2014 Meeting of the Southern Section of the American Society of Plant Biologists

In association with the University of Kentucky

Abstract Booklet

March 29-31, 2014
Hyatt Regency Lexington
Lexington, KY

General Session 1 **Sunday Morning**

8:05 AM

Microbial natural product-based bioprospecting: identification of acetobixan as an inhibitor of plant cellulose biosynthesis. *Ye Xia†, Lei Lei∞; Chad Brabham†; Jozsef Stork†; James StricklandØ,1; Adam Ladak‡; Ying Gu∞; Ian Wallace*; Seth DeBolt†. †Department of Horticulture, University of Kentucky, Lexington, KY; ∞Department of Biochemistry and Molecular Biology, Pennsylvania State University, State College PA; ØUnited State Department of Agriculture Forage-Animal Production Research Unit, N220 Agriculture Science Center North, University of Kentucky Campus, USDA-ARS; ‡Waters Waters Corporation 100 Cummings Center, Suite 407N, Beverly MA; *Energy Biosciences Institute, University of California, Berkeley, CA; 1 Current Address: Department Chair of Animal and Veterinary Sciences, Clemson University, Clemson, SC*

Microbially-derived natural products can influence both host and non-host metabolism and represent a source of untapped chemical biodiversity. Here it was reasoned that plant associated microorganisms secrete natural products that are capable of modifying plant cellulose biosynthesis, and that this could be systematically exploited to identify new bioactive compounds. To test this hypothesis, we examined the ability of small molecule secretions derived from a library of switchgrass (*Panicum virgatum* L.) endophytes to synergistically inhibit root growth in the cesA6prc1-1 mutant based on the knowledge that other cellulose biosynthesis inhibitors (CBIs) exhibit this effect. A member of the genus Bacillus (B. sp.-A) met these criteria and was metabolically fingerprinted by subtractive liquid chromatography and mass spectrometry. The complexity of natural small molecules limited our ability to isolate the most bioactive compound in the original B. sp.-A secretion. Despite this caveat, we were able to use differentially abundant compounds in this secretion as lead compounds to narrow the scope of our search for new CBIs from thousands of compounds to a single pharmacophore. Furthermore, we screened analogs of this original pharmacophore and identified a novel potent inhibitor of cellulose biosynthesis, which we referred to as acetobixan. Our results illustrate the power of this chemical genetic approach and serve as a paradigm for small molecule screens of natural chemical diversity.

8:20 AM

Identification and molecular characterization of a chlorophyll deficient non-photosynthetic Chlamydomonas reinhardtii mutant. *Mautusi Mitra, Katherine Smith[1], Tai Truong[1], Tashana C. Haye[1], Theresa Fuller[1] and Bernhard Grimm[2]. [1]University of West Georgia, Department of Biology, Carrollton, Georgia; [2]Humboldt University Berlin, Institute of Biology, Plant Physiology, Berlin, Germany.*

Photo-autotrophic growth under different light irradiances is modulated by a complex interplay that involves various physiological processes. These processes are: photosynthesis, carbon concentrating mechanisms, mitochondrial respiration, photosynthetic pigment biosynthesis and various photo-acclimatory and photo-protective processes. *Chlamydomonas reinhardtii* is a model green micro-alga. It has a short haplontic life cycle, possesses a photosynthetic apparatus very similar to higher plants, can grow photo-autotrophically and heterotrophically (can metabolize exogenous acetate as a carbon source), has well developed chloroplast and nuclear transformation techniques and possesses a completely sequenced genome. These attributes make it an elegant model organism to study all aspects of eukaryotic oxygenic photosynthesis. Our laboratory has generated a *Chlamydomonas* random insertional DNA nuclear mutant library by transforming the wild type strain 4A+ with the pBC1 vector that confers paromomycin (AphVIII) resistance to the mutants. This library was screened under heterotrophic, mixotrophic and photo-autotrophic growth conditions, under different light intensities. The screening has resulted in the isolation of 20 mutants that are either light sensitive, non-photosynthetic and/or photosynthetic pigment deficient to varying degrees under different light intensities in photo-autotrophic and mixotrophic growth conditions. We have identified a chlorophyll (chl) deficient mutant, 10E1, which is light-sensitive and non-photosynthetic under all light conditions. It can only grow heterotrophically and mixotrophically in the dim light. 77K fluorescence emission spectra studies show that 10E1 has an overall high PSI fluorescence compared to 4A+. It also shows a blue shift in the far red and in the red regions of the spectrum that suggests the uncoupling of the photosystem I (PSI) and photosystem II (PSII) chl antenna from the respective reaction centers. 10E1 displays an apparent abnormal PSII fluorescence signature, which is undocumented in any known *Chlamydomonas* mutants that have compromised state transition, photosynthetic electron transport chain, mitochondrial respiration and chlororespiration in the published literature. We have used TAIL-PCR to amplify the

Chlamydomonas genomic DNA, flanking the AphVIII end of the plasmid pBC1 that was used for mutagenesis. Currently we are purifying the genomic PCR products for DNA sequencing to identify the mutation locus in 10E1. We will be presenting our research on the physiological and molecular characterization of 10E1.

8:35 AM

Plant Gene Discovery by mPing-based Transposon Tagging. *C. Nathan Hancock[3], Lisa Kanizay[1], Hanh Ngyen[2], Tom Clemente[2], Wayne Parrott[1]. [1]Center for Applied Genetic Technologies, University of Georgia, Athens, GA; [2]Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE; [3]Department of Biology and Geology, University of South Carolina Aiken, 471 University Parkway, Aiken, SC 29801*

Genomics tools for identifying the genes responsible for specific traits are critical for making advances in plant biotechnology. Our goal has been to develop a transposon mutagenesis system based on the mPing miniature inverted repeat transposable element from rice. This element is relatively small (430bp) and is mobilized by the Ping or Pong transposase proteins in a cut and paste mechanism. *Arabidopsis* and soybean lines that were transformed with the mPing element and the appropriate transposase genes show active mPing transposition, resulting in heritable mutations. Characterization of mPing insertions in these plants indicated that mPing transposed to unlinked sites and preferentially inserted into euchromatin, with over 70% landing in or near (<2.5kb) annotated genes. Large numbers of soybean plants are being screened for mutant phenotypes, including a public workshop this summer in Nebraska. High-throughput methods for insertion site determination are also being developed to facilitate analysis of mPing mutagenized plants. Our current focus in the lab is to increase the effectiveness of this system by increasing the transposition rate and developing mPing-based activation tags. To increase transposition, we have screened modified transposase genes in a yeast transposition assay and performed tissue culture treatment of mPing containing plants. mPing-based activation tags designed to induce overexpression of nearby genes have been created and shown to be mobile in the yeast transposition assay. Together these advances will significantly improve the effective mutation rate of the system and greatly improve its usefulness. Transformation into additional plant species is currently underway.

8:50 AM

Rhodobacter capsulatus: An autotrophic platform for production of triterpene fuels. *S. Eric Nybo, Nymul Khan[2], Alex Rajangam[2], Dr. Wayne R. Curtis[2], Joseph Chappell[1]. 1Department of Chemical Engineering, The Pennsylvania State University, University Park, PA 16802; 2 Department of Pharmaceutical Sciences, University of Kentucky, Lexington, KY*

Rhodobacter capsulatus is a metabolically diverse phototrophic, gram negative bacterium. We have metabolically engineered this microorganism to produce botryococcene and squalene triterpenes by modulating carbon flux through its resident methyl erythritol phosphate (MEP) pathway. We have built several triterpene expression cassettes that strengthen accumulation of the fifteen carbon intermediate, farnesyl pyrophosphate (FPP). This C15 compound is then condensed into botryococcene (e.g. by botryococcene synthase, BS) or squalene (e.g. by squalene synthase, SS) in an NAD(P)H-dependent fashion. Expression of several MEP pathway components, including dxs, idi, and fps resulted in the accumulation of 7 mg L-1 squalene and 2 mg L-1 botryococcene in R. capsulatus engineered lines under heterotrophic growth conditions.

9:05 AM

A genome-scale resource for the functional characterization of Arabidopsis transcription factors. *Ghislain Breton, Jose L. Pruneda-Paz[1], Ghislain Breton[2], Dawn Nagel[1], Shin-Yong E. Kang[1], Katia Bonaldi1, Colleen Doherty[3], Stephanie Ravelo[1], Joseph Ecker[4] and Steve A. Kay[5]. (1) Division of Biology, University of California San Diego, La Jolla, CA; (2) Department of Integrative Biology and Pharmacology, University of Texas Health Sciences Center-Houston (UTHSC), Houston, TX: USA; (3) Department of Molecular & Structural Biochemistry, North Carolina State University, Raleigh, NC; (4) Plant Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA ; (5) Dornsife College of Letters, Arts and Sciences,University of Southern California, Los Angeles, CA.*

Extensive transcriptional networks play major roles in cellular and organismal functions. Transcript levels are in part determined by the combinatorial and overlapping functions of dozens of transcription factors (TFs) bound to gene promoters. TF-promoter interactions thus provide the basic molecular wiring of transcriptional regulatory networks. In plants, discovery of TF's functional role is limited by an increased complexity of network circuitry due to a significant expansion of TF families. Here, we present the construction of a comprehensive collection of *Arabidopsis* TFs developed for TF-centered approaches aimed at uncovering TFs and

their biological functions. We leveraged this collection to implement a high throughput DNA binding assay that identified direct regulators of a key clock gene called CCA1. TFs identified here provide molecular links between different plant signaling modules and the circadian clock. The resources introduced in this work will significantly contribute to better understand the transcriptional regulatory landscape of plant genomes.

9:20 AM

A systems level approach to dissect the regulation of photosynthetic carbon metabolism in rice. *Supratim Basu[2], Madana M. Ambavaram[1,†] Arjun Krishnan[1,‡], Ramegowda Venkategowda[2], Utlwang Batlang[1] , Lutfor Rahman[2], Niranjan Baisakh3 , Andy Pereira1,2* [1]Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA. 2Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, Arkansas. 3School of Plant, Environmental, and Soil Sciences, Louisiana State University Agricultural Center, Baton Rouge, LA.*

Photosynthetic carbon metabolism (PCM) harnesses solar energy and atmospheric carbon dioxide (CO2) into crop plants, which need to be improved to meet the growing global demand for food. PCM is affected by environmental stresses that are increasing with global warming and pose a serious threat to the growth and productivity of crop plants like rice, which feeds half the world. A systems level analysis of the regulation of PCM is essential to understand how plants are affected and can be improved for tolerance to environmental stresses. We have identified a rice transcription factor HYR (HIGHER YIELD RICE) that regulates essential PCM and morpho-physiological processes, and imparts superior photosynthetic capacity under elevated CO2 and light, as well as drought and high temperature stress. We used gene expression analysis and ChIP assays to identify a set of regulatory and other genes directly controlled by HYR, and subsequently their downstream genes. Our analysis shows HYR as the master regulator directly activating photosynthetic genes, cascades of transcription factors and other genes involved in carbon metabolism and stress tolerance.

