerance to environmental stress. The sorghum plants were inoculated with single or mixed AMF species and grown under greenhouse conditions. We measured plant above and below ground biomass, root morphology, percent colonization of AMF in roots, shoot P content and primary and secondary metabolites in roots. Our results revealed that when supplied with iron phosphate, plants inoculated with R. intraradices had higher biomass, percent root colonization and shoot P content than plants inoculated with G. gigantea. These results indicate that AMF species differ in their ability to increase plant productivity under apparently unavailable phosphorus conditions. These results will be further discussed using observed changes in plant metabolites due to association with different species of AMF.

Keywords: AMF, phosphorus, sorghum, plant response

CONCURRENT SESSION D – COMPETITIVE

CS D1. Elucidating the Role of the Pro-survival to Pro-Death Molecular Switch in the IRE1a Signaling Pathway in *Arabidopsis thaliana*

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In eukaryotic cells, biotic and abiotic stress causes the accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER) and subsequent ER stress. Which induces unfolded protein response (UPR). The most conserved UPR sensor amongst eukaryotes is Inositol-Requiring Enzyme 1 (IRE1), mitigate ER stress by up-regulating the cellular pro-survival pathway. However, under extreme conditions the infected portion will ultimately “switch” from pro-survival to pro-death in order to avoid further unfavorable circumstances for the plant as a whole. *Arabidopsis p*ossesses two homologs of major UPR sensor, IRE1a and IRE1b. In this study, we focused on IRE1a, which is the primary response pathway in the immune stress-triggered ER stress. Unlike mammals, the molecular mechanisms for turning off the pro-survival branch of IRE1a signaling in plants remain largely undefined. In *Arabidopsis*, upon ER stress IRE1a directly cleaves bZIP60 (basic leucine zipper 60 transcription factor) mRNA, leading to the production of an active transcription factor that activates cytoprotective genes. Throughout this study, the plant bacterial pathogen *Pseudomonas syringae* pv. tomato (Pst DC3000) was used as a biotic inducer for ER stress in an attempt to uncover regulatory mechanisms governing the regulation of bZIP60 expression during an immune response. Our findings suggest that novel *Arabidopsis thaliana* microRNA (miR5658; At4g39838) that can potentially target bZIP60, is specifically up-regulated upon acute ER stress. Moreover, quantification of miR5658 reveals that its induction coincides with bZIP60 mRNA suppression, suggesting that miR5658 might participate in the exquisite regulation of bZIP60 in a manner reminiscent of the mammalian X-box binding protein 1-miR-30c-2* regulatory mechanism. We also propose that the central plant immune regulator NPR1 (Non-Expressor of PR genes 1) plays an important role in regulating miR5658. Taken together, these results suggest that upon cell death-triggering stimuli, NPR1, via miR5658, may target bZIP60 mRNA to turn off the IRE1a-mediated pro-survival pathway.

Keywords: Arabidopsis, UPR, ER Stress, microRNA, IRE1

CS D2. Multi-omics Data and Multiple Problems

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A systems level understanding of cellular mechanisms requires knowledge of multilayered -omics including genomics, transcriptomics, interactomics, and metabolomics. Moreover, mathematical modeling and bioinformatics can translate the information pertinent to these multi-omics and map genotype-to-phenotype relationships. Here, we integrated structural and functional interactions resulted from co-expression networks, transcription factor-target interactions, interactomes and phenomics in *Arabidopsis*. Towards this, we curated a large-set of experimental datasets, mined publicly available databases, and incorporated predicted datasets. Our aim is not restricted to collection of dataset only but, to integrate them interactively, to interpret their role in a wide verity of biological systems and to develop the understanding of communication between different biological process based upon theses datatype.

Keywords: interactomics, network biology

CS D3. Fatty Acid Amide hydrolase in an Early Land Plant, *Physcomitrella patens*

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Fatty acid amide hydrolase (FAAH) belongs to a diverse class of enzymes in amidase signature family. In mammals, FAAH is targeted to affect neurological functions because it terminates endocannabinoid signaling by degrading anandamide, a 20C N-acylethanolamine (NAE 20:4). In higher plants, FAAH is known to modulate growth, development and stress tolerance by degrading 12-18C NAEs. Since anandamide was reported to exclusively occur in early land plants, we investigated its metabolic pathway in the moss Physcomitrella patens. Based on the highest identity with ratFAAH, we identified nine orthologs in moss, PpFAAH1 to PpFAAH9. Several bioinformatic tools were used to understand the structural basis of how catalytic residues fold for amidohydrolase activity. Based on these in silico analyses of PpFAAH homologs and their gene expression in response to saturated (NAE16:0) and unsaturated NAE (NAE 20:4) treatment, PpFAAH1 was selected for biochemical characterization. Heterologously expressed PpFAAH1 showed highest amidohydrolase activity at 37°C and pH 8.0. Both *in vivo* and *in vitro* studies showed that unsaturated NAE substrate is hydrolyzed faster than the saturated NAE (> 10-fold *in vivo* and 50-fold *in vitro*). Additionally, transgenic moss lines over expressing FAAH1 showed slower growth and disrupted gametophyte formation when compared to wild type. These data suggest that PpFAAH1-mediated NAE metabolism is likely involved in developmental transition in moss.

Keywords: endocannabinoid, FAAH, *P. patens*

CS D4. Determining the Role and Regulatory Control of POLTERGEIST and POLTERGEIST-LIKE1 in Plant Stem Cells

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All plant biomass originates from meristems, which contain discrete populations of stem cells. Maintenance of these stem cell populations, in *Arabidopsis*, is controlled by cell-cell signaling networks composed of a transmembrane receptor kinase (from the CLAVATA1 clade), a peptide ligand (from the CLE family), and a transcription factor (from the WOX family). The signaling cascade from this RK-WOX network is responsible

for limiting stem cell proliferation and allowing differentiation. The two redundant class 2C protein phosphatases, POLTERGEIST (POL) and POLTERGEIST-LIKE 1 (PLL1), negatively regulate the RK-WOX pathways. Mutation in one of these genes causes no noticeable phenotype, but the pol pll double mutant is seedling lethal, indicating its importance in development. It is currently unknown how these proteins negatively regulate the signaling network, or how they are regulated themselves. POL has two novel conserved domains which are enriched in serine residues. Many of these residues are evolutionarily conserved and phosphorylated. I hypothesize that the novel domains of POL are involved in regulation, post-transcriptionally, via phosphorylation. Using different approaches, including genetics and protein biochemistry, I hope to elucidate the function and regulation of the Poltergeist family of protein phosphatases in *Arabidopsis.*

Keywords: stem cells, development, phosphatases

CS D5. Development and Analysis of an Activation Tagging System in Wheat

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Transposable elements are DNA sequences that can excise from one location and reintegrate into a new place within the genome. Transposable elements can be used for mutagenesis because of their ability to induce changes to an organism's genetic sequence. Thus, mutagenesis is used as a tool for gene discovery by providing information on how specific genes effect the growth and development of an organism. A modified version of mutagenesis uses an activation tagging sequence, which shows the function of genes by causing their overexpression. A non-autonomous transposable element used for mutagenesis, known as mPing, was first discovered in rice and requires two proteins, ORF1 and Transposase, for mobilization. An activation tagging version of mPing, known as mmPing20F, was created by inserting an enhancer sequence from the promoter region of the figwort mosaic virus into a hyperactive version of mPing. Plant transformation was used to get mmPing20F:GUS and an ORF1/TPase expression construct inside the wheat genome. The first five generations after cross-pollination was completed were analyzed through PCR analysis and GUS staining to detect active transposition and determine transposition rates for each generation. The result of these experiments showed that homozygosity was achieved for the mmPing20F:GUS plasmid and that transposition of mmPing20F in transgenic wheat lines occurred when paired with both mobility proteins. Excision behavior of mmPing20F was also analyzed and demonstrated behavior similar to mPing in soybean, rice, and *Arabidopsis thaliana*. Further work will focus on increasing transposition rate per generation as well as cultivating plants that are homozygous for the ORF1/TPase expression construct.

Keywords: transposable elements, wheat, mutagenesis

CS D6. Identifying a Suppressor of the SUNN-1 Hypernodulating Phenotype through MutMap and Bulk Segregant Analysis

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Legumes, capable of forming nitrogen fixing nodules in symbiosis with rhizobia, control the number of nodules using a systemic signaling pathway referred as Autoregulation of Nodulation (AON). In *Medicago truncatula*, rhizobial infection triggers local signaling events resulting in MtCLE12 and MtCLE13 induction in the nodule meristem initiating AON. CLEs are translocated to shoot where they bind to a receptor complex containing the leucine-rich repeat receptor-like kinase SUNN, followed by subsequent signal transduction to roots resulting in termination of new nodule formation. Mutation of SUNN results in a 5-10 fold increase in nodule number. We undertook a forward genetic screen starting from EMS mutagenized seeds of sunn-1, an

allele harboring single amino acid change in kinase domain, to identify suppressor of sunn-1 (sos) lines. The suppressor screening was carried out using a weak allele with an amino acid change, sunn-1, with the potential of revealing novel genes whose protein product directly or indirectly interacts with SUNN to affect its function, or to identify a pathway component, or complementary protein in parallel pathway that alleviates the need for active SUNN protein in the pathway. We present phenotypic analysis of one of the suppressor lines, sos16, and our mapping strategy for identifying the cause of suppression by using a combination of MutMap and bulk segregant analysis. SNP data obtained from whole genome sequencing of 11 pooled backcrossed sos16;sunn-1 lines was used to identify the region of suppression, determined to be linked to sunn-1. Further confirmation of linkage comes from genetic markers data from a mapping cross with the A20 ecotype and we are testing SNP markers in bulk segregants to identify the cause of suppression. This work is supported by NSF IOS 14444 & #1733470.