9:35 AM

Glycoengineering of tobacco plants to produce asialoerythropoietin and erythropoietin. *Jiahua Xie, Farooqahmed S Kittur, Chiu-Yueh Hung, Elena Arthur. Department of Pharmaceutical Sciences, North Carolina Central University, Durham, NC.*

Plants have been extensively engineered to produce biopharmaceutical products and it is generally believed that plant-based expression system has several unique advantages for expressing biopharmaceuticals. For expressing human glycoproteins, the major hurdle for effective application of plants is their different glycosylation patterns compared to that in mammalian cells. Plant-produced glycoproteins carry plant-specific β1,2-xylose and α1,3-fucose residues while lack β1,4-galactose and terminal sialic acid residues, which are required for their stability and in vivo function. We are aimed at glycoengineering of tobacco plants to produce In our previous studies, we generated stable transgenic tobacco lines co-expressing human EPO and β1,4-galactosyltransferase (GalT) genes to produce asialo-rhuEPO lacking sialic acid glycan (Kittur et al., Plant Cell Rep. 2012, 31:1233-1243). Purified plant-produced asialo-rhuEPO (asialo-rhuEPOP) was shown to bind the EPO receptor with affinity similar to that of mammalian cell-produced rhuEPO (rhuEPOM). Recently, we further improved the expression level of asialo-rhuEPOP by 40-fold and purified sufficient amount of asialo-rhuEPOP for performing detailed N-glycan analysis, testing its cytoprotective effect in neuronal-like mouse neuroblastoma cells (N2A), and studying its mechanism of action (Kittur et al., PLoS One, 2013, e76468). Detailed N-glycan analysis using NSI-FTMS and MS/MS revealed that asialo-rhuEPOP bears 13 different N-glycan chains. In vitro cytoprotection assays showed that the asialo-rhuEPOP (20 U/ml) provides 2-fold better cytoprotection (44%) to neuronal-like mouse neuroblastoma cells from staurosporine-induced cell injury than rhuEPOM (21%). The cytoprotective effects of the asialo-rhuEPOP were found to be mediated by receptor-initiated activation of Janus kinase 2 (JAK2) and suppression of caspase 3 activation. Currently, we are testing cytoprotective functions of asialo-rhuEPOP in various cell-based models and further glycoengineering of sialylation pathway in tobacco plants to add sialic acid for expressing fully humanized recombinant EPO. Recombinant human erythropoietin (rhuEPO) and its derivative asialoerythropoietin (asialo-rhuEPO). RhuEPO is a glyco-hormone consisting of 166 amino acid long polypeptide chain and containing one O- and three N-glycan chains, which has hematopoietic and cytoprotective functions. Asialo-rhuEPO, a desialylated form of rhuEPO having cytoprotective function but lacking hematopoietic activity, has been reported to have broader protective effects in preclinical models of tissue injury and does not have side effects caused by hematopoietic activity. Therefore, asialo-rhuEPO could be developed as a broader cytoprotective agent.

9:50 AM Break

Graduate Oral Competition Session 1 **Sunday Morning**

10:15 AM

Determining the impact of endophytic bacteria on growth of bell pepper (*Capsicum annuum* L.).

Zheng Wang[1] and Timothy Coolong[2]. [1]Department of Horticulture, University of Kentucky, Lexington, KY; [2]Department of Horticulture, University of Georgia, Tifton, GA.

A total of 160 endophytic bacteria isolates were isolated from fruits, leaves, stems, and roots of seed surface-disinfected bell pepper plants which were grown under organic and conventional production systems applied with well-watered and drought stress treatments in 2011 and 2012. The BLAST results of 16S rRNA gene categorized these isolates (67 for 2011 and 93 for 2012) into 57 species and 3 phyla, Firmicutes, Proteobacteria, and Actinobacteria. These isolates, when re-inoculated into pepper plants in greenhouse conditions, were analyzed by their effects on promoting plant growth and resisting against water deficiency through evaluating 6 physiological indices, plant growth rate, leaf water potential, total leaf area, leaf dry weight, root and stem dry biomass. The final results indicated that there were 7 isolates from 2011 trial and 6 from 2012 that might promote or maintain plant growth under water deficit condition; whereas, there were 8 isolates from 2011 trial and 10 from 2012 that indicated promoting plant growth under well-watered treatment with the criteria of at least 2 out of 6 indices showing significantly greater than controls. The results suggested that the species isolated from the 2-year study were highly diverse, as well as having diversity when compared with different growing systems and irrigation regimes. In addition, endophytic bacteria may not only serve as a plant growth promotion bacteria that existed inside plants with adequate irrigation, but could also exist in abiotic-stressed plants which may potentially modulate plant growth to cope with adverse environments.

10:30 AM

Interacting Partners of the SUNN Symbiotic Regulatory Kinase. *Ashley Crook, Elise Schnabel and Julia Frugoli. Clemson University, Department of Genetics & Biochemistry, Clemson, SC 29634*

The control of nodule number, or autoregulation of nodulation (AON), exhibited by nodule-forming legume species involves a complex signaling pathway encompassing molecules that act in both the root and the shoot. SUNN, a leucine rich repeat receptor-like kinase, is a key regulatory kinase in the AON pathway. High homology to the Arabidopsis LRR-RLK, CLAVATA1, suggests that SUNN is a membrane-bound receptor that likely acts in a multi-protein complex. To address subcellular localization of SUNN, we transiently co-expressed SUNN and a plasma membrane protein (AtPIP2) in the epidermal cells of *Nicotiana benthamiana*. Our results indicate SUNN is localized to the plasma membrane and some experiments suggest plasmadesmatal localization. We have undertaken steps to identify protein-protein interactions that involve the SUNN kinase utilizing transgenic *Medicago truncatula* carrying a YFP/ Hemagglutinin-tagged SUNN gene driven by the 35S CaMV promoter in a sunn-4 (null) background. Co-immunoprecipitation of the tagged SUNN kinase was used to isolate interacting partners that will be identified using a LTQ Orbitrap mass spectrometer. Additionally, we are producing recombinant SUNN in *Pichia pastoris* for use in Bio-Layer Interferometry experiments examining binding of substrates. This work is supported by NSF IOS#1146014 and a Clemson University Wade Stackhouse Fellowship to A.C.

10:45 AM

RDNs/HPATs and CLEs: A role in the autoregulation of nodulation. *Stephen Nowak[1], Tessema Kassaw[2], Elise Schnabel[1] and Julia Frugoli[1]. [1]Clemson University, Dept of Genetics & Biochemistry, Clemson SC; [2]Colorado State University, Department of Biology, Fort Collins, CO.*

Legumes form a root based symbiotic relationship with nitrogen fixing Rhizobia in the soil, housing the bacteria in nodules formed on the roots. Autoregulation of Nodulation (AON) is an important pathway controlling nodule number based on nitrogen status of the plant and nodule development already underway. AON involves both local and long distance signaling within the plant. Our previously published work with *Medicago truncatula* identified a gene Root Determined Nodulation1 (RDN1) as a component of the AON pathway; mutations in this gene cause loss of nodule number regulation resulting in increased nodulation. The gene is part of a multi-gene family conserved in all green plants and localized in the Golgi. Another group has demonstrated that homologues of RDNs in Arabidopsis, Hydroxyproline Arabinosyl Transferases (HPATs), are responsible for the addition of arabinose residues onto the hydroxyproline of some members of a family of small signaling peptides known as Clavata like/Extensins (CLEs). MtCLE12 and MtCLE13 have been shown to be involved in AON; constitutive expression of either of these genes suppresses nodulation. We are

expanding our analysis of the role of RDNs and CLEs in AON by using RNA interference (RNAi) to knockdown the two other genes in the *M. truncatula* RDN family, RDN2 and RDN3. Additionally, we report progress on a screen of mutagenized plants constitutively expressing MtCLE12 and MtCLE13 for suppressors of the non-nodulating phenotype. The intent is to identify additional genes involved in processing CLEs affecting AON in order to determine the role of RDNs and CLEs in root to shoot signaling. This work is supported by NSF IOS#1146014.

11:00 AM

Chemical inducers of systemic acquired resistance in plants. *Mohamed El-Shetehy, Gah-Hyun Lim, Shine M. B., Keshun Yu, Aardra Kachroo and Pradeep Kachroo. Department of Plant Pathology, University of Kentucky, Lexington, KY.*

Systemic acquired resistance (SAR) is a highly desirable form of resistance that protects against a broad-spectrum of pathogens (1-4). SAR involves the generation of a mobile signal at the site of primary infection, which moves to, and arms distal portions of a plant against subsequent secondary infections. The last decade has witnessed considerable progress and a number of signals contributing to SAR have been isolated and characterized. Among the signals contributing to SAR are salicylic acid (SA) and several components that feed into SA pathway including the methylated derivative of SA (MeSA), the nine carbon dicarboxylic acid azelaic acid, the phosphorylated sugar glycerol-3-phosphate (G3P), and two lipid transfer proteins (LTPs) DIR1 (Defective in Induced Resistance) and AZI1 (AA insensitive). The diverse chemical natures of the SAR inducing molecules have led to the growing belief that SAR might involve the interplay of multiple diverse and independent signals. More recent evidence suggests that coordinated signaling from diverse signaling components facilitates systemic immunity in plants. Relationship among recently identified mobile inducers of SAR will be discussed.

11:15 AM

Mammalian herbivory on fourteen experimentally planted native hardwood tree seedlings of the Kentucky Bluegrass savanna-woodland community. *James D. Shaffer[1] Scott K. Gleeson[1], John J. Cox[2], John M. Lhotka[2]. [1]University of Kentucky, Department of Biology, [2]University of Kentucky, Department of Forestry*.

Savanna communities are strongly influenced by disturbance regimes that affect plant composition and structure. It has been hypothesized that after the precipitous post-1500 decline in Native American populations and use of fire as a habitat management tool, the now globally endangered Kentucky Bluegrass savanna-woodland community may have been primarily maintained by large mammal (elk [*Cervus canadensis*] and American bison [Bison bison]) herbivory and other physical disturbances (e.g. trampling). Although these megaherbivores have been regionally extirpated, browsing by white-tailed deer (Odocoileus virginianus), eastern cottontail (*Syvilagus floridanus*) and various small rodent species (*Microtus* spp., *Peromyscus* spp., and *Reithrodontomys* sp.) appears to be a current limiting factor to the regeneration of remnant Bluegrass stands by impacting the establishment, survival, and growth of native hardwood tree seedlings. We established a large scale, long-term tree restoration project at the largest savanna-woodland remnant (Griffith Woods Wildlife Management Area, Harrison Co., KY) to understand how herbivory affects the establishment and growth of fourteen native hardwood tree species (n= 6,168 seedlings) common to Central Kentucky. We compare herbivory among tree seedling plots using a block design that included individual tree seedling protectors and herbaceous competition control using mowing and herbicide.

11:30 AM

Identification and quantitative analysis of the polyphenolic compounds in Fragaria spp. mutants. *Sutapa Roy and Douglas D. Archbold. Department of Horticulture, University of Kentucky.*

N-Acylethanolamines (NAEs) are fatty acid derivatives in plants that negatively regulate seedling growth. N-Lauroylethanolamine (NAE 12:0), one type of NAE, inhibits root length, increases radial swelling of root tips and reduces root hair numbers in a dose dependent manner in *Arabidopis thaliana* L. (ecotype Columbia). We initiated a forward genetics approach by screening a population of T-DNA "activation-tagged" lines for NAE resistance to identify potential genes involved in NAE signaling events in *Arabidopis thaliana* L. (ecotype Columbia). Seeds of the activation tagged lines developed by the Salk Institute were grown at 0, 25, 30, 50, 75 and 100 µM NAE 12:0. Ten mutant individuals which displayed NAE resistant (NRA) seedling phenotypes, compared with wild-type (Columbia, Col-0) seedlings were identified. We have focused on one mutant line, identified as NRA 25, where the resistance to NAE 12:0 appears to be mediated by a single dominant, nuclear gene. Thermal asymmetric interlaced (TAIL) PCR identified the location of the T-DNA insert as 3.86 kbp upstream of the locus At1g68510. Quantitative PCR indicated that the transcript level corresponding to

At1g68510 is upregulated by 10-12 fold in the mutant relative to wildtype. To determine whether the NAE tolerance in NRA 25 is mediated by overexpression of At1g68510 we have created overexpressing lines of At1g68510 with and without GFP fusions behind the 2X35S CaMV promoter. Confocal images of the fusion proteins suggest that GFP-At1g68510 is concentrated in the nucleus and this was confirmed by counterstaining with 4\', 6-Diamidino-2-phenylindol (DAPI). As might be predicted, preliminary results with overexpressing lines of At1g68510 exhibit enhanced resistance to NAE when compared with the wildtype. Next steps are to identify the association of At1g68510 with specific genomic regions or interacting proteins that may be additional components of NAE signaling in plants.

Graduate Oral Competition Session 2 — Sunday Afternoon

1:00 PM

Effects of fatty acid desaturase 7, lipoxygenase C and Hydroperoxide lyase on performance of potato aphids, *Macrosiphum euphorbiae* in tomato. *Jiamei Li, Carlos A. Avila, Fiona L. Goggin*, Harry J. Klee, and Denise M. Tieman. *corresponding author, Entomology Department, University of Arkansas, Fayetteville, AR*.

C6 volatiles in plant have a variety of defense-associated functions to insects. In tomato, C6 volatile aldehydes and alcohols are mainly synthesized from linoleic and linolenic acids through the successive action of the enzymes lipoxygenase (LOX) and hydroperoxide lyase (HPL). Lipoxygenase C (LOXC), an isoform of tomato lipoxygenase, is specifically involved in the generation of a fatty acid-derived C6 volatile compounds. Hexanal and hexanol are derived from linoleic acid, and (Z)-3-hexanal and (Z)-3-hexanol are derived from linolenic acid. Fatty acid desaturase 7 (FAD7) is an omega-3 FAD that desaturates linoleic acid into linolenic acid. The aphid-resistant tomato spr2 mutant with impaired FAD7 functions emit more hexanal and hexanol and less (Z)-3-hexanal and (Z)-3-hexanol than wild-type controls because of higher linoleic acid and lower linolenic acid levels. To investigate if variation of fatty acid-derived C6 volatiles contribute to spr2 tomato resistance to potato aphid (*Macrosiphum euphorbiae*), this study measured and compared the settling behavior, survival and fecundity of potato aphids in mutant tomato lines with impaired functions of FAD7, antisense suppression of LOXC or HPL and their respective wild-type controls. The results indicate that suppression of HPL increased aphid host preference, but did not impact aphid population growth. Suppression of LOXC had no significant effect on aphid performance, whereas loss function of FAD7 significantly inhibited the settling behavior, survival and fecundity of potato aphids. All of these three mutant lines had a lower level of (Z)-3-hexenal than their controls, but (Z)-3-hexenal levels did not correlate with aphid performance across the genotypes tested. Therefore, it is likely that aphid resistance in the spr2 tomato mutant is due to factors other than fatty acid-derived C6 volatiles. This study advances our understanding of biochemical basis of aphid resistance.

1:15 PM

Gene Expression and Physiological Analyses to Study Grain Filling in Oryza sativa Japonica varieties Cypress and LaGrue Subjected to High Nighttime Temperatures. *Nicholas Lawson, Lacy Nelson, Paul Counce[1], Karen Moldenhauer[2], Terry Siebenmorgen, Kenneth Korth[1]. Department of Plant Pathology, University of Arkansas, Fayetteville, AR; [1]Rice Research and Extension Center, Department of Crop, Soil and Environmental Sciences, University of Arkansas, Stuttgart, AR; [2]Rice Research and Extension Center, Stuttgart, AR.*

Night is a key period for starch accumulation in rice endosperm. Average nighttime temperatures have been rising more rapidly than average daytime temperatures globally. Starch properties and structure are important factors in grain quality. Rice producers around the world, including here in Arkansas, have observed a reduction in yield and quality correlated with high nighttime temperatures (HNT). Imperfect starch granule packing leads to chalky appearance in the center of endosperm and is a major effect of HNT. Along with environment, genotype has a strong influence over starch composition and certain cultivars tolerate HNT better. Tolerant cultivars produce grain with a lower percentage of chalk and higher yields than susceptible cultivars. To examine how rice might overcome stress from HNT, two ideas were explored. The first was to compare gene expression between a tolerant (cv. Cypress) and a susceptible (cv. LaGrue). Gene expression analysis was carried out using DNA gene strip arrays with tissue isolated from plants grown in temperature-controlled conditions. The susceptible cultivar had more genes that varied in expression than did the tolerant. The number of genes found to have differential expression between cultivar did not vary between high and low temperature treatments. The second was to assess physiological differences between the two cultivars by measuring daytime photosynthetic

rates. Gas exchange measurements were collected from field-grown plants and different photosynthetic rates observed in cultivars examined. This research illustrates an important link between physiological and gene expression characteristics and tolerance of rice to high nighttime temperatures.

1:30 PM

Identification of proteins that regulate virus resistance in soybean. *Hexiang Luan, Haijian Zhi and Aardra Kachroo. University of Kentucky, Lexington, KY.*

Soybean Mosaic Virus (SMV) is one of the most destructive viruses that affects seed quality and production of soybean. The SMV genome comprises a single ORF that produces 11 mature proteins. At least three SMV proteins have been identified as important for virus virulence on the soybean host. We used a yeast two-hybrid screen to identify soybean proteins that interact with two SMV virulence proteins and tested the roles of the identified proteins in SMV pathogenesis. We used a previously described bean pod mottle virus-based vector to silence genes encoding the target proteins and analyzed the response to SMV in the various silenced lines. Results related to the functional characterization of two such proteins will be discussed. We show that a soybean elongation factor 1A and a vacuolar ATPase are important for SMV virulence in susceptible soybean backgrounds. Preliminary analysis indicates these proteins also regulate soybean defense to other viruses and may be involved in the cell death responses.

1:45 PM

Enzymes from fungal and plant origin required for chemical diversification of loline alkaloids in grass-Epichloë symbiota. *Juan Pan[1], Minakshi Bhardwaj[2], Padmaja Nagabhyru[1], Robert B. Grossman[2], Christopher L. Schardl[1]. [1]Department of Plant Pathology, University of Kentucky, Lexington, KY; [2]Department of Chemistry, University of Kentucky, Lexington, KY.*

The lolines are a class of bioprotective alkaloids that are produced by Epichloë species, fungal endophytes of grasses. These alkaloids are saturated 1-aminopyrrolizidines with a C2 to C7 ether bridge, and are structurally differentiated by the various modifications of the 1-amino group: -NH2 (norloline), -NHCH3 (loline), -N(CH3)2 (N-methylloline), -NCH3Ac (N-acetylloline), -NHAc (N-acetylnorloline), and -NCH3CHO (N-formylloline). Other than the LolP cytochrome P450, which is required for conversion of N-methylloline to N-formylloline, the enzymatic steps for loline diversification have not yet been established. Through isotopic labeling we determined that N-acetylnorloline is the first fully cyclized loline alkaloid, implying that deacetylation, methylation, and acetylation steps are all involved in loline alkaloid diversification. Two genes of the loline alkaloid biosynthesis (LOL) gene cluster, lolN and lolM, were predicted to encode an N-deacetylase (acetamidase) and a methyltransferase, respectively. A knockout strain lacking both lolN and lolM produced N-acetylnorloline but not the other lolines, and complementation with the two wild-type genes restored production of N-formylloline and N-acetylloline. These results indicated that lolN and lolM are involved in the steps from N-acetylnorloline to other lolines. The function of LolM as an N-methyltransferase was confirmed by its heterologous expression in yeast resulting in conversion of loline to N-methylloline. One of the more abundant forms, N-acetylloline, was only observed in some but not all plants with symbiotc *Epichloë siegelii*, and when provided with exogenous loline, asymbiotic meadow fescue (Lolium pratense) plants produced N-acetylloline. We conclude that, although most loline alkaloid biosynthesis reactions are catalyzed by fungal enzymes, both fungal and plant enzymes are responsible for the chemical diversification steps in symbio.

2:00 PM

Occurrence and Implications of Anandamide (a mammalian neurotransmitter) in the Moss *Physcomitrella patens*. *Richard R. Sante[1], Sunitha Shiva[2], Ruth Welti[2] and Aruna Kilaru[1]. [1]Department of Biological Sciences, East Tennessee State University, Johnson City, TN; [2]Kansas Lipidomics Research Center, Division of Biology, Kansas State University, Manhattan, KS.*

N-acylethanolamines (NAEs) with C12-C18 acyl chain are ubiquitous in seed plants and play a role in mediating abscisic acid (ABA)-dependent or -independent responses to stress. The moss *Physcomitrella patens* has a unique lipid profile and shows high tolerance in abiotic stresses. Protonema tissues amongst three developmental stages of moss had the highest occurrence of NAE metabolites and NAE pathway gene expression. Moss showed a variation in membrane major and minor polar lipid composition compared to Arabidopsis. Anandamide, N-arachidonylethanolamide (NAE 20:4), is an endocannabinoid receptor ligand unique to animals, in which it influences a wide range of physiological and behavioral functions. Endocannabinoid receptor-mediated interactions, similar

to that of animals, have not been elucidated for plants. Using selective lipidomics approach, we recently identified occurrence of anandamide or NAE 20:4 and its precursor in moss plants. Anandamide showed higher growth inhibitory effects than NAE 12:0 on moss growth when applied exogenously. *Physcomitrella patens* provides us with a unique opportunity to address if early land plants, such as mosses, retained NAE-mediated signaling mechanism that is akin to animals but not to vascular plants and if such distinctive NAE profile and mechanisms by which it may function in moss plants is responsible, in part, for their natural ability to resist high temperatures and tolerate osmotic and salt stresses and dehydration. Our current studies are focused on characterization of anandamide metabolic pathway and its functional role in the development of moss. Insights into unique lipid composition and signaling pathways that mosses acquired naturally, during their successful transition from water to land, may lead to development of tools necessary to enhance abiotic stress tolerance in other plants.

2:15 PM Break

Graduate Oral Competition Session 3 Sunday Afternoon

2:45 PM

Arabidopsis Seed-filling Regulatory Association Network Analysis. *Chirag Gupta[1], Eva Collakova[2] and Andy Pereira[1]. [1]Department of Crop, Soil and Environmental Sciences, University of Arkansas, Fayetteville, AR; [2]Virginia Tech, Blacksburg, VA.*

Global co-expression networks are aimed at determining the functional modules and gene clusters for their putative role in biological pathways. However, the expression pattern of genes and their transcriptional regulators in the cell are governed by various environmental cues, developmental stages and tissues. The predictive power of global co-expression networks is limited when tested for specific biological context. Here, we present a transcription factor (TF) regulatory association network in Arabidopsis which encompasses association of every TF and all other genes in the genome. This network was queried with TFs which are expressed during different stages of seed development to identify regulators of central carbon metabolism during development and seed filling. Interestingly, the predicted targets of three MYB transcription factors were significantly enriched for the term 'central carbon metabolism'. So far, two MYB TFs have not been functionally validated for their role in seed development and seed filling. The knockout mutants of these TFs are being characterized.