Keywords: autoregulation of nodulation, suppressor, SUNN kinase, MutMap, bulk segregant analysis

CONCURRENT SESSION E – COMPETITIVE

CS E1. Identification of Novel Nematode Resistance Strategies in Wild Soybean

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The domestication process of crop plants often involves selection for agronomic traits against the plant's intrinsic resistance strategies. Thus, domestication processes decrease genetic variation, making crop plant varieties more susceptible to pests than their wild relatives. Domestication of the wild soybean (*Glycine soja*) accounts for a major loss is genetic diversity. The *G. soja* gene pool is indisputably more diverse than the cultivated soybean (*Glycine max*) due to a primary loss of nucleotides in domestication and continued loss due to selection and modern breeding practices. Therefore, we dissect the diversity contained in the wild soybean population, which has been going through differential stress from varying environments, as a naturally adapted source of resistance. In this study, resistance to Soybean Cyst Nematodes (SCN) is investigated in a newly identified SCN resistant ecotype. In order to investigate the global gene expression changes, we compare RNA seq-based transcriptomes of the novel SCN-resistant wild soybean ecotype vs SCN-susceptible ecotype, SCN-resistant cultivar and SCN-susceptible cultivar under both control and nematode-treated conditions. All accessions were inoculated with SCN HG type 2.5.7. High-throughput Illumina total RNA sequencing of root tissue is used to produce expression profiles to compare transcriptomes of all samples from treatment and control experiments. This project identified candidate genes and associated pathways involved in SCN resistance and advances the long-term goal to develop SCN resistant soybean cultivars, which has crucial significance to agriculture and environmental sustainability.

Keywords: Glycine soja, wild soybean, soybean cyst nematode, SCN

CS E2. Interactions between IRE1 and AGB1 in Pathogen-Induced Unfolded Protein Response

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In eukaryotic cells, the accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER) results in ER stress that induces a cascade of reactions called the unfolded protein response (UPR). In *Arabidopsis*, the most conserved UPR sensor, Inositol-requiring enzyme 1 (IRE1), responds to ER stress by directly cleaving bZIP60 (basic leucine zipper 60) mRNA in response to both abiotic and biotic stresses. This leads to the production of an active transcription factor that promotes the expression of multiple ER stress-responsive genes. AGB1 is the only G-protein -subunit encoded by the *Arabidopsis* genome and is involved in

numerous signaling pathways. AGB1 has previously been shown to control a distinct UPR pathway independently of IRE1. Preliminary data from transcript and pathology analyses using double and triple knockout mutants suggest that both IRE1 and AGB1 contribute additive effects to pathogen resistance and bZIP60 splicing, and analyses of bZIP60 splicing levels in agb1 ire1a ire1b triple mutant upon biotic stress-inducing stimuli, such as treatments with salicylic acid and flagellin. Moreover, we examine a potential link to the master immune regulator NPR1 through a series of genetic and biochemical analyses using combinatorial higher order loss-of-function mutants of AGB1, IRE1a, IRE1b and NPR1.

Keywords: ER stress, IRE1, UPR, AGB1, NPR1

CS E3. Unraveling the Role of Vacuolar Invertase in the Establishment of Plant Disease Susceptibility

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University of Alabama at Birmingham

Throughout time, plants and their corresponding pathogens have been locked in an evolutionary arms race. The results of this race can be observed on multiple levels, from molecular genetics to metabolic processes. Plants have evolved an innate system to recognize potentially pathogenic microbes via microbe-associated molecular patterns which are common epitopes across of variety of pathogens. As a result, pathogens secrete effectors and toxins into plants to dampen plant immunity, while plants trigger MTI, ETI or other immune responses to defend against pathogen attacks. Besides the battle over the plant's immune response, both plants and pathogens require nutrition to survive and provide energy for cellular activities. Sucrose, as the main form of assimilated carbon, is not only essential for plant growth and development, but also involved in plant defense by activating plant the immune response as a signaling molecule. Under infection, pathogens rearrange carbon partitioning in plants, which results in a sugar content shift and triggers plant defense. As key players of hydrolyzing sucrose, plant invertases, including cell wall invertase, cytosolic invertase and vacuolar invertase, have been reported to participate in plant-pathogen interactions. While cell wall invertase is induced during early stage of pathogen infection, vacuolar invertase accumulates at later stages. Here we show the induction of ATVI2, a vacuolar invertase, at both transcript and protein levels after Pst DC3000 infection, but not under Pst DC3000 hrcC- infection. Furthermore, compared to wild type, atvi2 mutant exhibits resistance to DC3000, while pathogen growth of hrcC- remains the same. These results imply ATVI2 contributes to pathogenesis in an effector-dependent manner. We predicted several transcription factors which may regulate ATVI2 expression. Next, we are going to test the direct binding of TF-DNA using EMSA in vitro and ChIP-qPCR in vivo, analyzing the roles of these transcription factors in plant-pathogen interaction and ATVI2 regulation.

Keywords: plant-pathogen interaction, effector, vacuolar invertase

CS E4. Accumulation of Iso-Flavonoids and Phenolic Acid Conjugates in Response to Soybean Cyst Nematode in Wild Soybean (*Glycine soja*)

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Plants produce a wide range of biologically active metabolites to protect themselves against attacking pests. Elucidating the key metabolites and associated pathways underlying defense responses is critical in understanding the molecular mechanisms of plant chemical defense. Non-targeted metabolomics analysis has emerged as a useful strategy to increase our understanding of the resistance-related (RR) metabolites and pathways in plant-pathogen interactions. In this study, we performed a non-targeted metabolomic analysis to determine and compare the roles of key metabolites and pathways in response to infection by the soybean

cyst nematode (SCN, Heterodera glycines) in wild soybean (*Glycine soja*). SCN is the most devastating pest causing significant losses in soybean yield. A comparison of the metabolic profiles among SCN-resistant (S54) and susceptible (S67) genotypes showed clear differences, mirroring the effects of isoflavonoids (daidzein, daidzin, malonyl daidzin, formononetin, and iso-formononetin), as well as phenolic acids and phenolic acids-derived hydroxyl and methylated glucoside esters, in defense. To the best of our knowledge, these findings uncover the first metabolomics-based network for defending against SCN HG type 1.2.5.7 (SCN-2). The results of the present research can facilitate the future metabolic engineering to develop novel and diverse soybean cultivars with enhanced SCN resistance and/or improved nutraceutical value.

Keywords: metabolomics; resistance; soybean cyst nematode (SCN); wild soybean (*Glycine soja*); phenolic acids

CS E5. Regulation of *Arabidopsis* Floral Organ Development by the Transcription Factor AINTEGUMENTA-LIKE6

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Flowers originate around the periphery of the inflorescence meristem. The ABCE model of flower development in *Arabidopsis* proposes that discrete floral organ identities are specified by several classes of homeotic genes. Classes B and C (BC) are needed to establish petal, stamen and carpel identity. Two members of the AINTEGUMENTA-LIKE/PLETHORA (AIL/PLT) transcription factor family, AINTEGUMENTA (ANT) and AINTEGUMENTA-LIKE6 (AIL6), may help activate these genes in stage 3 flower primordia. ant ail6 double mutant flowers lack petals, stamens and normal gynoecium, and exhibit reduced BC gene expression in stage 3 flowers. ChIP-qPCR using an AIL6 epitope tagged line in a synchronous floral induction system (AIL6m:gAIL6-VENUS AP1:AP1-GR ap1 cal) shows that AIL6 directly binds to regulatory sequences of the class B genes APETALA3 (AP3) and PISTILLATA (PI) and to the class C gene AGAMOUS (AG) in stage 3 flowers. A steroid inducible AIL6-GR line in the double mutant background (AIL6m:gAIL6-GR ant ail6) was also created to determine how quickly expression of the homeotic genes respond to induction of AIL6 activity. Phenotypically, activation of AIL6-GR partially restores petal, stamen and carpel identity in these double mutant flowers, indicating that homeotic gene activity is restored in these plants. RT-qPCR on mock and dexamethasone treated AIL6m:gAIL6-GR ant ail6 inflorescences did not show increased expression of the homeotic genes at four and twenty-four hours after steroid treatment. Because stage 3 flowers contribute little tissue to the entire inflorescences harvested for these experiments, we are also examining AP3 and AG expression in these plants by in situ hybridization. Furthermore, we are generating plants that contain AIL6m:gAIL6-GR ant ail6 in the synchronous floral induction system (AIL6m:gAIL6-GR ant ail6 AP1:AP1-AR ap1 cal) so that RT-qPCR can be performed on tissue composed solely of stage 3 flowers.

Keywords: flower, *Arabidopsis*, meristem, floral organ development, homeotic

CONCURRENT SESSION F - COMPETITIVE

CS F1. Improved Network Biology Approach Discovers High-Confidence Candidate Host Targets of Pathogen TAL Effector Target Genes in Bacterial Leaf Streak of Rice

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Network biology has proven instrumental for the identification of key players in different biological systems including plant-pathogen interactions. Bacterial leaf streak (BLS) of rice, caused by Xanthomonas oryzae pv. oryzicola (Xoc), is an important disease that is becoming more prevalent with climate change. Xoc takes advantage of highly conserved transcription activator-like (TAL) effectors delivered into host cells to manipulate diverse biological processes including nutrient transport and defense response pathways by direct transcriptional activation of target genes. Available rice protein-protein interaction (interactome) and transcriptome datasets provide the opportunity to explore the complex landscape of interplay between host and pathogen during bacterial leaf streak. We applied network structural centralities and weighted k-shell decomposition methods to reveal the most influential players in a rice computational protein-protein interactome (RicePPInet), which encompasses 16,895 proteins and 708,819 interactions. We found that highly connected nodes (hubs) as well as nodes that situate in the center of the network (internal layered proteins) are enriched for candidate Xoc TAL effector target genes identified in a previous study. Further, we constructed a large rice response-to-pathogen co-expression network and integrated with RicePPInet to generate a Rice Immune Protein-Protein Interaction Network (RIPIN). Inner layered proteins in RicePPInet are enriched in RIPIN, and involved in several important biological processes. In summary, the results of our multi-omics, integrative network systems biology approach reveal that network centrality and structural properties may be highly predictive of TAL effector targets. This finding parallels the results of our previous effector target discovery in *Arabidopsis-Pseudomonas* interactions, suggesting that the approach is broadly translatable to other pathosystems.

Keywords: network systems biology, host-pathogen interaction, rice immunity, protein-protein interaction, network centrality

CS F2. Comparative *In Silico* Analysis of WRINKLED 1 Paralogs in Angiosperms

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WRINKLED 1(WRI1), a member of AP2/EREBP class of transcription factors regulates carbon allocation between glycolytic and fatty acid biosynthetic pathway. Additionally, among the four WRI1 paralogs in *Arabidopsis*, WRI3 and 4 but not WRI2, are also able to increase fatty acid content in different tissues. While the role of WRI1 is well established in seeds, the potential or WRI1 or its paralogs as master regulators in oil-rich nonseed tissues is poorly understood. Recent transcriptome studies of avocado (*Persea americana*) mesocarp revealed that the ortholog of WRI2, along with WRI1 and WRI3 was highly expressed during oil accumulation. Through transient expression assays, we further demonstrated that both PaWRI1 and PaWRI2 can accumulate oil in tobacco leaves. We conducted a comprehensive and comparative *in silico* analysis of WRI paralogs from a dicot, monocot and a basal angiosperm to identify distinct features associated with function. These data provide insights into the possible evolutionary changes in WRI1 homologs and allow for identification of new targets to enhance oil biosynthesis in diverse tissues.

Keywords: WRINKLED

CS F3. The Exploitation of the Glyoxylate Cycle in *Arabidopsis thaliana* by *Pseudomonas syringae* for Glucose Procurement

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While the life histories of organisms differ from one another, the driving force of life is to acquire nutrients, so organisms can grow and reproduce. To achieve this purpose, divergent evolution has created various nutritional modes such as photoautotrophs, herbivores, carnivores, parasites, etc. For example, parasitic

microbes invade their host organism to acquire nutrients. Here I show how the phytobacterium, *Pseudomonas syringae*, hijacks the plant's metabolic machinery, specifically the glyoxylate cycle, to withdraw the maximum amount of monosaccharides from the host cell. Carbohydrates, such as glucose, serve as the predominant energy source for a significant portion of organisms. While plants utilize carbohydrates, resulting from photosynthesis, they are also capable of breaking down lipids to fuel gluconeogenesis for the creation of glucose, for example, during seed germination. Peroxisomal citrate synthase (CSY2 and CSY3), the key enzymes downstream of β-oxidation that initiate the glyoxylate cycle by converting acetyl-CoA and oxaloacetate to citrate, are upregulated within 24 hours of a Pseudomonas syringae pv. tomato DC3000 infection. Moreover, the phytohormone, salicylic acid, which is accumulated during a hemibiotrophic pathogen attack, perturbs the interaction of CSY2 and CSY3. Additionally, we see an overall increase in the enzymatic activity of citrate synthase. By triggering this predominantly dormant cycle in mature leaves, the pathogen causes the plant to make available additional nutrients. Understanding the pathogen's strategy to exploit the host's metabolism can lead to novel agricultural strategies against bacterial diseases.

Keywords: Arabidopsis thaliana, defense, glyoxylate cycle, metabolism, citrate synthase

CS F4. Using Lasercapture Microdissection and RNASeq to Produce a Tissue by Time Transcriptome of Nodulating *Medicago truncatula* Roots

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The legume-rhizobial symbiosis involves a complex signal exchange between host plant and bacteria to initiate the symbiosis, leading to the formation of root nodules in which the bacteria fix nitrogen for the plant. Signal transduction events occur between the host cell layers in tissues, organs, and across time. For example, bacterial infection threads pass through the epidermis and outer cortical cells towards the inner cortical cells. The inner cortex and pericycle cells become mitotically active before the arrival of the infection thread. The vasculature is separated from inner cortical cells by the pericycle and endodermis and signaling passes across these tissues to the infection thread advancing across the cortex. Previous transcriptomic analyses used whole roots to identify host genes involved in nodule development, but this does not capture unique transcription events in specific tissues. To address this, we are performing transcriptome profiling of specific root tissues during nodule development by using laser capture microdissection (LCM) to isolate the tissues for RNA extraction. This is followed by RNA-seq analysis of libraries made from epidermal, vascular, inner and outer cortical cells at 0, 12, 24, 48 and 72 hours post inoculation. We are able to gather enough tissue to make unamplified libraries, but a comparison of unamplified to amplified libraries revealed minimal differences, except for time involved in capturing the tissue. We proceeded with amplified samples and to date we have completed all unfixed controls and five tissue libraries from fixed, inoculated tissue samples. We are able to detect expression of around 20,000 different transcripts from individual tissues, suggesting good depth of coverage. Tissues also showed enrichment of tissue-specific marker genes, demonstrating effective separate tissue types by LCM. We will use the data generated to uncover distinct tissue gene expression profiles during nodule development. This work is supported by NSF IOS 14444.

Keywords: symbiosis, lasercapture micro dissection, RNASeq, *Medicago truncatula*

CS F5. Photosynthetic Responses of Grain and Forage Sorghum to Temperature Stress

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As sorghum is one of the major crops of the Southern Great Plains and constitutes for the major part of food production, it is vital to understand the photosynthetic responses to temperature stress due to the projected climate extremities. Two species of sorghum: grain and forage sorghum were considered for the study. The total number of days with temperatures greater than 33 ˚C has been increasing in the SGP according to National Oceanic and Atmospheric Administration and National Weather Service. Temperature stress decreased the photosynthetic rate and leaf stomatal conductance. The crops are grown season-long in six controlled growth chambers, the temperatures of which are maintained at (daytime/nighttime) 20/12, 24/16, 28/20, 32/24, 36/28 and 40/32 and relative humidity maintained at 60%. The light and CO_2 response curves are studied and respective parameters are derived with an open gas exchange LI-6400 photosynthesis systems (LICOR, Lincoln, Nebraska, USA) fitted with a 6400-40 leaf chamber fluorometer (LCF), which determine photosynthetic efficiency. These response curves help in determining the enzymatic functions and further enable to understand the physiology of the crop plant, hence preparing them for future adverse climatic conditions. The activity of the enzyme Rubisco and PEPC are the major contributors for effective photosynthesis in C4 plant species. The carbon dioxide assimilation increased with increasing temperatures till it reaches the optimum and with further increase in temperature, the assimilation efficiency has decreased. This is due to the corresponding effects in the activity of enzymes contributing to photosynthesis. Further analysis is needed to understand the underlying process of photosynthesis which is the life-sustaining process on the planet. In this, we present and discuss the response to the temperature of photosynthesis to leaf internal CO_2 concentration and ambient light levels.

Keywords: photosynthesis, light and CO_2 response curves, sorghum

POSTER PRESENTATIONS

P1.*The Effects of Clinorotation on Growth of Different *Arabidopsis thaliana* Genotypes

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Multiple stressors can affect plant development, but some genotypes are more resistant to stress than others. Variation across plant genotypes allows us to identify those that are more resistant to a specific stress factor, such as gravitational acceleration. Plants experience stress from altered gravity conditions during space flights or reduced gravity if grown on the Moon and Mars. Resistant plant genotypes are crucial for possible distant space exploration by humans in the future. Thus, the goal of this study is to find *Arabidopsis thaliana* genotypes that are resistant to gravitational stresses. One hundred wild-type genotypes from different origins were tested. To simulate gravitational stress, we placed plates with surface sterilized seeds on to a rotating 2D-clinostat for seven days, while the control plates were kept vertically oriented. Morphological parameters such as shoot length, primary and secondary root length, and the number of root hairs were recorded from images using ImageJ software. For each genotype, growth parameters from clinorotated and vertical seedlings were compared with t-tests. Our preliminary data from ten genotypes analyzed suggest that clinorotated seedlings have reduced shoot growth compared to seedlings grown vertically. Two genotypes have similar or increased growth for main root and total root length, number of secondary roots, and root hairs, compared to the controls. Once we have assayed all one hundred genotypes, we will focus on identification of genes involved in resistance to gravitational stress.

Keywords: gravitational stress, microgravity, clinorotation, growth responses, resistant genotypes

P2. Genes Regulating Growth, Phototropic, and Gravitropic Responses in *Arabidopsis thaliana*

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Gravitropic and phototropic responses play an important role in terms of plants adapting to their environment. However, the specific mechanisms for these tropisms are unknown. Only in the absence of 1g gravity conditions that are available during a space-flight (i.e., microgravity), the pure phototropic responses can be characterized. With DNA transcriptome data from our space-flight experiments, we identified six genes that are activated in microgravity. Wild-type (Columbia) *Arabidopsis thaliana* seedlings and six knockout mutants (of the identified genes), AT1G22270, AT5G25070, AT5G53650, AT4G06534, AT4G37650, AT2G02550, were used to test their gravitropic and phototropic responses. Vertical light-grown as well as dark-grown 4-day-old seedlings were reoriented 900 and their gravitropic responses were assayed and compared to a wild-type. Similarly, seedlings were treated with unidirectional blue or red light to see differences in their phototropic responses. Preliminary data from the gravitropism experiments showed that some mutants have altered gravitropic responses in comparison to wild-type seedlings. Interestingly, our results to date suggest that a neurofilament light protein, associated with the actin cytoskeleton, plays a role in the attenuating of gravitropism in roots.

Keywords: knockout mutants, shoot and root curvature, shoot and root growth

P3. * Analysis of Phototropism and Gravitropism in *Arabidopsis thaliana* in a Ground-based Control Experiment

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Plant adaptations to light and gravity are crucial for their survival and fitness. Since gravitropism and phototropism work simultaneously, microgravity is needed to separate the effects of two environmental factors. In microgravity, during spaceflight experiments with *Arabidopsis thaliana*, novel root and shoot responses have been observed that are not seen on Earth. For instance, roots had positive responses to blue and red light, and shoots grew towards unilateral red light. The goal for this study is to perform a ground-based control experiment that simulates the effects of microgravity by using a 2D clinostat and to compare plant responses. Thus, we want to compare spaceflight data to studies with clinostats, which can be considered microgravity analysis. Similar to the space-flight experiment, surface sterilized seeds of the wild-type genotype, Landsberg, and two phytochrome lacking mutants are plated on growth medium and placed vertically under white light. Vertically oriented seedlings are then put onto the clinostat with unidirectional blue or red light treatments for 44 hours. Shoot and root responses are video recorded every 30 minutes and are analyzed with ImageJ software. Results to date suggest that 2D clinostat treatments partially simulate microgravity effects. A future implication to this project is to understand the mechanisms of plant tropisms and how this can be applied for plant growth development in microgravity conditions, on the Moon, or Mars.