3:00 PM

Towards development of an Ac-Ds activation tagging system in tomato. *Ipeleng Randome[1], Supratim Basu[1], Anuj Kumar[1], Dragana Avirovik[2] and Andy Pereira[1]. [1]Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR; [2]Department of Biology, Virginia Tech, Blacksburg, VA.*

In plants transposable elements can alter transcription of adjacent genes thereby leading to changes in gene expression and thereby in phenotypic variation. The Ac-Ds system is a maize transposon system consisting of the autonomous Ac transposable element that encodes the transposase and a non-autonomous Ds element that can be stabilized by segregation of the Ac transposon. The maize Ac-Ds system has been widely used to create mutants in many plants including Arabidopsis and rice. We have developed an Ac-Ds transposon 'activation tagging' (ATag) system to be able to excise and transpose transposon inserts bearing a strong 35S-enhancer element all around the genome. We used the tomato (*Solanum lycorpesicum*) cultivar M82 that has an erect determinate habit suitable for greenhouse and field screening, to transform the Ac-Ds ATag constructs. We have screened putative tomato transformants of T1 progeny harboring the Ac-Ds ATag cassette by genomic PCR using Ac and Ds element specific primers to identify transformants. These lines are being studied for excision and transposition of Ds-ATag elements through TAIL-PCR and resulting product sequences aligned to tomato genome. The objective is to identify plant lines with active transposition in order to create a library of Ds-ATag insertion lines and identify mutants in tomato that are altered in development of plant architecture and fruit development. Along with the tomato genome sequence, these lines will be a good resource for plant development and tomato breeding.

3:15 PM

Abiotic stress tolerance in rice mediated by coordinate regulation of lignocellulose biosynthesis pathway. *Ritu Mihani[1] Supratim Basu[1], Niranjan Baisakh[2], Andy Pereira[1]. [1]Cell and Molecular Biology Program, Department of Crop, Soil and Environmental Sciences, University of Arkansas. Fayetteville, AR; [2]School of Plant, Environmental and Soil Sciences, Louisiana State University, Baton Rouge, LA.*

Abiotic stresses like salinity, drought and cold pose a serious threat to the yield and productivity of crop plants, especially rice that feeds half the world population. Exposure of plants to abiotic stresses activates several defense responses like stomatal closure, maintenance of root water uptake, and synthesis of osmoprotectants. Previously we have seen that overexpression of AtSHN2 in rice (Nipponbare) causes a 34% increase in cellulose and a 45% reduction in lignin content. The rice AtSHN lines also exhibit an altered lignin composition correlated with improved digestibility, with no compromise in plant strength and performance. In this research we have overexpressed OsSHN in rice. OsSHN transgenic lines showed sense suppression (ss) and exhibited no secondary cell wall thickening when examined by scanning electron microscope. Furthermore OsSHN-ss rice lines showed the up regulation of lignin biosynthetic genes while down regulation of genes involved in cellulose biosynthesis. Moreover, the transgenic rice plants were tolerant to salinity stress (200 mM). PEG-mediated drought stress and cold stress (4C). Gene expression analysis by qPCR showed up-regulation of stress tolerance genes. The results from the present experiments shows that stress tolerance of OsSHN-ss transgenic rice plants are mediated by shift in carbon flux to lignin biosynthesis pathway and may be in turn related to wax biosynthesis.

3:30 PM

Genetic Dissection of N-Acylethanolamine (NAE) Signaling Pathway in *Arabidopsis thaliana* L. *Bikash Adhikari[1], Cheol-Min Yoo[2], Lionel Faure[1], Elison B. Blancaflor[2], and Kent D.Chapman[1] . [1]Center for Plant Lipid Research, Department of Biological Sciences, University of North Texas, Denton, TX; [2]Plant Biology Division, Samuel Roberts Noble Foundation, Ardmore, OK.*

N-Acylethanolamines (NAEs) are fatty acid derivatives in plants that negatively regulate seedling growth. N-Lauroylethanolamine (NAE 12:0), one type of NAE, inhibits root length, increases radial swelling of root tips and reduces root hair numbers in a dose dependent manner in *Arabidopis thaliana* L. (ecotype Columbia). We initiated a forward genetics approach by screening a population of T-DNA "activation-tagged" lines for NAE resistance to identify potential genes involved in NAE signaling events in *Arabidopis thaliana* L. (ecotype Columbia). Seeds of the activation tagged lines developed by the Salk Institute were grown at 0, 25, 30, 50, 75 and 100 µM NAE 12:0. Ten mutant individuals which displayed NAE resistant (NRA) seedling phenotypes, compared with wild-type (Columbia, Col-0) seedlings were identified. We have focused on one mutant line, identified as NRA 25, where the resistance to NAE 12:0 appears to be mediated by a single dominant, nuclear gene. Thermal asymmetric interlaced (TAIL) PCR identified the location of the T-DNA insert as 3.86 kbp upstream of the locus At1g68510. Quantitative PCR indicated that the transcript level corresponding to At1g68510 is upregulated by 10-12 fold in the mutant relative to wildtype. To determine whether the NAE tolerance in NRA 25 is mediated by overexpression of At1g68510 we have created overexpressing lines of At1g68510 with and without GFP fusions behind the 2X35S CaMV promoter. Confocal images of the fusion proteins suggest that GFP-At1g68510 is concentrated in the nucleus and this was confirmed by counterstaining with 4\', 6-Diamidino-2-phenylindol (DAPI). As might be predicted, preliminary results with overexpressing lines of At1g68510 exhibit enhanced resistance to NAE when compared with the wildtype. Next steps are to identify the association of At1g68510 with specific genomic regions or interacting proteins that may be additional components of NAE signaling in plants.

3:45 PM Break

4:00 PM

Poster Session

Undergraduate Poster Competition*

P1* **Symbiotic endophytes could improve crop production**. *Katelyn Knight, Caitlyn Bonham, Hayden Armuelles, Dr. Mustafa Morsy. Department of Biological and Environmental Sciences, University of West Alabama, Livingston, AL.*

Global climatic change, particularly heat and drought stress significantly affects agricultural production every year. In 2012 the Federal Crop Insurance Program paid out a record-breaking $17.3 billion in crop losses due to the worst drought in the United Sates in the last 50 years. With the current economic crisis and the growing population of the world, there is a dire need to overcome the

effects of drought and heat on crop production. We may be able to solve this crisis by simply learning how wild plants survive extreme environments. Most naturally growing plants serve as unique ecological hosts for diverse communities of cryptic symbiotic fungi that exist within plant tissue without causing any apparent symptom to their host. The symbionts often contribute multiple benefits, from improving tolerance against biotic and abiotic stresses to enhancing plant growth by increasing water and nutrient uptake efficiency. The fungal endophyte Curvularia protuberate carrying the double stranded RNA virus (CThTV) enables the native host *Dicanthelium langinosum* to survive soil temperatures up to 65 oC. This fungus also provides a tolerance to many crop plants such as tomato, corn, rice and wheat. We conducted greenhouse and field trials to examine the role of the fungus in enhancing tomato growth under normal farming conditions. The tomato plants harboring the fungal/viral symbionts had better vegetative growth compared to the non-infected tomatoes. Variation in fruit production and mass was also observed. We expect that the fungal endophyte C. protuberate may contribute to the improvement of crops environmental stress tolerance

P2* **A Molecular Approach to the Autoregulation of Nodulation: Genes and Hormones**. *Kaylee Kotwis, Lucy Rummler, Ashley Crook, Elise Schnabel and Julia Frugoli. Clemson University, Department of Genetics & Biochemistry, Clemson, SC.*

Legumes form a symbiotic relationship with nitrogen fixing Rhizobia in the soil. The bacteria are housed in nodules formed on the roots. Autoregulation of Nodulation (AON) is an important pathway controlling nodule number based on previous nodule development and nitrogen and carbon availability. Our lab exploits the model legume system *Medicago truncatula* to understand the pathway leading to nodule number regulation. We have identified multiple genes affecting the process, including the SUNN receptor kinase, mutation of which results in altered auxin flux. Here we report results of two projects focused on SUNN and AON. In one, we examine auxin distribution during wild type nodule development in transgenic *M. truncatula* carrying a DR5:eRFP promoter fusion over the course of several days after inoculation with Rhizobia. DR5 is responsive to auxin, and cells responding to auxin are clearly visible by red fluorescence in living plants. In addition to the expected strong fluorescence in the growing root tip, both emerging nodules and emerging lateral roots show an auxin response signal concurrent with development of these organs, and the signal is similar but not identical. Future work will examine this signal in AON mutants such as sunn. As part of the characterization of SUNN, we created a transgenic wild type plant carrying a tagged version of SUNN driven by the 35S promoter. This plant was then crossed into the sunn-4 null mutant and progeny homozygous for sunn-4 carrying the transgene were selected. After determining these plant nodulated normally (the transgene rescued the phenotype) we wish to select a sunn-4 line homozygous for a single copy of the transgene for future work. We report the results of using Real Time PCR to determine copy number of the transgene. This work is supported by NSF IOS#1146014 and Clemson University's Creative Inquiry Program.

P3* **The role of Hydroxyproline Arabinosyl Transferases (HPATs) in Arabidopsis**. *Benjamin Flanagan[1], Tessema Kassaw[2], Elise Schnabel[1] and Julia Frugoli[1]. (1) Clemson University, Department of Genetics & Biochemistry, Clemson, SC 29634; (2) Colorado State University, Department of Biology, Fort Collins, CO.*

Our lab works on the Autoregulation of Nodulation in *Medicago truncatula*, a local and long distance signal transduction pathway in which legumes regulate the number of nodules they form to host rhizobial symbionts that fix nitrogen. Our previously published work identified a gene Root Determined Nodulation1 (RDN1) as a component of the AON pathway; mutations in this gene result in loss of nodule number regulation resulting in increased nodulation. The gene is part of a multi-gene family conserved in all green plants and localized in the Golgi. Another group has demonstrated that genes homologous to RDNs in *Arabidopsis*, Hydroxyproline Arabinosyl Transferases (HPATs), are responsible for the addition of an arabinose residue onto the hydroxyproline of some members of a family of small signaling peptides known as Clavata like/Extensins (CLEs). Encoded by three genes in both *M. truncatula* and Arabidopsis, these enzymes appear to be expressed throughout the plant. However, the group reporting HPAT activity for these molecules reported no phenotypes in Arabidopsis plants carrying a T-DNA insertion in a single member of the gene family, perhaps because they did not report any observations of roots. Here we report insertions in Arabidopsis HPAT3 result in shorter roots and increased lateral root density, consistent with the role of *M. truncatula* RDN1 in nodule development. We are creating double and triple mutants and assaying for root length, lateral root density, and vascular organization to determine the roles of arabinosylation of CLE peptides in Arabidopsis. This work is supported by NSF IOS#1146014 and Clemson University's Creative Inquiry Program.

P4* Determining the role of target site duplication sequences on the transposition of miniature inverted repeat transposable elements. *David Gilbert[1], Catherine Bridges[2], C. Nathan Hancock[1]. 1)Department of Biology and Geology, University of South Carolina Aiken, Aiken, SC; 2)Marine Biology Graduate Program, College of Charleston, Charleston, SC.*

DNA transposons are sequences within the genome that are mobilized by transposase proteins, which excise and re-insert the element back into the genome. Some transposons, including miniature inverted transposable elements (MITEs), do not encode transposase proteins, but are mobilized in trans. Some MITEs have been found to reach very high copy number in plants and are thought to play a large role in genome evolution. MITE insertion produces identical target site duplications (TSDs) flanking the element. The focus of our research is to determine how the TSDs influence the transposition of the element and excision site repair. To address this question, we are using the Tourist-like MITE mPing and the Stowaway-like MITE 14T32-T7 from rice. mPing insertion creates a 3bp TSD, which is then repaired precisely upon excision (reverting the site of insertion back to the original). 14T32-T7 creates a 2bp TSD and but leaves behind the TSD and a little bit of the end of the element upon excision. To determine the role of the TSD in transposition, we have mutated single bases in the TSDs of both mPing and 14T32-T7 and performed yeast transposition assays. For both elements, we observed that some mutations in the TSD severely inhibit transposition rates. Interestingly, when the two TSD sequences on either end are not matching, the transposition rate is also reduced. To further determine cause of this reduction, we have created a strain of yeast that is unable to repair double-stranded DNA breaks by non-homologous end joining, but can still perform homologous repair. By performing assays in this strain, we will be able to separate the role of excision and repair and determine which transposition step is responsible for this phenomenon.