Keywords: microgravity, clinostat, blue light, red light, shoot curvature, root curvature

P4.* Investigation of AIL6 Regulation of the Floral Homeotic Genes AG and AP3

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Floral organ development is a complex process that requires numerous genes, including the floral homeotic genes, which act in different regions of a flower primordium and in distinct combinations to specify sepal, petal, stamen and carpel identities. Two other genes required for the establishment of floral organ identity as well as other aspects of floral organ development are AINTEGUMENTA (ANT) and AINTEGUMENTA-LIKE6 (AIL6), two members of the AINTEGUMENTA-LIKE/PLETHORA (AIL/PLT) transcription factor family in *Arabidopsis* thaliana. While loss of AIL6 function alone shows no obvious phenotype and loss of ANT function primarily affect floral organ growth, ant ail6 double mutant flowers do not produce petals, stamens, or normal carpels. This phenotype is correlated with reduced expression of the floral homeotic genes, APETALA3 (AP3) and AGAMOUS (AG), and we hypothesize that these genes may be direct targets of ANT and AIL6 regulation. To investigate this possibility, we created transgenic lines expressing an ethanol inducible artificial microRNA that downregulates AIL6 expression in the ant mutant background (35S:AlcR/AlcA:amiR-AIL6 ant). Reduced levels of AIL6 mRNA were detected in a 35S:AlcR/AlcA:amiR-AIL6 ant line treated with ethanol for 24 hours. In addition, we observe several phenotypes (smaller and fewer petals) consistent with AIL6 knockdown. AG and AP3 mRNA levels were reduced in the ethanol treated plants suggesting that the expression levels of these genes respond quickly to reductions in AIL6 expression, consistent with our hypothesis that these genes may be directly regulated by AIL6.

Keywords: floral, homeotics, floral organ development

P5. Investigating the Functions of AINTEGUMENTA Target Genes in *Arabidopsis* Flower Development

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The *Arabidopsis* inflorescence meristem gives rise to floral meristems which give rise to floral organ primordia. These floral organ primordia adopt one of four fates (sepal, petal, stamen and carpel) as specified by four different classes of floral homeotic genes that work in different combinations to specify distinct organ identities as described in the ABCE model. The AP2/ERF transcription factor AINTEGUMENTA plays an important role in floral organ growth, development and specification, acting both upstream and downstream of the floral homeotic genes. To better understand the roles of ANT in floral organ development, I am investigating the function and expression of four genes that appear to be direct targets of ANT regulation. I am investigating the function these genes by creating transgenic lines that overexpress each gene under the constitutive 35S promotor from the cauliflower mosaic virus and generating CRISPR mutant alleles. Overexpression of each of these four genes does not lead to any obvious changes in flower development, although homozygous lines have not been obtained for most lines yet. I am also generating promoter:GUS reporter gene constructs to examine the spatial expression of these genes during flower development.

Keywords: AINTEGUMENTA, flower development

P6.* A Study of Endosperm Developmental Shift in *Arabidopsis* Using the Ectopic Expression of InvINH1 by KRS Promoter

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The endosperm and embryo are the results of double fertilization in flowering plants. Each structure has its

own mechanisms for growth and development, but they rely on each other for successful seed development. In the early stages of *Arabidopsis* seed development, the endosperm grows rapidly while the embryo grows slowly. After endosperm cellularization, this growth pattern changes so that the endosperm grows slowly while the embryo grows quickly. This shift in growth is likely due to nutrients shifting from the endosperm to the embryo. We hypothesized that invertase, an enzyme that breaks down sucrose, is one of the primary mechanisms for this nutrient shift. Invertase Inhibitor 1 (InvINH1) was identified in our lab as being specifically expressed before endosperm cellularization. Therefore, the presence of InvINH1 is correlated with slow embryo growth. To further investigate the effects of InvINH1 on embryo growth rate, KRS promoter was selected to ectopically express InvINH1 after endosperm cellularization. To construct the pKRS-InvINH1 chimeric gene, the coding region of InvINH1 was first amplified and cloned into a binary vector. Positive clones were verified by sequencing. Then, the 2048bp KRS promoter region was amplified from genomic DNA and cloned in front of InvINH1 coding region. This will allow us to express InvINH1 after endosperm cellularization. The 2048bp KRS promoter region was also cloned in front of a gene encoding the Green Fluorescent Protein, which will allow us to verify that KRS promoter is only active in the cellularized endosperm. After the correct clones are verified by sequencing, the two constructs will be used to transform *Arabidopsis* plants via Agrobacterium-mediated floral dip method. For transgenic plants carrying the pKRS-InvINH1 transgene, we expect to see a delay in embryo growth, which will support our hypothesis that the function of InvINH1 is to suppress embryo growth before endosperm cellularization.

Keywords: Arabidopsis, ectopic expression, invertase inhibitor, transgene

P7.* Modeling Metal Uptake in *Arabidopsis thaliana* Plants Exposed to Kanamycin

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Plants take up essential nutrients from the soil through their roots, and in the process absorb substances released by soil microorganisms such as antibiotics. Although research in this area is sparse, it is expected that the natural propensity of organisms to fight off unwanted influences drives plants to develop resistance against these antibiotics. One well known example of this phenomenon is observed in *Arabidopsis thaliana* plants, which possess the Atwbc19 gene that confers resistance to the antibiotic kanamycin. Atwbc19 mutants are very sensitive to kanamycin and their Zn uptake is compromised under normal conditions. In addition, Fe uptake in control plants declines when they are exposed to kanamycin. These preliminary findings suggested a link between antibiotics and metal uptake. Here, we propose and experimentally validate a model that explains the connection between metal uptake and antibiotic resistance. .The metal transporter IREG1 allows Fe transport into the xylem, and is shut down in the presence of kanamycin. Atwbc19 on the other hand transports Zn-NA and serves as an alternate route for Fe transport during kanamycin exposure, as Fe-NA. Data was collected by plating Atwbc19 mutant as well as control plants in media containing varying levels of iron for a period of seven, eleven and thirteen days. Plants were harvested, dried and sent for metal analysis. For control plants we observed an overall reduced uptake of iron both in the presence and absence of kanamycin only when citrate was present in the media. On the other hand Atwbc19 mutants showed a drastic decrease of iron and zinc content when exposed to kanamycin. After estimating parameters using a subset of data, the resulting model predicted experimental results well. Thus the results support our model based on a modified metal homeostasis, namely the inhibition of the iron transporter IREG1 in the presence ofkanamycin.

Keywords: antibiotic resistance, metal transport, modeling

P8.* Study on the Effect of Ectopically Expressing InvINH1 by RGP3 Promoter on Embryo Growth Rate in *Arabidopsis*

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Appropriate seed development in *Arabidopsis thaliana* involves the coordinated growth between two main components, the embryo and the endosperm. The first stage of endosperm development, known as the syncytial phase, involves the division of the nuclei without forming a cell membrane and cell wall. Following this stage is the cellularization of the endosperm. After cellularization, the growth rate of the endosperm decelerates while embryo growth accelerates. Consequently, the endosperm is eventually absorbed by the embryo. The acceleration of embryo growth suggests that sugar is reallocated from the endosperm to the embryo. Invertase is an enzyme that converts sucrose into fructose and glucose. Invertase has been shown to regulate the movement of sugar from maternal tissues to the endosperm. Our lab identified an invertase inhibitor (InvINH1) that is expressed in the endosperm before cellurization. When a ZOU promoter was used to express InvINH1 in the cellularized endosperm, a subtle delay in embryo growth is observed. To strengthen this phenotype, a RGP3 promoter is used to drive the expression of InvINH1. RGP3 promoter is a stronger promoter than that of ZOU. RGP3 interconverts UDP-Arabinopyranose and UDP-Arabinofuranose in the endosperm, which is essential for the formation of plant cell wall. To enhance the delayed embryo growth phenotype, promoter RGP3 was first cloned in front of the InvINH1 coding region to create vector pRGP3-InvINH1. To confirm the expression pattern of RGP3 promoter, the promoter was also cloned in front of a green florescent protein (GFP) reporter. After sequence verification, both constructs will be transformed into *Arabidopsis* plants. After transgenic plants carrying the pRGP3-InvINH1 transgene are identified, detailed phenotypic analysis will be carried out to determine whether using RGP3 promoter to express InvINH1 in the cellularized endosperm could enhance the delayed embryo growth phenotype.

Keywords: Arabidopsis thaliana, RGP3, invertase inhibition, InvINH1, endosperm, embryo

P9.* Delayed Cotyledon Greening Regulates phyB Signaling in *Arabidopsis*

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Phytochrome B (phyB) is an important photoreceptor that perceives red light and regulates growth and maturation in plants. Levels of the active form of phyB in plants are regulated by light conditions such as direct sunlight and shade, as well as light-independent processes like dark reversion, in which phyB is converted from its active form to its inactive form. In this study we characterize a newly identified protein Delayed Cotyledon Greening1 (DCG1) and a homologue DCGH that are presumed to interact with phyB. These interactions demonstrate enhanced light sensitivity in plants. Upon generating overexpression lines of both DCG1 and DCGH, we found that cotyledon greening is delayed, and gravitropism in the hypocotyls of seedlings is inhibited. However, light-regulated genes including CAB3, RBCS, and FedA are upregulated in DCG1 and DCGH overexpression lines and down-regulated in their mutants. These phenotypes likely occur due to the association of DCG1 and DCGH with phyB, but the molecular functions of these proteins as well as the mechanism by which they interact with phyB is still unknown. Thus, DCG1 and DCGH are new proteins that regulate light-responsive gene expression and adaptation of plants to their environment through affecting phyB activity.

Keywords: Arabidopsis, phytochrome, cotyledon

P10.* Evaluation of mPing Transposition in *Arabidopsis thaliana* DNA Methylation Mutants

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Transposable elements (TEs) are mobile DNA sequences that move from one area in a genome to another through excision and insertion. TE movement is catalyzed by the activity of transposase enzymes. In this project we are testing the TE known as mPing, originally from rice, in the model plant *Arabidopsis thaliana*. *Arabidopsis* is a suitable for this project because of its small size and the ease of manipulating its DNA. Our overall question was whether changes in chromatin structure, specifically DNA methylation effects transposition behavior. A mPing transposition construct was transformed control, met1, and ddm1 mutant plants. In order to observe the transposition of mPing, the plants are being tested for GFP using fluorescence microscopy and PCR. This should allow us to determine if the loss of DNA methylation and its associated loosening of the chromatin structure allows for more transposition.

Keywords: transposable element, methylation

P11. Exploring a Potential Interaction between α–Amylase3 and the Catalytically Inactive β–Amylase9 in *Arabidopsis thaliana*

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Starch, a complex sugar, is the principle energy storage molecule in plants. This supply of sugar ensures that the organism is able to continue its metabolic processes through the night, when energy from the sun is unavailable. These molecules are composed of two components: amylose and amylopectin. The former consists of simple sugars joined 1,4 in an unbranched chain, which is hydrolyzed by amylase enzymes. β–amylase (BAM) enzymes are a specific type of amylase that have been identified as principle catalysts in this degradation of transitory starch. However, most plant genomes include BAM genes coding for proteins that are predicted to have no enzymatic activity. One such non-catalytic protein is BAM9, although initial research suggests that this protein may function as a pseudoenzyme and is regulating the activity of another protein. There is evidence to propose that BAM9 specifically interacts with α–amylase3, a catalytic enzyme known to function in starch degradation. To further explore this potential interaction, two different assays are being used. The first is a pull-down analysis using labeled proteins, where the presence of both proteins in the final solution indicates that there is a specific interaction between the amylases. Secondly, a colorimetric assay is being used to quantify the catalytic activity of AMY3 alone and in the presence of BAM9. Any difference in the absorbance of the solution indicates a change in the amount of starch degraded by AMY3. Together, these data will allow us to better explain the results of earlier experiments and develop a more comprehensive understanding of the role played by BAM9 in starch degradation.

Keywords: amylase, starch, enzyme, *Arabidopsis thaliana*

P12.* Sequence Analysis of a miRNA-Induced *Arabidopsis thaliana* Mutant

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Understanding gene function is essential to solving genetic problems or beneficially altering gene expression. An important tool for determining gene function is gene silencing, because it allows you to see how the organism behaves when the targeted protein is absent. The method we used to randomly decrease gene

expression of *Arabidopsis* genes was miRNA-induced gene silencing. This method involves attaching a microRNA target sequence to an mRNA sequence and inducing the production of tasiRNAs. The tasiRNAs subsequently degrade homologous sequences. We transformed a naturally occurring *Arabidopsis thaliana* miRNA, called miR173, into random positions of the genome. One of the resulting plants was a mutant that exhibits altered leaf shape, delayed flowering, and reduced seed set in a dominant manner. We extracted DNA from plants with the mutant phenotype and are currently working to prepare a DNA library for nanopore sequencing. Nanopore sequencing is a relatively new technique that sequences long strands of DNA through a protein nanopore. This method provides high-throughput sequencing results, but also provides long sequencing reads. We will analyze the nanopore sequencing results by conducting a BLAST search for the transgene sequence using Geneious software, and then analyze adjacent sequences to identify candidate genes. To verify that the identified candidate genes are responsible for the mutant phenotype, we will test for changes in gene expression using quantitative reverse transcriptase (RT) PCR to analyze changes in the mRNA levels. A decrease in mRNA levels in the mutant would confirm that the mutant phenotype is due to gene silencing.

Keywords: nanopore sequencing, TasiRNA

P13.* Natural Variation and Stress Tolerance of IRE1a Gene of *Arabidopsis thaliana*

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In modern plant biology, the evolution of ecophysiological traits with regards to environmental pressures is of key interest. In plants, such as the model plant *Arabidopsis thaliana*, the perception of abiotic and biotic stresses first occurs at the molecular level and is perceived by cellular receptors. Many stress responses increase the demand on protein translation and folding machinery, which can cause exceedingly high burden on the endoplasmic reticulum (ER), a phenomenon termed ER stress. The accumulation of ER stress can activate the unfolded protein response (UPR). The inositol-requiring protein 1a (IRE1a), a key element in UPR, alleviates ER stress by activating a transcription factor bZIP60 through an extremely rare event of ribonuclease-catalyzed cytoplasmic splicing. Considerable variation has been observed in the levels of expression of the IRE1a gene in natural accessions of *Arabidopsis*. Here, we attempted to examine the differences in abiotic and biotic stress tolerance of two natural accessions of *Arabidopsis* that are previously shown to have the largest difference in IRE1a basal gene level. Thus, the diverse geographic range of *Arabidopsis* inhabitation elucidates the relation of natural selection to the development of IRE1a variation, and in turn, different levels of stress tolerance.

Keywords: IRE1a, bZIP60, natural variation, *Arabidopsis*, abiotic stress, biotic stress

P14.* Identification of a New Mutant in the Autoregulation of Nodulation Regulatory Pathway in *Medicago truncatula*

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Legumes utilize a long-distance signaling pathway to regulate the number of nitrogen-fixing symbiotic nodules that form on their root systems. In the current model for this process in *Medicago truncatula*, two CLE peptides (MtCLE12p and MtCLE13p) produced in developing nodules travel via the xylem to the shoot where they interact with the receptor-like kinase SUNN which triggers a return signal to the roots putting a halt to further nodulation. The isolation of mutants defective in the autoregulation process has also led to the identification of additional components of the pathway including the hydroxyproline arabinosyltransferse RDN1, which is necessary for modification of the CLE12 peptide in roots, and the pseudokinase CRN, which

acts along with SUNN in the shoot, potentially in complex with the receptor-like protein CLV2. We report the characterization of another autoregulation mutant, 8XV1, which was generated by fast neutron bombardment. We determined through grafting that the lesion in 8XV1 acts in the root to increase nodule number, although it is not clear if it influences signaling to the shoot or response to signals from the shoot. We mapped the 8XV1 lesion to the middle of chromosome 2 and have identified a deletion that includes a candidate gene. We report attempts to rescue the mutant phenotype with a functional copy of the candidate gene using transformation of mutant roots by Agrobacterium rhizogenes. This work is supported by NSF IOS # 14444 and # 1733470 and a Clemson University Creative Inquiry grant.

Keywords: legumes, nodulation, long distance signaling

P15. Gene Expression Pattern of NCR150, a Nodule-Related Gene in Two Supernodulation Mutants and Wild-Type

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Most legumes form lateral organs called nodules on roots to accommodate symbiotic bacteria known as rhizobia for obtaining fixed nitrogen to grow efficiently in N-deprived environments. An autoregulatory feedback mechanism controls the number of nodules that form. Transcriptome analyses of early nodulation stages in the model legume *Medicago truncatula* provide the possibility of identifying genes involved in this regulatory process. Here, we performed gene expression profiling over five post inoculation time points (0, 12, 24, 48, 72 hours) in supernodulation mutants sunn-4 and rdn1-2, which are defective in nodule number regulation, and the wild type A17 through an RNA-Seq approach. Through comparing the expression level of nodulation-related genes in wild-type with the supernodulation mutants, we identified a gene named NCR150 which showed a spike in expression 12h after inoculation in A17 but less so in supernodulation mutants. NCR genes encode small secreted peptides which strikingly are mostly expressed only in nodules. Previous studies have shown that some NCR peptides are suspected to impact rhizobial development and have antimicrobial activity during nodule development. NCR150 is among the very few NCR genes detected outside of nodules, being found in epidermal cells responding to rhizobia as well as in roots responding to mycorrhizae. To verify the difference in expression of NCR150 in the supernodulation mutants we are using qRT-PCR on samples collected from 0 to 12 hours after inoculation.

Keywords: nodulation, autoregulation of nodulation, *Medicago truncatula*, transcriptome, signaling peptides

P16. Using a Molecular-genetic Approach to Investigate the Interactions between Rice and Nitrogen-fixing Bacteria, *Azospirillum brasilense*

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Major non-legume crops can form beneficial associations with nitrogen-fixing bacteria like Azospirillum brasilense. Our current understanding of the molecular aspects and signaling that occur between important crops like rice and these nitrogen-fixing bacteria is limited. In this study, we used an experimental system where the bacteria could colonize the plant roots and promote plant growth in wild type rice and symbiotic mutants (dmi3 and pollux) in rice. Our data suggest that plant growth promotion and root penetration is not dependent on these genes. We then used this colonization model to identify regulation of gene expression at two different time points during this interaction: at 1day post inoculation (dpi), we identified 1622 differentially expressed genes (DEGs) in rice roots and at 14dpi, we identified 1995 DEGs. We performed a

comprehensive data mining to classify the DEGs into the categories of transcription factors (TFs), protein kinases (PKs), and transporters (TRs). Several of these DEGs encode proteins that are involved in the flavonoid biosynthetic pathway, defense and hormone signaling pathways. We also identified genes that are involved in nitrate and sugar transport and are also implicated to play a role in other plant-microbe interactions. Overall, findings from this study will serve as an excellent resource to characterize the host genetic pathway controlling the interactions between non-legumes and beneficial bacteria which can have long-term implications towards sustainably improving agriculture.

Keywords: RNA-seq, rice, plant growth promoting bacteria, *Azospirillum*

P17. Chilling Induced Alteration of Warm Growing Degree Hour and Base Temperature for Floral Bud Break in Peach

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Bud break timing in peach [*Prunus persica* (L.) Batsch] is determined by the fulfillment of a chilling requirement (CR) and a heat requirement (HR) for development. Genotypic variation in CR has been well-characterized in peach. Potential variation in HR among varieties has received less attention, in part due to the overlap of effective temperatures for CR and HR and dynamic modification of HR by continued chilling accumulation beyond the minimum threshold CR for bud break. HR could vary in the magnitude of growing degree hours (GDH) and/or the base temperature for accumulating GDH. We estimated the GDH and base temperature for floral bud break by forcing replicate stem cuttings at constant temperatures of 13, 14, 15, 16, 18, and 20 ^{o}C and analyzing the effect of temperature on the inverse of hours accumulated to reach median bud break (defined by appearance of sepal or petal coloration). We evaluated varieties with different CR through a range of partial to fully satisfied chilling accumulations. Varieties differed in their chill saturated GDH and base temperatures for floral bud break. Within a variety, chilling accumulation reduced GDH while minimally affecting the base temperature for GDH accumulation. Improved descriptions of variety specific dynamics of GDH and base temperature response to chilling may allow improved bloom date modeling in peach.