P5* Targeted Insertion of the Transposable Element, mPing, by Manipulation of Transposase Proteins. *Ashley Strother and C. Nathan Hancock. Department of Biology and Geology, University of South Carolina Aiken, Aiken, SC.*

Transposable elements, like mPing, are mobile pieces of DNA that move throughout the genome of a cell through a cut-and-paste mechanism. The rice transposon, mPing, is mobilized by two proteins, ORF1 and Transposase, encoded by the autonomous transposons, Ping and Pong. This element preferentially inserts in gene-rich regions and has high transposition activity, making it a great tool for disrupting genes to determine gene function. My goal is to modify the ORF1 and Transposase proteins to produce targeted insertion of mPing. If the transposon's insertion can be targeted to specific sequences in the genome, specialized mutagenesis applications could be performed. To determine if targeted insertion of mPing is possible, I added a GAL4 DNA binding domain to the N-terminus or C-terminus of the ORF1 and Transposase proteins. The transposition rate and quality using the modified proteins was tested using a yeast transposition assay. The results indicated that addition of the GAL 4 binding domain to the proteins reduces transposition frequency to different degrees depending on where it is located. However, these modified proteins show increased frequency of insertion near a GAL target sites compared to control proteins. These results suggest that targeted insertion of the mPing element is possible, providing a potentially new mechanism for plant genome modification.

P6* Identifying sequences responsible for the high transposition rate of a Tourist MITE. *Daymond Parrilla, Kristian Pickrell, C. Nathan Hancock. Department of Biology and Geology, University of South Carolina Aiken, Aiken, SC.*

Transposable elements are repetitive sequences, which have the ability to move throughout the genome. These elements are very useful because they can be used as tools to for mutagenesis and gene discovery. The focus of this study is mPing, a 430-bp deletion derivative of the natural occurring Ping element from the rice genome. Miniature inverted repeat transposable elements (MITEs) like mPing, potentially exhibit a very high transposition activity and can reach very high copy number in plants. For comparison we constructed, mPong, an artificial deletion derivative of the natural occurring Pong element that shares approximately 80% identity to mPing. The mPong element shows very low transposition activity, compared to mPing. The question we are trying to address is how one naturally occurring MITE is mobilized very well, while the other is not? To answer this, we compared chimeric constructs of mPing with mPong. By performing yeast transposition assays on the different constructs, we were able to identify a region that promotes transposition in mPing. The next step after determining the transposition promoting region is to identify the specific sequences required for high transposition. To do this, I performed mutagenesis of mPing using manganese error-prone PCR. We are screening these mutants to identify lines with altered transposition activity. By comparing the sequences of the mutant mPings with mPing we hope to determine the sequences responsible for high transposition. Identification of these sequences should allow us to further understand the behavior of MITEs and allow us to develop more useful mutagenesis tools.

P7* **Optimizing germinal transposition of mPing in Arabidopsis thaliana**. *Curtney Burckhalter, Keifer Richardson, C. Nathan Hancock. Department of Biology and Geology, University of South Carolina Aiken, Aiken, SC.*

Transposable elements (TE) are repetitive sequences that are able to move throughout the genome. Some types of TEs, including mPing from rice, are mobilized by a cut and paste mechanism catalyzed by transposase proteins. The overall goal of our research is to develop mPing into an efficient mutagen for gene discovery in plants. To be effective, mPing must produce heritable insertions that disrupt gene function. Previous studies have shown that mPing preferentially inserts near genes and can cause mutant phenotypes in plants. However, the transposition of mPing in A. thaliana has only rarely produced germinal transposition when the 35S promoter was used for driving expression of the transposase genes. Recently, germinal mPing transposition was detected when the constitutive RPS5A promoter was used to drive the expression of the Pong ORF1 and Pong TPase LA proteins. Also, a chimeric ORF1 (ORF1S C1) made from the Pong and Ping ORF1 and a nuclear import signal was shown to produce drastically increased transposition of mPing in yeast assays. The objective of this project is to test novel mPing mutagenesis constructs to determine if they increase the germinal mPing transposition rate in plants. Our hypothesis is that using two different constitutive promoters and the ORF1S C1 protein will increase transposition rate and germinal transposition in A. thaliana. Constructs with the RPS5A promoter driving ORF1S C1 and the GmUbi promoter driving Pong TPase LA were transformed into Arabidopsis using the floral dip method. The transposition in the T1 generation was monitored by the use of a GFP reporter gene. A high percentage of these plants were found to have large sectored areas of GFP, suggesting that they will produce germinal transposition events. The next generation will be analyzed to determine the germinal transposition rate. When complete, this research should provide more information about how to optimize using mPing as a mutagenesis tool.

P8* **Nitric Oxide-mediated Retrograde Signaling in Plants.** *Jared Gabbert, Keshun Yu, Qing-ming Gao, Pradeep Kachroo, Aardra Kachroo. Department of Plant Pathology, University of Kentucky, Lexington, KY*

The conserved cellular metabolites nitric oxide (NO) and oleic acid (18:1) are well known to regulate disease physiologies in diverse organisms. While NO and 18:1 are important regulators of plant disease physiology, their relationship remains uninvestigated. Interestingly, like in humans, plants too regulate NO levels via 18:1 even though they utilize distinct activities. A reduction in 18:1 levels, via a genetic mutation in the 18:1 synthesizing stearoyl-ACP desaturase, induces NO accumulation. Furthermore, reducing 18:1 and NO application induces the expression of similar sets of nuclear genes, majority of which were expressed in a salicylic acid-independent manner. The constitutive defense signaling phenotype of low 18:1 containing plants is partially restored by a single mutation in the Nitric Oxide Associated (NOA) 1 gene, or completely restored by double mutations in NOA1 and either of the nitrate reductases, NIA1 or NIA2. The altered defense-related phenotypes in ssi2 plants can be rescued by restoring the 18:1 levels via second site mutations in genes encoding a glycerol-3-phosphate (G3P) acyltransferase, a G3P dehydrogenase, and an acyl carrier protein. We have also identified additional suppressors that restore defense signaling without affecting 18:1 levels in ssi2 plants and these are being characterized for ssi2-triggered phenotypes. Detailed characterization of one of these suppressors will be presented.

P9* **High Throughput Phenotyping of High Vitamin C Tobacco Lines.** *Earl Morris[1], Zachary Campbell[1], Gabriela Rodriguez[2], Argelia Lorence[1,3] 1 Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR; 2 Department of Biology, University of Puerto Rico, Mayagüez, Puerto Rico; 3 Department of Chemistry and Physics, Arkansas State University, Jonesboro, AR.*

Vitamin C (L-ascorbic acid or AsA) is the most abundant water soluble antioxidant in animal and plant cells. Humans are unable to synthesize their own vitamin C and must acquire it from the diet. Elevating AsA levels in plants has been shown to not only increase their nutritive value, but also lengthen shelf life and positively affect tolerance to abiotic stresses. In plants, AsA functions in the regulation of redox potential in biochemical processes, serves as an enzyme cofactor, and has been shown to be involved in cell signaling events attributed to environmental stress acclimation. Four different pathways have been proposed for the biosynthesis of AsA in plants. The protein of interest in this work is myo-inositol oxygenase (MIOX), the first enzyme in the inositol route to vitamin C. Previous work by the Lorence Laboratory identified a myo-inositol oxygenase (MIOX) gene in chromosome 4 of Arabidopsis after investigating the role of myo-inositol as a precursor to the biosynthesis of AsA. The Lorence group has shown that the level of MIOX4 gene expression is directly correlated to the AsA content in the leaves of Arabidopsis plants. Arabidopsis lines that over-express MIOX4 have higher foliar AsA content compared to wild type (WT) plants growing under similar conditions. The over-expression of MIOX4 has been shown to lead to enhanced growth and biomass accumulation of aerial and root tissues in Arabidopsis lines. The

Lorence group has also shown that MIOX4 Arabidopsis over-expressers are tolerant to salt, cold, heat, and environmental pollutants such as thrichloroethylene and pyrene. Despite the substantial work done on growth and stress tolerance of MIOX4 over-expressing Arabidopsis lines, little is known about the effect of over-expressing this enzyme in other plant models. The goal of this project is to investigate the effects of AtMIOX4 over-expression in the growth and abiotic stress tolerance (particularly salt and drought) of tobacco plants (*Nicotiana tabacum* var. Xanthi). The phenotypic characteristics of interest are leaf area, convex hull area, caliper length, compactness, color classification, in planta water content, and in planta chlorophyll content. One important aspect of this research plan is access to a highly sophisticated phenotyping instrument (Scanalyzer HTS, LemnaTec). This instrument uses visible, fluorescence, and near infrared cameras to precisely measure phenotypic characteristics of plants. Using high-throughput screening, this nondestructive method allows for much more precise phenotyping of our tobacco lines than preliminary measurements and will allow for multiple quantitative measurements within the growth cycle of the tobacco plants. Scientific advances in the understanding of AsA metabolism in tobacco may provide new opportunities for the development of healthier tobacco crops.

P10* Discovery of Symbiotic Fungal Endophytes and Their Effects on Agricultural Plants. *Brandon S. Nelson, Nicole Davis, Fannetta Dancy, Tamaya Tompson,. Stephanie Shoup, and Mustafa Morsy. University of West Alabama Station 7, Bibb Graves Hall, Livingston, AL*

Many plants survive extreme environments via symbiotic relationship with fungi. We conducted an experiment during a discovery based Cell Biology class at the University of West Alabama to identify possible beneficial fungal symbionts from a healthy plant in a harsh environment. Our aim, is to identify fungi that can have useful applications in crop production. We collected healthy plants growing in harsh environments around UWA campus, we sterilized the upper root system and isolated pure cultures of fungal endophytes residue within these plants. We have isolated fifty different fungal isolates. All fungi were identified as Ascomycetes based on their ITS region of rRNA using PCR followed by DNA sequencing. We have also tested the soil samples in which these plants were growing in the presence of macronutrients, pH and salt level. All soils were very poor in the major macronutrients, and some soil samples have an extreme pH (9.2). Despite the poor nutrients and extreme pH, these plants looked healthy, suggesting a possible role of the fungal endophyte reside within them in increased their adaptation. Our ongoing research includes testing some of the isolated fungal endophyte in tomato plants (our model system) to check if any of these fungi can provide plants with the benefit of better growth or adaptation to harsh environments such as salt stress, extreme pH, drought, or poor soil nutrients.

P11* Characterization of *Epipremnum aureum* EaF82 by expressing in Arabidopsis Plants. *Keely N Wharton, Chiu-Yueh Hung, Jiahua Xie. Department of Pharmaceutical Sciences, North Carolina Central University, Durham, NC.*

'Golden Pothos' (Epipremnum aureum) is a naturally variegated plant making it ideal material for the study of chloroplast biogenesis and development by direct comparison between green and white sectors. EaF82 is a gene found in 'Golden Pothos' plants, which is differentially expressed in variegated leaf tissues (Hung et al., J Exp Bot, 2010, 61:1483-1493). Its homologue could not be found in any gene databases, thereby leaving its true function(s) unknown. To determine its role in plants, the chimeric gene EaF82-sGFP driven by 35S promoter or EaF82 promoter were cloned into an expression vector pBI121, respectively. They were introduced into Arabidopsis plants by Agrobacterium-mediated transformation to observe the effects of EaF82 on plant development and to study its localization. In our previous study, we found that EaF82 is co-localized with IAA (Hung et al., Physiol Plantarum, 2014, accepted). Therefore, GFP was expected to be observed where auxin level is high in various growth stages or specific tissues of transgenic plants. In the plant development study, transgenic Arabidopsis plants overexpressing EaF82 under 35S promoter were grown side by side with wild type plants to observe differences in growth under various conditions (temperature, light cycle, etc). We found the transgenic plants were more sensitive than wild type to higher temperature and as a result, a weak silique development was observed. In the localization study using transgenic Arabidopsis plants expressing EaF82-sGFP under EaF82 promoter, we observed GFP accumulation in the root tips, cotyledon, and new leaves of the transgenic seedlings. Further growth stages are still under observance.

P12* Characterization of Two Genes in Arabidopsis That Control Lipid Droplet Formation. *Kevin T. Mutore, Chris N. James, Charlene Case, Kent D. Chapman. Center for Plant Lipid Research, Department of Biological Sciences, University of North Texas, Denton, TX.*

Fat specific protein of 27 kDa (FSP27) is a protein identified in mammalian cells that mediates the process of lipid droplet (LD) growth by acting as a channel that allows the directional transfer of lipids from a smaller to a larger LD. This transfer occurs at lipid droplet contact sites - and demonstrates the dynamic properties of LDs (Gong et al., J Cell Biol. 2011 Dec 12; 195(6):953-63). Since many of

the genes that are involved in LDs synthesis and metabolism are conserved between plants and animals, our research looked to uncover candidate homologous genes for FSP27 in the Arabidopsis thaliana genome. Identification and characterization of LD biosynthesis genes in plants will be important in understanding how LDs form in plant tissues and the extent of evolutionary conservation of this process across kingdoms. A basic local alignment search tool (BLAST) search uncovered two candidate proteins that shared regions of sequence homology with FSP27. Unexpectedly, early characterization studies have shown that T-DNA disruptions of these genes, AT1G80810 (Tud) and AT5G39580 (P62), led to a 12- and 7-fold increase in LDs in leaf tissue, respectively. Furthermore, seed oil levels were increased with the Tud mutant lines, whereas the P62 knockout lines showed no change in lipid levels in seeds (compared with the Col(0) wild type background). On the other hand, the size of the LDs in the P62 lines was 4 times larger on average than those in wild-type seedling leaves. Although the loss-of-function of these two different genes appeared to markedly influence lipid droplet ontogeny and lipid storage in the Arabidopsis system, these effects were opposite of what would be expected if these were functional homologues of FSP27. Further work is needed to understand how these proteins function in the compartmentalization of storage lipids in plants, and their relationship to lipid biosynthesis genes in other organisms.

P13* Spadix Function in *Pinellia pedatisecta*. *T. Marshall, M. Davis, A. McCaskill and F. Corotto. University of North Georgia, Dahlonega, GA*

Pinellia pedatisecta produces inflorescences that include a protruding, apical spadix. Male and female flowers are attached to the base of the spadix within the spathe, an enclosing, leaf-like sheath. We hypothesized that the spadix serves to attract pollinators to the plant. To test this hypothesis, and better understand the function of the spadix in *Pinellia* reproduction, a series of treatments were applied to the spadix. Removal of the spadix, male flowers, or both revealed that the spadix does have a significant impact on fruit set. The presence of either male flowers or the spadix is sufficient to produce fruit. In the absence of both, however, hardly any fruit is set. This indicates that the spadix attracts pollinators to facilitate cross-fertilization while the male flowers allow for self-fertilization.

Poster Session

P14 Grass-fungal endophyte symbioses effects on nitrogen fixation and dynamics in a Kentucky pasture. *Lindsey Slaughter, R. L. McCulley, E. Carlisle, J. Nelson. University of Kentucky, Lexington, KY.*

Tall fescue, an important forage species of pastures in the eastern U.S., can associate with an endophytic fungus, *Neotyphodium coenophialum*. However, endophyte presence in tall fescue can negatively impact animal production through production of toxic alkaloids; therefore, non-toxic 'novel' strains of the endophyte are being adopted by forage producers. In this study, the impact of grass: endophyte symbiosis on nitrogen fixation and dynamics was investigated by measuring 15N natural abundance in plant and soil samples. The study site consisted of tall fescue that was either infected with the common toxic strain of the endophyte, infected with one of two non-toxic strains, endophyte-free, or contained an equal mixture of endophyte treatments. To assess the effect of endophyte presence and strain on the amount of nitrogen derived from biological fixation via legume symbiosis and nitrogen use in co-occurring tall fescue, δ15N natural abundance was measured in red clover (RC), tall fescue associated with red clover (TF+RC), and tall fescue not associated with clover (TF-RC) collected from plots of each of the tall fescue – endophyte treatments. In addition, δ15N natural abundance was measured in bulk soil samples from each plot over a period of 4 years in order to gain insight into long-term changes in N-cycling in this system. Differing endophyte effects influenced δ15N in both tall fescue samples and red clover, but not in soil over time. The results of this study indicate that endophyte infection and strain have varying effects on nitrogen dynamics in both tall fescue and neighboring red clover.

P15 A Forward Genetic Approach to Unraveling a Genetic Pathway: Mapping Suppressor Screen Mutants. *Sophie Altamirano, Ashley Crook, Elise Schnabel and Julia Frugoli. Clemson University, Department of Genetics & Biochemistry, Clemson, SC.*

Autoregulation of nodulation (AON), exhibited by nodule-forming legume species involves a complex signaling pathway encompassing molecules that act in both the root and the shoot that regulate the number of nodules that form during the symbiosis. In *Medicago truncatula*, SUNN, a leucine rich repeat receptor-like kinase, is a key regulatory kinase in the AON pathway, with orthologs isolated by mutation in pea, soybean, bean, Lotus japonicas and *M. truncatula*. SUNN has high homology to the Arabidopsis CLAVATA1, which acts in a multi-protein complex. Building on work done with CLV components in Arabidopsis, we undertook a forward genetics approach to identify components of the AON signaling pathway. Utilizing a mutant suppressor screen

of sunn-1, we have identified five individuals carrying mutations that suppress the supernodulation phenotype of sunn-1. We report the phenotypes and the progress on mapping these mutations, which is in various stages of completion. This work is supported by NSF IOS#1146014, a Clemson Diversity Fellowship to S.A. and a Clemson University Wade Stackhouse Fellowship to A.C.

P16 Single seed selection for low phytate soybeans. *Maythem Al-Amery, David Hildebrand, Hirotada Fukushige. University of Kentucky, Lexington, KY*

Most seed Phosphorus (P) is bound in phytate which is unavailable to monogastric animals depriving them of P and causing eutrophication from P in animal waste. It is valuable to reduce the phytate levels of seeds used for food and feed. Determination inorganic phosphate (Pi) concentrations in soybean (*Glycine max* (L.) Merr.) Low phytate soybean mutants are known such as MIPS (D-myo-inositol 3-phosphate synthase) mutants with correspondingly increased Pi. Measurement of seed Pi levels is an established technique for screening for low phytate mutants but to date it has not been performed non-destructively from single seed samples. A protocol was developed reducing sample size dramatically thereby reducing the cost and time and saving a generation in the genotyping, especially using 96 well plates and plot reader which accelerating the whole process. The new technique was tested using 1~2 mg samples, 96 well plates with 8 MIPS mutants, 8 wild-type and 8 seeds segregating for a MIPS mutation. The Pi concentrations for GM-lpa-TW1 ranged between 2.1 and 3.1 mg/g and 0.3 and 0.5 mg/g for wild type seeds and between 0.3 and 3 mg/g for the segregating seeds. Genotyping by Pi measure this way was confirmed by DNA analysis doe limited seeds.

P17 Transcriptional Regulators of Triacylglycerol Biosynthesis in Nonseed Tissues. *Parker Dabbs, Carlee Haas, Aruna Kilaru. Department of Biological Sciences, East Tennessee State University, Johnson City, TN*

Triacylglycerols (TAGs) play an important role in plants not only as an energy reserve in seeds, to allow for germination, but also in nonseed tissues as a resource to seed dispersers. Humans also utilize TAGs for consumption, chemical and industrial feed stocks, and production of biofuels. While much of the research has focused on understanding TAG biosynthesis in seed tissues, very little is known of its regulation in nonseed tissues. Here, we have taken comparative transcriptomics approach to identity transcription factors that are likely involved in TAG biosynthesis in nonseed tissues. Comparison of RNA-seq data for oil-rich nonseed tissues (mesocarp of avocado and oil palm) with that of oil-rich seed tissues (rapeseed and castor) and oil-poor tissues (oil palm leaf, mesocarp of date palm) revealed transcription factors that are highly conserved among oil-rich species and also those that are tissues nonseed-specific. Among the conserved transcription factors, Wrinkled 1 was highly expressed in seed and nonseed oil-rich tissues, irrespective of the species. Interestingly, avocado mesocarp also showed abundant expression levels for homologs of WRI2 and WRI3. WRI3 was shown to be associated with TAG accumulation in arabidopsis seed tissues but WRI2 function is yet to be determined. Cloning and characterization of putative WRI 1, 2, and 3 of avocado, using transient tobacco leaf expression assay and complementation analysis of Atwri1 mutants is underway. Ten other potential transcription factors that are likely specific to nonseed oil biosynthesis were also identified and select candidates are being validated by transient expression assays. Results from these studies are expected to provide insight into differential regulation of oil biosynthesis in various plant tissues.

P18 Characterization of anandamide metabolic pathway in moss. *Swati Swati, Richard Sante, Brent Kinser, Aruna Kilaru. Department of Biomedical Science, Biology Department, ETSU, TN*.

N-Acylethanolamines (NAEs), including anandamide (NAE 20:4) are fatty acid ethanolamides, which in mammals bind to cannabinoid receptors and act as neuromodulators for a variety of physiological processes. Although C12-C18 NAEs are ubiquitous in plants, identification of anandamide, a 20C, polyunsaturated omega-6 fatty acid ethanolamide, in P. patens but not in higher plants, has opened the possibility that NAEs in early land plants may play a role that is similar to animals and beyond what is understood in flowering plants. Presence of certain unconventional lipids in bryophytes may have enabled their successful transition from water to land and imparted them with natural ability to resist high temperatures and tolerate osmotic and salt stresses and dehydration. Therefore, it is hypothesized that mosses may have evolved to retain unique NAE metabolites, such as anandamide, and mechanisms by which they mediate stress tolerance. To address this premise, three main objectives are being pursued, using P. patens: 1) Biochemical and molecular characterization of NAE metabolic pathway, 2) Generation and phenotypic characterization of NAE metabolite mutants, and 3) Elucidation of the physiological role of NAEs in abscisic acid-mediated dehydration tolerance. Here, identification and cloning of putative genes that likely encode for NAE synthesis and hydrolysis are discussed. A long-term goal of the project is to elucidate the mechanisms by which mosses maintain tolerance to abiotic stress, which perhaps may have been altered

or lost in vascular plants. This study is expected to reveal novel functional and evolutionary insights into lipid-mediated biological responses in plants.