Keywords: prunus, phenology, development

18. Global Metabolomics Elucidates the Differential Regulation and Inducibility of Plant Stress Responses across Biotypes with Contrasting Glyphosate Susceptibilities

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The primary driver of glyphosate resistance (R) in the agricultural weed Palmer amaranth (*Amaranthus palmeri*) is the amplification of the gene that codes for 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, the target enzyme of herbicide glyphosate. EPSPS is the penultimate enzyme of shikimate pathway, which through the production of phenylalanine, drives the phenylpropanoid pathway. EPSPS amplification could result in a higher capacity for R-biotypes to produce phenylpropanoids, and thus to withstand environmental stress. The lack of fitness penalty associated with maintenance and expression of EPSPS amplification indicate that the resistance mechanism might be induced after herbicide application rather than being constitutively expressed. We tested the innate and induced cellular physiology of two glyphosate susceptible (S) and three glyphosate resistant (R) biotypes of A. palmeri exposed to herbicide by contrasting their global

metabolome. Global metabolite profiling using ultra-high resolution accurate mass (UHRAM) mass spectrometry identified more than 40,000 mass features, which were curated to 72 primary metabolites and 11,267 secondary metabolites, which were further processed using molecular ion network and path analysis. In the absence of herbicide the cellular physiology of R-biotypes were innately different from that of the S-biotypes- the former was abundant in TCA metabolites and carbohydrates, whereas the latter had a higher relative abundance of intermediaries of shikimate pathway. From more than 200 phenylpropanoids that were identified, the R-biotypes had a proportionately greater abundance of flavonoids, especially glycosylated flavanols. Though similar in numbers, the molecular identity of compounds upregulated under glyphosate stress was dissimilar between the S- and R-biotypes, with S-biotypes upregulating monophenolics, whereas the R-biotypes preferentially upregulating flavonoids. Our results highlight the strength and complementarity of metabolomics to gauge and predict the innate and perturbed cellular physiology by capturing the global metabolome, and indicate that the resistance mechanism in A. palmeri may partly be induced.

Keywords: glyphosate, *Amaranthus palmeri*, amaranth, metabolomics, resistance, stress, EPSPS

19.* Lysing *Bacillus cereus* with Phage Amidase

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Bacteriophage are viruses that attack specific types of bacteria. Scientists have shown that purified amidase proteins encoded by some bacteriophage can destroy the cell walls of specific bacteria. *Bacillus cereus* is a species of bacteria that infects human intestines and implicated in food poisoning cases. Development of phage-based strategies for controlling pathogens like *Bacillus cereus* will allow for more effective treatment of bacterial infection without traditional antibiotics. We plan on using amidases from two different bacteriophage, BPS13 and DIGNKC, in order to lyse or break down the cell wall of, Bacillus cereus, without lysing other bacteria. The first step of our project is to create expression constructs for both BPS13 and DIGNKC. These constructs were verified by PCR and sequencing. The two amidase constructs were then transformed into Agrobacterium to allow for transformation into Arabodopsis thaliana plants. We predict that our plant produced amidases will successfully lyse *Bacillus cereus* in culture. We have successfully transformed *Arabidopsis* with the DIGNKC amidase. We will test transgenic *Arabidopsis* leaf tissue to determine if our construct can produce enough amidase to inhibit growth of *Bacillus cereus* in culture. We will also test BPS13 in the future.

Keywords: amidase, *Arabidopsis*

P20. Enhanced Unusual Fatty Acid Accumulation in Exotic Euphorbiaceae Species Requires Specialized Lysophosphatidic Acid Acyltransferases

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Tung tree (*Vernicia fordii*), castor bean (*Ricinus communis*) and several other Euphorbiaceae sp. accumulate oils rich in structurally unusual fatty acids (UFA), many of which are valuable industrial feedstocks. Transgenic production of such fatty acids often results in various adverse plant characteristics, however,

resulting in low seed yields, poor resistance to abiotic stresses, etc., thus limiting agronomic exploitation of these plants. UFA production in high yielding non-food oil crops would provide new robust sources for these valuable 'green petrochemicals'. Previous studies has shown that multiple oil metabolism genes must be coexpressed to achieve efficient UFA incorporation into seed storage lipids. Here we use demonstrate that lysophosphatidic acid acyltransferases from two Euphorbiaceae species have high selectivity for incorporation of their respective unusual fatty acids into phosphatidic acid, an intermediate of oil biosynthesis. These results are consistent with the hypothesis that UFA accumulation arose in via co-evolution of multiple oil metabolic enzymes that cooperate to enhance selective fatty acid incorporation into seed oils.

Keywords: acyltransferase, triacylglycerol, biotechnology, Euphorbiaceae, unusual fatty acid

21.* Isolating an Autoinducer that Regulates Quorum Sensing in *Chlamydomonas reinhardtii*

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Quorum sensing (QS) is a process by which microorganisms utilize low-molecular weight signals, generically classified as autoinducers (AIs), to couple phenotypic switching to population density. While QS is well-established in prokaryotes, there is growing evidence that unicellular eukaryotes such as yeast and fungi are also capable of this phenomenon. Detecting, observing, and controlling this intercellular communication system in eukaryotes has a wide variety of potential applications and could significantly impact our understanding of microbial ecology. However, one challenge is to determine relevant phenotypes which are likely to be QS regulated. Motility is a good candidate for QS regulation in unicellular eukaryotes, as it would allow these organisms to search for new resources prior to nutrient scarcity. We previously confirmed a density dependent effect on motility in the model unicellular algae Chlamydomonas reinhardtii. Through a series of media swaps and culture extractions we now provide significant evidence for the presence of an AI which increases the swimming velocity obtained by these unicellular algae. Utilizing LC-MS as well as known AIs in other eukaryotes we have attempted to identify the AI in C. reinhardtii. Our findings expand our understanding of QS among unicellular eukaryotes and has opened a new area for further research.

Keywords: quorum sensing, *Chlamydomonas reinhardtii*, algae, autoinducer

22.* Understanding the Responses of Poplar to Its Most Damaging Pathogen, *Sphaerulina musiva*

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Populus spp. are among the fastest-growing temperate trees, with important commercial uses in pulpwood, engineered lumber, and biofuel. In anticipation of shrinking petroleum reserves and a reduced land-base to produce forest products, interest in short-rotation, intensive culture of poplar trees as an alternative fuel and fiber resource has increased. However, widespread adoption of short-rotation, intensive poplar production is hindered by the occurrence of several damaging diseases, with stem canker caused by *Sphaerulina musiva*

(previously named *Septoria musiva*) being the most damaging to the economy in North America. This pathogen can also cause leaf spot in poplars. Severe *Sphaerulina* leaf spot outbreaks can reduce the photosynthetic area and cause premature defoliation, thereby decreasing annual growth. Stem cankers reduce growth, predispose the tree to colonization by secondary organisms, and cause severe girdling and breakage of the main stem. Because chemical and biological management of this disease has had only limited success, planting resistant species and clones offers the best long-term strategy for sustainable disease management and a reduction of the chemical footprint in the ag-ecosystem. However, successful and efficient breeding for resistance to S. musiva requires a molecular-level understanding the mechanism underlying the host's response to this pathogen, which currently is not available. In this project, we aim to study candidate genes involved in host response to the pathogen by sequence analyses and transformation.

Keywords: biotic stress, gene expression, host/pathogen interaction, poplar

P23. Small Stem Assay for Chestnut Blight Resistance in Segregating Full-Sib Families of F2 Chestnut Trees

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Restoration efforts of the American Chestnut began in the 1920"s with attempts of introducing resistance into the species by hybridizing the American chestnut with an Asian species of the same genus, *Castanea mollissima*. In 1981 Charles Burnham hypothesized that back crossing hybrids, which have been selected for blight resistance, with American chestnut would conserve the alleles for blight resistance and introduce American chestnut morphology into these blight resistant hybrids. The American Chestnut Foundation was founded in 1983 to help with Burnham's proposal. In recent years many chapters of the foundation are participating in a small stem assay to screen these hybrids for blight resistance. This is the inoculation of chestnut seedlings during their first growing season. The small stem assay allows for early progeny testing of hybrids. The application of the small stem assay for progeny testing in the breeding program has also been of controversy to the foundation. The question being is this approach a useful tool for progeny testing? Is the inoculation of trees at such young age too severe to see the expected results? In the past there has also been an issue with seedlings not being successfully inoculated with the techniques of the small stem assay. This study focuses on the method of inoculation and the results of a small stem assay on a population of straight F2s, F1s, American and Chinese controls. The American and Chinese exhibited significant differences in resistance. The F2s displayed a full range of resistance from highly resistant to low resistance to the effects of chestnut blight. 70 American chestnut trees died out of the 70 that were inoculated. Out of the 46 Chinese seedlings that were inoculated only 4 died. 407 F2s were inoculated and 62 of those seedlings survived the inoculation.

Keywords: small stem assay, Chestnut Blight resistance

P24.* Exploring the Unique Beta-amylase2 and Its Novel Potential Binding Partner

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To utilize stored energy, plants break down starch, a common carbohydrate, into maltose through the use of proteins called amylases. One of the primary amylases in plants are Beta-amylases (BAMs). Multiple BAMs are involved in starch degradation; however, the role of certain BAMs, including BAM2, are not known. The purpose of this project in to established whether BAM2 is acting with previously identified potential binding proteins, Mgn1 and Mgn2. As a first step to check for a protein-protein interaction BAM2-His was over expressed and purified through affinity chromatography. Each Mgn, tagged with strep, is currently being

cloned and placed in expression vectors. Once over expressed each Mgn-strep will be assayed via affinity pull-downs with BAM2-His to check for interactions. Identifying a binding partner for BAM2 is essential for understanding the enzyme's function and purpose and could increase understanding of using starch as an energy source.