P19 Identification of the WRKY Transcription Factor Family from the Medicinal Plant *Catharanthus roseus*. *Craig Schluttenhofer[2], Sitakanta Pattanaik[2] and Ling Yuan[1,2], Department of Plant and Soil Sciences 1 and Kentucky Tobacco Research and Development Center 2, University of Kentucky, Lexington, KY.*

The medicinal plant *Catharanthus roseus* produces pharmaceutically valuable terpene indole alkaloids (TIAs) used in the treatment of cancer and hypertension. Currently production of these compounds for pharmaceuticals is limited. Manipulation of transcription factors (TFs) to increase expression of TIA pathway genes is one methods to potentially improve TIA production. WRKY transcription factors are involved in stress tolerance to biotic pathogens and potential regulate TIA biosynthesis. We identified 48 *Catharanthus* WRKY TFs using recently available transcriptome data. To identify candidate regulators of the TIA pathway CrWRKYs were compared to WRKYs previously published as regulating natural products in other species. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to measure CrWRKY transcript levels after treatment with methyl jasmonate (MeJA), a potent elicitor of the TIA biosynthesis and accumulation. Based on known functions of homologs in other species we provide a model for those *Catharanthus* WRKY which are potential regulators of the TIA pathway.

P20 Identification and Characterization of DGAT1 and PDAT1 involved in TAG biosynthesis in Avocado. *Md Mahbubur Rahman, Ha-Jung Sung, Jay Shockey and Aruna Kilaru. Department of Biomedical Sciences, Department of Biological Sciences, East Tennessee State University, Johnson City, TN and USDA-ARS, New Orleans, LA.*

Triacylglycerol (TAG) is a predominant storage lipid in plants. Accumulation of TAG in nonseed tissue is highly important because of their enormous biomass (e.g. mesocarp, leaves, roots etc.) relative to seed tissue. A minute increase in TAG accumulation in the nonseed tissue can contribute to enhanced biofuel production, industrial feedstock and nutritional supply for human consumption. To accomplish this goal, it is pertinent to understand the regulation of rate-limiting reactions involved in TAG accumulation in nonseed tissue. Avocado (*Persea americana*), a basal angiosperm, stores up to 70% oil in the form of TAG in nonseed tissue (mesocarp). RNA-Seq and Q-PCR analysis of developing mesocarp of avocado revealed higher expression for diacylglycerol acyltransferase (DGAT1) and phospholipid: DAG acyltransferase (PDAT1), coinciding with the period of TAG accumulation. Therefore, we hypothesize that DGAT1 and PDAT1 are responsible for catalyzing the terminal step in TAG biosynthesis in avocado mesocarp. Using the transcriptome data, we identified full-length coding sequences for DGAT1 and PDAT1. These acyltransferases will be characterized for their enzyme activity and substrate specificity, subsequent to their expression in yeast. Complementation of Arabidopsis dgat1 and pdat1 mutants and phenotypic characterization will also be carried out using Gateway-cloning technique; transgenic lines will be assayed for TAG content in seeds. Our study is expected to provide basic understanding of TAG accumulation in avocado mesocarp tissue.

P21 Towards development of an Ac-Ds activation tagging system in tomato. *Ipeleng Randome, Supratim Basu, Anuj Kumar, Dragana Avirovik2 and Andy Pereira, Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR; 2Department of Biology, Virginia Tech, Blacksburg, VA.*

In plants transposable elements can alter transcription of adjacent genes thereby leading to changes in gene expression and thereby in phenotypic variation. The Ac-Ds system is a maize transposon system consisting of the autonomous Ac transposable element that encodes the transposase and a non-autonomous Ds element that can be stabilized by segregation of the Ac transposon. The maize Ac-Ds system has been widely used to create mutants in many plants including Arabidopsis and rice. We have developed an Ac-Ds transposon 'activation tagging' (ATag) system to be able to excise and transpose transposon inserts bearing a strong 35S-enhancer element all around the genome. We used the tomato (*Solanum lycorpesicum*) cultivar M82 that has an erect determinate habit suitable for greenhouse and field screening, to transform the Ac-Ds ATag constructs. We have screened putative tomato transformants of T1 progeny harboring the Ac-Ds ATag cassette by genomic PCR using Ac and Ds element specific primers to identify transformants. These lines are being studied for excision and transposition of Ds-ATag elements through TAIL-PCR and resulting product sequences aligned to tomato genome. The objective is to identify plant lines with active transposition in order to create a library of Ds-ATag insertion lines and identify mutants in tomato that are altered in development of plant architecture and fruit development. Along with the tomato genome sequence, these lines will be a good resource for plant development and tomato breeding.

P22 ER Localized Arabidopsis SEIPIN Proteins Regulate Lipid Droplet Number and Morphology. *Yinggi Cai and Kent D. Chapman. Center for Plant Lipid Research, Department of Biological Sciences, University of North Texas, Denton, TX.*

The lipodystrophy protein SEIPIN (BSCL2 in humans) localizes at the junctions of lipid droplets (LDs) and the endoplasmic reticulum (ER) and is important in LD biogenesis in humans, mice, Drosophila, and yeast (Cartwright and Goodman, 2012; Szymanski et al., 2007). However, essentially nothing is known about the functions of SEIPIN homologues in plants. There are three putative SEIPIN homologues in Arabidopsis, designated SEIPIN 1, 2 and 3. Here, yeast (*S. cerevisiae*) SEIPIN-deletion-mutant strains and tobacco (*N. benthamiana*) leaves were used to test the functions of Arabidopsis SEIPIN homologues in the process of LD biogenesis. The LD phenotype in yeast cells and tobacco mesophyll cells assessed by confocal microscopy suggested that the three Arabidopsis SEIPINs have different effects on the number and size of LD. Arabidopsis SEIPIN1 increased the size of LDs, while SEIPIN2 and especially SEIPIN3 increased the number of smaller sized lipid droplets. Similar to yeast SEIPIN, Arabidopsis SEIPIN2 and SEIPIN3 could partially reverse the aggregated ER membrane phenotype in the yeast SEIPIN-deletion strain. On the other hand, over-expressed, ER-localized Arabidopsis SEIPINs disrupted the normal reticulated ER organization and produced aggregated ER that was co-localized with LD in tobacco mesophyll cells. Lipidomics of yeast cells and tobacco leaves expressing Arabidopsis SEIPINs suggested that Arabidopsis SEIPINs elevated the amount of triacylglycerol (TAG) and altered the fatty acid composition in neutral lipids. SEIPIN2 and SEIPIN3 expressed in yeast SEIPIN-deletion-mutants reduced the proportion of unsaturated fatty acids and increased the proportion of saturated fatty acids in TAG. In tobacco leaves expressing Arabidopsis SEIPINs, the neutral lipids had more palmitic acid and less linolenic acid than untransformed "mock" controls. Overall our results suggest that Arabidopsis SEIPINs are part of the conserved machinery in eukaryotes for LD biogenesis and that these plant proteins may cooperate in the storage of neutral lipids in different plant tissues and at different stages of plant development by regulating the number and size of lipid droplets. These findings provide new insights into neutral lipid compartmentation in plants, and may offer novel strategies to manipulate the accumulation and packaging of neutral lipids in plant tissues.

P23 Using variegated 'Golden Pothos' Plants to Study Nuclear Genes Involved in Chloroplast Biogenesis. *Laquitta Thomas, Chiu-Yueh Hung, Jiahua Xie, Department of Pharmaceutical Sciences, North Carolina Central University, Durham, NC.*

Chloroplast is a critical organelle in plants responsible for photosynthesis and production of amino acids, lipids, hormones and many other metabolites. However, the total number of genes required for chloroplast biogenesis and function remains unclear. Chloroplasts develop from proplastids by a series of steps, which require 2,500 to 3,500 proteins encoded by genes from both nuclear and chloroplast genomes. Although chloroplast requires so many proteins, its genome only encodes less than 100 proteins while the remaining over 95% of proteins are encoded by nuclear genes. Given that chloroplast proteins are encoded by two separate genomes, understanding the number of genes and their coordinated expression is both of great fundamental and practical importance. To study these nuclear genes encoding chloroplast proteins, a novel functional assay system is being created. In this system, regenerated pale yellow plants from the naturally variegated *Epiprenum aureum* 'Golden Pothos' (Hung and Xie, Biol Plantarum, 2009, 53: 610-616) were used whose color defection has been confirmed resulting from low expression of EaZIP, a nuclear gene encoding the Mg-protoporphyrin IX monomethyl ester cyclase in the chlorophyll biosynthesis pathway (Hung et al., J Exp Bot, 2010, 61:1483-1493). The assay is based on compensating the loss of EaZIP under an inducible condition. First, the genetic cassette containing EaZIP driven by an inducible promoter was created and overexpressed in pale yellow 'Golden Pothos' using Agrobacterium-mediated transformation. It is expected that the transgenic pale yellow plants overexpressed EaZIP will develop normal chloroplasts under induced conditions. Then the assay system can be used to study those nuclear genes encoding chloroplast proteins and their coordinate expressions.

P24 Comparative study of varying M. truncatula genotypes and phenotypes as they relate to saponin defenses against insect pests. *Audra Harris, Lacy Nelson, Brynn Alford, Kenneth Korth. Department of Plant Pathology, Division of Agriculture, University of AR.*

Herbivorous insects have long been some of the most damaging plant pests. Saponins are triterpene-glycosides that in some cases have a defensive role in plants. We assessed performance of the beet armyworm, *Spodoptera exigua*, feeding on accessions of the legume *Medicago truncatula*. Insect growth and fecundity of beet armyworm following ad libero feeding were measured on four distinct plant accessions, selected because of their varying profiles of leaf saponins. Experiments were designed to test for differential insect performance that might be based on these metabolite differences. Insect growth rate and weights are higher on an insect artificial diet than on *M. truncatula* leaf diets. Insects feeding on accessions ESP105 and GRC43 gained weight more quickly

and had lower mortality, whereas insects feeding on accessions A17 and PRT178 experienced slow weight gain and increased mortality. Accession ESP105 has very low levels of saponins in foliar tissues, whereas the others have relatively high levels. Although it is the case for ESP105 and PRT178, we did not observe a correlation between poor insect performance and high saponins in all accessions. Saponins from each accession will be extracted and tested for impact on insect performance via addition to artificial diets. Transcripts of genes encoding enzymes in the biosynthetic pathway for saponins were induced by insect feeding, and show variation between genotypes. This includes transcripts for beta-amyrin synthase, along with putative cytochrome P450 enzymes predicted to be involved in saponin aglycone synthesis. By combining bioassays, metabolite profiling and gene expression data, we hope to determine the role of saponins as plant defenses, and the genetic regulation of saponin biosynthesis in a forage legume.