Keywords: protein-protein interactions, beta-amylases, starch degradation, affinity chromatography

P25. Proteomics of Pierce's Disease Tolerant and Susceptible Grape Xylem Sap Ra

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Pierce's disease (PD), caused by bacterium *Xylella fastidiosa*, represents a significant threat to grape cultivation and industry. The bacterium is transmitted by the xylem sap-feeding sharpshooter, which clogs xylem vessels by the formation of biofilm and results in the wilting of plant. Xylem sap is known to contain signal transduction proteins, stress-related proteins and pathogen-related proteins. However information regarding the total protein composition of *Vitis* xylem sap is limited. Hence, the study was carried out to investigate xylem sap proteome of different *Vitis* species to understand the genetic diversity and various metabolic pathways associated with PD tolerance. In this study, we include *V. vinifera* (bunch), Florida Hybrid bunch grape (FH) and *V. rotundifolia* (muscadine) cultivars to characterize differentially expressed and unique proteins. Comprehensive LC MS/MS approach identified a total of 402 xylem sap proteins among all *Vitis* species of which, 185 proteins were common to all species. Bunch, FH, and muscadine sap showed 52, 53, and 30 unique proteins respectively. The cluster dendrogram analysis the proteome showed that all of the *Vitis* species are bifolious. Florida hybrid bunch and muscadines are more closely related to each other than the bunch grape. Functional analysis revealed that carbohydrate proteins are abundant in bunch grape, while defense related proteins are more abundant in FH and muscadine grape. Proteins such as beta-1, 3-glucanase, WD repeat-containing protein, peroxidase, and receptor-like protein kinase feronia-like are involved in defense responses and contribute to the plant defense mechanism against bacterium and oxygen generation are uniquely found in the muscadine xylem sap. We conclude that, major functions of sap proteins in Bunch, FH, and Muscadine grape are carbohydrate metabolic process and proteolysis (23%), protein phosphorylation (38%), and oxidation and reduction process (16%), respectively. Proteins involved in the defense and peroxidase activity, are present in abundant levels in FH and muscadine xylem sap and in reduced levels in bunch xylem sap indicate possible role conferring the PD tolerance to Florida hybrid and muscadine cultivars.

Keywords: grape, proteomics, *Xylella*

P26.* Determining If *Arabidopsis*-Produced Phage Proteins Can Inhibit *Erwinia Amylovora*

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Fire blight is a devastating disease known to affect a host of fruit trees across Europe and North America. This bacterial infection, caused by *Erwinia amylovora*, affects many important species, including apple, cherry, plum, pear, and rose. Our overall strategy was to use lytic proteins from *Erwinia*-killing bacteriophage to kill blight-causing bacteria. Our intention was to generate recombinant DNA coding for lytic transglycosylase proteins and express them in *Arabidopsis*. These bacteriophage-derived enzymes induce fatal lysis of cells and are bacteria specific because they function by breaking down specific types of peptidoglycan. We

hypothesized that expression of viable lytic proteins in plants is possible and could allow inexpensive production of large volumes of protein. We also predict that if blight-susceptible species produced their own bacteria-fighting proteins, they would become blight resistant. This could potentially open many opportunities for producing crops and trees with the ability to flourish in previously "unusable" environments (out of range), opening many acres of land up for food production. If orchards must be planted with high tree density, trees producing bacteria-fighting proteins will be critical for success. We have successfully synthesized two bacteriophage constructs and transformed them into Escherichia coli. One of these constructs has been transformed into *Arabidopsis*. We are in the process of PCR-verifying the transformed plants, and upon verification of plasmid integration will perform assays to test the ability of plant-produced lytic proteins to inhibit the growth of *Erwinia amylovora*. At this point, our results suggest that bacteriophage sequences can be transformed into *Arabidopsis.*

Keywords: fire blight, bacteriophage

P27.* A Transient Receptor Potential Ion channel is Involved in the *Chlamydomonas reinhardtii* CO_2 Concentrating Mechanism

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The carbon dioxide concentrating mechanism (CCM) is a key feature of green algal photosynthesis. The CCM is induced in response to limiting CO_2 conditions. Calcium-dependent signaling has been shown to play a role in this acclimation process to low inorganic carbon. One type of Ca^{2+}-channel, the transient receptor potential (TRP) family of ion channels, is specific to the green alga lineage and are not found in land plants. TRP channels generally mediate the flux of Ca^{2+} ions in response to environmental perturbations and one recent study has revealed the role of Ca^{2+} signaling in acclimation to limiting CO_2 in the green alga, *Chlamydomonas reinhardtii*. The TRP gene G13, annotated as a Ca^{2+}-channel was identified through bioinformatics analyses as having a role in acclimation of algal cells to low CO_2. Transcript levels for this gene are significantly upregulated under low CO_2 conditions, and mutant cells lacking G13 show an impaired growth phenotype when cells are cultured in low CO_2. In this presentation we show that a calcium binding protein/transcription factor in *C. reinhardtii*, CAS, is significantly downregulated in the G13 mutant along with other genes under the control of CAS. The preliminary results suggest that G13 is involved in Ca^{2+} signaling mechanisms associated with *C. reinhardtii* cells acclimation to low CO_2.

Keywords: calcium channel, *Chlamydomonas reinhardtii*, TRP, carbon concentration mechanism, CCM, bioinformatics, algae, CO_2 regulation

P28.* The Effect of Fungal Endophytes on Plant Production Under Abiotic Stress

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An increasing human population and global climate changes, combined with limited water resources, arable land, and depleted plant nutrients, challenge the sustainability of current agricultural practices. Thus, a major obstacle facing 21st century innovators is to develop more environmentally friendly and sustainable agricultural systems. Our lab has identified a large collection of fungal endophytes isolated from wild plants growing under stressful environmental conditions, including drought and salt stress, in Alabama. We hypothesize that some of these fungal endophytes can be beneficial to plant growth and stress tolerance. Specifically, we assumed that corn plants colonized with endophytic fungi will have increased productivity compared to a non-symbiotic (NS) control plants under field conditions. Field trials were conducted during the summer of 2018 growing season. The field trials were conducted in uncontrolled environment as in

regular farming conditions. Plants were watered for a week after planting and were dependent on rainfall the rest of growing season. Data shows that most treatments compared to NS showed great results. Endophytes W11, W14, J, 8A, and 1D had dramatic increase in corn ears production, up to 34% increase. On the other hand, endophytes coded 3B and W1 had lower production compared to NS. Overall, the field trials were successful for showing that corn production can be maintained and in some cases be better in harsh conditions. After the proof of concept is achieved, our lab will focus on understanding the physiological and molecular mechanisms controlling the relationship between corn and endophytic fungi leading to increased productivity.

Keywords: fungal endophyte, crop production, abiotic stress

P29.* Fungi: Bio-Fertilizer to Improve Crop Productivity

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There are thousands of people around the world that go hungry every day, but this number will grow exponentially by the year 2050. Scientists are predicting a food shortage and one of the ways to combat this shortage is through the use of biofertilizer to boost the crop yields. One method to apply such biofertilizers is to restore the beneficial microbes that was lost from crop plants due to the overuse of chemical fertilizers, pesticides and insecticides over the years. Our lab focuses on the discovery of beneficial endophytic fungi present within wild plants growing under harsh environments. We apply these endophytes to crop plants in order to improve productivity and the ability to withstand harsh conditions such as drought and soil salinity. While traditional fertilizers can have harmful effects on the environment by running off into rivers and other bodies of water, fungi live within the crop plants and do not wash away easily and supply nutrients to the plants. We tested 14 endophytic fungi on tomato plants under greenhouse conditions and our results concluded a dramatic increase in tomato yields. They not only increased the number of tomatoes produced but also their weight. Two treatments had a significant increase in the amount of tomatoes produced over the non-endophytic control treatment, with W8 having a 98% increase and W12 having an 89.1% increase. Our controlled condition testing has proved these endophytes to be beneficial, next is to test these endophytes under field conditions. The ultimate goal of the project is to develop our fungal technology into a product that serves the farmers for various crops. In addition, we plan to understand the mechanisms by which endophytes contribute to plant production and survival.

Keywords: fungal endophyte, crop production, abiotic stress

P30. 3D Printer Solutions for the Plant Biology Laboratory

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3D printers have recently become portable, affordable and user-friendly. 3D printed lab materials can offer both cost-effective as well as specialized 'need-based' solutions to research challenges. In our lab, we began by utilizing 3D printers to fabricate several simple, one-piece devices such as microcentrifuge tube stands and round bottom flask rings. More recently, we have developed multi-piece devices including a miniature hydroponic system. Here we will focus on the development of these mini-hydroponic boxes in support of our kin recognition studies in *Arabidopsis thaliana*. These boxes allow us to functionally connect the root exudates of two plants while limiting physical contact and the potential of interference from mechanical (touching) effects. In all cases, we were able to move quickly between the planning, coding, printing, and testing phases

with minimal material costs. Here we present the development cycle of this device as an example of how 3D printing can quickly and easily provide viable laboratory solutions.

Keywords: 3D printing, hydroponics, Kin Recognition, *Arabidopsis thaliana*

P31.* Plants Display Age Specific Responses to Bacterial Quorum Sensing Molecules, N-acyl-Homoserine Lactones (AHLs) on Adult *Arabidopsis thaliana*

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AHLs (N-acyl homoserine lactones) are produced by many Gram-negative bacteria as signaling molecules. These molecules are used in quorum-sensing (QS) pathways that indirectly sense bacterial cell density and significantly impact the outcomes of host-pathogen and mutualist interactions. Given the potential impact of phenotypes such as virulence factor and biofilm production on prospective hosts, many eukaryotes have evolved to detect and respond to AHLs, along with the release of exudates capable of influencing QS. Prior studies have established that AHLs can alter root development in *Arabidopsis thaliana* seedlings. These effects appear dependent upon AHL amidolysis by a plant-derived fatty acid amide hydrolase (FAAH) to yield L-homoserine. However, FAAH expression varies as a function of plant age leading us to hypothesize that AHL responses may well be dependent on the life stage of a given plant. In this experiment, we have employed wild-type and FAAH overexpression lines in conjunction with other reporters to test this hypothesis. Our findings have important implications to our understanding of host-microbial associations as a function of plant age.

Keywords: quorum sensing, AHLs, FAAH

P32.* Evaluating Structure-Activity Relationships in Reactive Oxygen Associated Molecules at the Root Surface of *A. thaliana*

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The chemical environment of the root surface and the immediate surroundings, the rhizosphere, are crucial for maintaining proper plant-plant and plant-microbial associations. In many cases the chemical signals which comprise this complex network are exuded from the roots of plants. However, in some cases the process of signal generation is more active. For example, in the parasitic plant *Striga asiatica*, hydrogen peroxide (H_2O_2) generated at the growing root tip are capable of oxidizing the cell wall phenolics of prospective hosts into p-benzoquinones (pBQs). The active production of these signals are necessary and sufficient to trigger the transition between vegetative growth and parasitism in this obligate parasitic angiosperm. This 'active' signaling process is known as semagenesis ('sema' signal 'genesis' origins) and while originally discovered in parasitic plants, increasing evidence now suggests that the phenomenon plays an important role at any site in which reactive oxygen species (ROS) are in contact with cell wall phenolics. The accumulation of these Reactive Oxygen Associated Molecules (ROAMs) can significantly impact root system architecture, calcium dynamics, and more. However, our understanding of how these signals are perceived remains poorly understood. In *Striga asiatica*, perception of these ROAMs has been coupled to a specific receptor and we have attempted to determine if such a receptor mechanism is conserved in non-

parasites. In the present study, we have conducted a series of structure-activity relationship assays investigating the effects of these compounds on root elongation, hormone production, calcium dynamics, ROS production and more in the model angiosperm *Arabidopsis thaliana*. We report our findings within the context of our understanding of ROS regulated development and the evolution of parasitism.

Keywords: rizosphere, *Striga asiatica*, *p*-benzoquinones, angiosperm, semagenesis, reactive oxygen species, structure-activity relationship, *Arabidopsis thaliana*

P33.* RNASeq Analysis of Wild Type, *sunn-4* and *rdn1-2 Medicago truncatula* plants During Early Nodule Development

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During the early stages of the development of the nitrogen-fixing symbiotic nodule in legume roots there are a series of changes that take place in the root: the root hairs of the epidermis interact with the rhizobial partner, cells of the inner cortex and pericycle begin responding, infection threads grow, and eventually nodules emerge. The plant monitors the extent of nodulation and regulates the number of mature nodules that form. The process involves the generation of peptide signals in the early nodule primordium that travel to the shoot where they are perceived and trigger a response signal that restricts further nodulation in the root system. *Medicago truncatula* plants that are defective in this process fail to properly send the signal to the shoot (*rdn1-2*) or fail to properly perceive the signal in the shoot (*sunn-4*) resulting in a hypernodulation phenotype. We have used RNA-seq analysis to assess gene expression changes in the nodulating zone of the root over the first 72 hours of nodule development caused by disruption of *RDN1* and *SUNN*. We have identified a group of genes that respond during nodulation of wild type plants and have assessed their expression in the hypernodulation mutants with the goal of identifying genes involved in the nodule regulation process. Although many of the genes display similar or increased expression in the mutants (reflective of the higher number of nodules), we have identified a small group of genes that appear to be misregulated in *rdn1-2* and *sunn-4*. This work is supported by NSF IOS ##14444.

P34.* Presentation title: Analyzing the Effect of Promoter 5' Deletions on InvINH1 during Early Seed Development

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Endosperm is a tissue produced inside the seed of flowering plants. It surrounds the embryo and provides nutrients in the form of starch, proteins, and oils. Endosperm is an important source for human diet. It also acts as a food storage for the developing embryo and provides signals to regulate embryo growth. Endosperm development consists of two phase, the syncytial phase and the cellularization phase. Interestingly, embryo growth is faster in the cellularization phase than the syncytial phase. An invertase inhibitor, InvINH1, is highly expressed during the syncytial phase. Since InvINH1 inhibits invertase, an enzyme that promotes growth, the expression pattern of InvINH1 agrees with the slower embryo growth rate during the syncytial phase. The goal of the project is to identify the cis-elements in InvINH1 promoter that are responsible for its specific expression in the syncytial endosperm. To achieve this goal, I generated 5' promoter deletion constructs for the 1100 bp InvINH1 promoter. Two InvINH1 promoter fragments (500bp, and 700bp) were amplified by PCR, digested with restriction enzymes XbaI and BamHI, and cloned into vector pUC-GUS via ligation. Post-ligation, the plasmid was transformed into *E. coli* cells. The clones containing the expected construct were analyzed and validated by restriction digestion. After further validate by sequencing, the promoter deletion constructs will be used in protoplast transient expression system to identify the region within the promoter that are bound by transcription factor AGLs.

Keywords: early development; invertase; endosperm

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KH1. A Chemical Genetic Roadmap to Improved Tomato Flavor

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Modern commercial tomato varieties are substantially less flavorful than heirloom varieties. To understand and ultimately correct that deficiency, we quantified flavor-associated chemicals in over 500 modern, heirloom and wild accessions. A subset of these accessions were evaluated in consumer panels, identifying the chemicals making the most important contributions to flavor and consumer liking. Modern commercial varieties contain significantly lower amounts of many of these important flavor chemicals than older varieties. Whole genome sequencing and a genome-wide association study allowed us to identify genetic loci affecting most of the target flavor chemicals, including sugars, acids and volatiles. Together, these results provide an understanding of the flavor deficiencies in modern commercial varieties and the information necessary for recovery of good flavor through molecular breeding.

Keywords: fruit quality, molecular breeding

KH2. Medicines from Plants— ANexus of Biodiversity and Biotechnology

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The chemical diversity of plant natural products has provided humans with a variety of intriguing structures and biological activities. The biological function of most plant natural products remains unstudied, but in general their presence is believed to increase organismal fitness. Commonly, many natural products are thought to play a role in communication of the plant with its environment, as these compounds possess an array of biological activities. Due to these biological activities, 25% of medicines today are either derived directly from plants or are structural modifications of plant natural products. An understanding of how these molecules are formed would serve a dual role to enable a study of the in planta function, as well as development of a synthetic biology production platform. Natural products typically do not accumulate to high levels in the plant. If the source plant for a novel drug is not amenable to cultivation, drug development can be precluded. Engineering of a natural product biosynthetic pathway into an easily cultivated host plant can result in a sustainable supply of a drug. The first obstacle to this approach, however, is knowledge of the underlying biosynthetic genes. Biochemical pathway elucidation in non-model systems has often taken decades to complete. A prominent example is the well-known plant natural product morphine produced by the opium poppy Papaver somniferum. Though discovered in the early 1800's, the biosynthetic pathway to morphine was not completely elucidated until 2014. Next-gen sequencing technology enables revolutionary new approaches to biochemical pathway discovery in the non-model system. A combination of bioinformatics and next-gen sequencing has the potential to shorten natural product pathway discovery in non-model systems from several decades to several years. Presented herein are results obtained to date elucidating and refactoring a pathway to steroid alkaloids.

Keywords: biosynthesis, alkaloid, *Camelina sativa*, next-gen sequencing

KH3. High Value Enzymes and Bio-Pharmaceuticals Made in Chloroplasts

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Chloroplasts are ideal bioreactors for production of enzymes and bio-pharmaceuticals. High level expression of the largest human protein (891 kDa)- pentameric form of human Blood Clotting Factor VIII was demonstrated using synthetic biology approaches (Plant Biotechnology Journal 16: 1148). In addition, selectable marker genes have been removed to facilitate regulatory approval after expression of therapeutic, food or feed enzymes or proteins in lettuce chloroplasts. Recent advances in this field including commercial scale production of human therapeutic proteins in FDA-approved cGMP facilities (Biomaterials 70: 84-93), development of tags to deliver protein drugs to targeted human cells or tissues (Biomaterials 80: 68-79), methods to quantify in planta drug dose using proteomic quantitation by parallel reaction monitoring analysis (Plant Physiology 172:62-77), long-term stability of proteins/enzymes at ambient temperature (Molecular Therapy, 24: 1342-1350), testing human drug doses in large animals (Molecular Therapy 25: 512-522), toxicology, pharmacokinetic and pharmacodynamics studies to obtain regulatory approval will be presented (Annual Review of Genetics, 50: 595-618; Genome Biology 17:134). Five newly launched leaf-products has been compared here with 23 commercial microbial-enzyme products for textile, detergent or juice industries. Crude leaf-extract enzymes are functional at low concentrations without protease inhibitors. Contact-angle water droplet absorption by the FAMAS bioscouring videos exceeds 3-second industry requirements. Leaf-lipase/mannanase crude-extracts remove chocolate/mustard oil stains more efficiently at 70 °C than commercial enzymes (<10% activity). Endo/exoglucanase crude leaf-extracts remove dye efficiently from denim surface and de-pilled knitted fabric. Leaf-pectinase powder efficiently clarified orange juice pulp. Thus, leaf-production platform offers a novel low-cost approach by elimination of fermentation, purification, concentration, formulation and cold-chain to revolutionize healthcare and enzyme industries. Results of recent investigations will be presented to stimulate plant biologists seeking career opportunities in interdisciplinary fields of research.

Keywords: chloroplast, bioreactors, biopharmaceuticals, enzymes, career opportunities

KH4. Biotechnology and Gene Editing Approaches to Improving the Protein and Oil Content of Oilseed Crops

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Output Traits increase the value of seeds by improving the quantity or quality of their components, such as protein and oil, and can create value for growers, processors, food companies and consumers. Corteva's oil seed output trait research program is aligned with our strategic intent of innovating to feed the world and provides an opportunity to create highly differentiated soybeans, canola and other oil seeds. Initial metabolic engineering efforts have been directed towards meeting the increasing demand for soy protein by increasing the protein content of beans while also increasing the oil content. Commodity soybean seeds contain approximately 40% protein and 20% oil and are an important source of protein for animal feed and oil for food and industrial uses including bio diesel. By manipulating the expression of key genes in the developing soybean embryo we have been able to significantly increase both the protein and oil content of the bean at the expense of undesirable carbohydrates. The result is a soybean with a composition that is unique and that has the potential to displace current commodity soybeans in the US, Canada and Brazil. We are using the knowledge generated by this approach to further improve the bean composition using gene editing techniques and further extend these improvements to other oil seeds such as canola.

Keywords: oilseeds, protein, oil, metabolic engineering, gene editing, CRISPR-cas