P25 Formation of hormone-induced nodule-like structures in cereals. *Ryan Hiltenbrand, Hannah Posey, Arijit Mukherjee. Department of Biology, University of Central Arkansas, Conway, AR.*

Availability of nutrients, and especially nitrogen, is a major constraint for crop productivity and sustainable agriculture. Over the last decades, there has been an excessive dependence on nitrogen fertilizers. Unfortunately, this has caused many negative consequences at the economic and environmental level. One option for improving crop yields while maintaining the sustainability of our agriculture systems is to take advantage of naturally occurring beneficial plant-microbe symbioses. The two most efficient plant-microbe symbioses are those occurring with arbuscular mycorrhizal (AM) fungi, and with nitrogen-fixing bacteria, rhizobia. More than 85% of land plants (including cereals, legumes etc.) can form a symbiotic association with AM fungi that benefits the host plant in improved nutrient (especially phosphorus) uptake from the soil in exchange for carbohydrates. The more recent plant-microbe association is efficient in atmospheric nitrogen fixation and occurs between legumes (soybean, alfalfa, peas) and rhizobia. In this process, the rhizobia fix atmospheric nitrogen for its host plant inside specialized root structures called nodules. Over the last decades genetics in model legumes identified several plant genes that are required for the establishment of these associations. Interestingly, some of these genes are required for both legume-rhizobia and AM symbioses. This has led to the concept of the common symbiotic pathway (CSP). Some of these CSP genes are also present in non-leguminous species, including cereals. Furthermore, reverse genetic studies have shown that these genes are conserved in rice during AM symbiosis. This means that some components required for legume-rhizobia symbiosis are already present in cereals and the future of improving nitrogen-fixing associations in cereals looks quite promising. Other studies revealed that classical plant hormones such as auxins, cytokinins and ethylene play key roles in establishment of legume-rhizobia and mycorrhizal symbioses. For instance, auxin transport inhibitors and cytokinins have been shown to induce the formation of nodule-like structures (NLS) in roots of *Medicago sativa* and *Medicago truncatula* even in the absence of bacteria. In addition, transcriptomic studies in *M. truncatula* revealed plant genes that lead to the formation of these NLS. Interestingly, in cereals such as wheat and corn, addition of auxin stimulates the formation of similar root structures. It was also reported that nitrogen fixation by some nitrogen-fixing endophytes increased inside these NLS. Unfortunately, our knowledge of NLS formation in cereals is still fragmentary. For example, the host genes controlling the formation of these nodule-like structures in cereal roots are still unknown. Our long-term goal is to investigate the host plant genes controlling the formation of these hormone-induced nodule-like structures in cereals. Towards that goal we have already optimized the formation of these structures under different conditions. We are currently getting our samples ready for a transcriptomic study during NLS formation in rice. In addition, we are comparing NLS formation between *Medicago truncatula* and rice.

P26 Engineering high value oil production into biofuel crops. *Zuodong Jiang, Chase Kempinski and Joe Chappell. Plant Biology Program, University of Kentucky, Lexington, KY.*

Assuming biofuels generated via the fermentation of sugars derived from cellulosic and non-cellulosic constituents of biofuels crops will provide a substantial contribution to our future energy needs, augmenting and amending the productivity of these biofuel crops is now a major research thrust worldwide. One way of enhancing these biofuels crops will be to engineer them for value-added components such as oils that can be used for efficient fuel production and the manufacturing of other high-value products currently derived from petroleum oils. Towards this end, we are engineering optimized production of long, branched-chain hydrocarbon biosynthesis into plants suitable as biofuels crops. Branched chain hydrocarbons, like methylated triterpenes, are readily cracked into paraffins and naphthenes that can either be distilled to combustible fuels (gasoline, jet fuel and diesel), or can be used directly for the synthesis of plastics, nylons, paints and other oil-derived products manufactured by diverse chemical industries.

P27 Double-stranded RNA-binding protein 4 is required for resistance signaling against viral and bacterial pathogens. *Gah-Hyun Lim, Shifeng Zhu, Keshun Yu, Aardra Kachroo, Pradeep Kachroo. Department of Plant Pathology, University of Kentucky, Lexington, KY.*

Species-specific immunity is induced when an effector protein from a specific pathogen strain is perceived by a cognate resistance (R) protein in the plant. In Arabidopsis, the R protein HRT, which confers resistance to turnip crinkle virus (TCV) (1,2), is activated upon recognition of the TCV coat-protein (CP), a potent suppressor of host RNA silencing. Recognition by HRT does not require RNA silencing suppressor function of CP and is not associated with the accumulation of TCV-specific small-RNA. However, several components of the host RNA silencing pathway participate in HRT-mediated defense against TCV. For example, the double stranded RNA binding protein (DRB) 4 interacts with the plasma membrane localized HRT, and is required for its stability (3). Intriguingly, TCV infection promotes the cytosolic accumulation of the otherwise primarily nuclear DRB4, and this in turn inhibits HRT-DRB4 interaction. These data together with differential localization of DRB4 in plants inoculated with avirulent and virulent viruses, suggests that sub-cellular compartmentalization of DRB4 plays an important role in activation of HRT.

P28 Presence of Atrazine in Surface Waters of North Louisiana. *Om Devkota, Stephen W. Banks, M. Cran Lucas, Dalton R. Gossett. Department of Biological Sciences, Louisiana State University, Shreveport, LA.*

Like many pesticides, recent studies indicate that in areas where atrazine has been used, significant amounts of this herbicide can be detected in surface water. In areas where atrazine has been used extensively, it is possible to detect atrazine in groundwater aquifers and surface water bodies such as streams, lakes and rivers. Atrazine is widely used in the United States where corn and sorghum is grown commercially. It is one of the most frequently applied herbicide in agriculture for the selective control of broad leaf and grassy weeds. In this study, surface water was sampled at nine different sites in Shreveport/Bossier: Lake Bistineau, Cross Lake, Flat River, The Red River, Bickham Dickson Lake, Red Chute Bayou, and the LSU Red River Research Station constructed wetland. All Samples were collected after rainfall in these areas. The research required the development of a multi-residue analytical methodology allowing atrazine to be extracted and quantified using the techniques of Solid Phase Extraction (SPE) and Ultra High Performance Liquid Chromatography (U-HPLC). The procedure required the pre-concentration of 500 ml water samples via a supelcean ENVI-18 SPE cartridge. After elution of the SPE cartridge with methanol, samples were dried down under nitrogen and re-suspended in 70/30 methanol/deionized water. The final volume of the re-suspended sample was 100µL.U-HPLC analysis was performed using a Dionex Ultimate 3000 U-HPLC Instrument Array using a Dionex Acclaim C-18 HPLC column. This study has revealed the presence of atrazine at all the sites examined, however the levels detected at each site varied seasonally.

P29 Screening and characterization of diverse rice genotypes for water use efficiency and drought tolerance. *Anuj Kumar, Ramegowda Venkategowda, Supratim Basu, and Andy Pereira. Department of Crop, Soil, & Environmental Sciences, University of Arkansas, Fayetteville, AR.*

Rice is a primary food source for about half of the world's population. The production and productivity of rice is severely affected by abiotic stresses like drought especially at reproductive stage as rice production requires approximately 30% of the global freshwater, which is a dwindling and unpredictable natural resource. Consequently, increasing water use efficiency (WUE) of crop production is a sustainable solution to the growing global water scarcity. Previously improved WUE has been reported through individual transgene expression in Nippponbare but it is necessary to exploit the existing natural genetic variability in rice germplasm to screen for improved WUE. In the present study, we have screened diverse rice genotypes from the USDA mini-core collection for altered biomass, instantaneous WUE (iWUE) and photosynthesis related parameters by applying controlled drought stress using a gravimetric approach. Screening showed that the genotypes exhibited variation in biomass, photosynthesis and iWUE under controlled drought stress with most of them showing significant reduction in the measured parameters compared to control condition. Drought screening identified susceptible and tolerant genotypes. The tolerant and susceptible genotypes are being crossed to generate mapping populations which will be used to develop RILs for mapping of QTLs related to drought tolerance traits.

P30 Role of a nodule-specific membrane transporter during symbiotic nitrogen fixation in legumes. *Christina Wyman, Vagner A. Benedito [1], Carroll P. Vance [2,3], 1 Laboratory of Plant Functional Genetics, Plant and Soil Sciences Division, West Virginia University, Morgantown, WV, USA; 2 Plant Science Research Unit, USDA-Agricultural Research Service, St. Paul, MN, USA; 3 Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN.*

Legumes are an important part of the human diet and account for 1/3 of the world's primary crop production. Legumes are vital for sustainable agriculture due to their ability to fix nitrogen by reducing atmospheric nitrogen to ammonia when in symbiotic

association with rhizobial bacteria. Within nodules, symbiosomes are organellar structures formed through endocytosis of bacteria into infected nodule cells. Symbiosomes enclose differentiated bacteroids capable of fixing nitrogen. Active nutrient exchanges between the legume plant and the endosymbiont are critical to symbiotic nitrogen fixation and must pass through the symbiosome and bacteroid membranes via transporters. Branched chain amino acids are required for full development of bacteroids and steadfastness of nitrogen fixation. The APC transporter encoded by Medtr8g089360 locus is being studied to understand its physiological function during nodule development and nitrogen fixation. This transporter is closely related to the Arabidopsis AAT1 and CAT5 basic amino acid transporters. It is expressed exclusively in nodules, starting early in development (4 dpi), with highest expression 10 days post inoculation. In mature nodules, expression is highest in the nitrogen fixation zone and surrounding tissues. This data supports our current hypothesis that APC supplies branched-chain amino acids to bacteroids. APC has 600 amino acid residues and spans 14 transmembrane domains, typical for members of this family. Current work is focused on substrate specificity and subcellular localization as part of our aim to understand physiological function during symbiotic nitrogen fixation.

P31 Magnetic Growth Stimulation of Microalgae for Biofuel. *Jeff Zabolotney, Timothy Sherman, Kelly Major. Department of Biology, University of South Alabama, Mobile, AL.*

Global atmospheric carbon concentration has shown dramatic increase since the industrial revolution. The use of a practical, renewable biofuel can result in a cleaner, more responsible community and environment. Microalgae offer great promise as this source of sustainable energy to match our ever increasing demand. Rapid growth rates, high light efficiency, and adaptability are just a few of the many characteristics that put microalgae ahead of land based crops in the race for an optimum source of biofuel. Current limitations for microalgal fuel production do not arise from these marvelous organisms, but rather in our methods of their growth and energy extraction. Recent research has identified low levels of magnetic exposure as a novel method of significantly increasing microalgal growth and biomass. The mechanisms by which this growth stimulation occurs, however, are not known. My research focuses on elucidating the pathways through which magnetic exposure stimulates the growth of microalgae. I am currently comparing growth characteristics of microalgae grown at different levels of magnetic flux density produced by permanent iron magnets and electromagnets. Exposure time to magnetic fields is also being investigated to determine optimum magnet growth environments. By understanding the principles by which photosynthetic organisms respond to magnetic radiation, approaches to algal growth and magnetic exposure can be optimized to obtain significantly more biomass for renewable biofuel.

P32 Efficiency and Structure of Repair Site after I-SceI Mediated DNA Excision in Rice. *Clinton Greub and Vibha Srivastava 1, 2. 1 Department of Crop, Soil, & Environmental Sciences, and 2 Department of Horticulture, University of Arkansas, Fayetteville, AR*.

Genetic engineering of several desired traits, such as new biochemical pathways, are not possible with a single gene; these complex traits may involve coordinated expression of multiple genes. These genes must be linked or stacked to ensure they do not segregate out in later generations. There are many ways to accomplish gene stacking, including cross-hybridizing and co-transformation. Our lab focuses on multi-gene cassette stacking using site specific recombination. However, since gene introduction relies on the use of selectable marker genes (SMG), and SMG should be recycled and removed from the final product, a gene-stacking method should involve directed integration followed by SMG excision. I-SceI is a “homing” endonuclease from *Saccharomyces cerevisiae* that induces site-specific double strand breaks (DSB). I-SceI has already been efficiently used in Arabidopsis for gene excision; however, little is known about the efficiency of SMG excision by I-SceI and the structure of the repaired site. Determining the efficiency and structure of the repair site can help us better utilize I-SceI in SMG removal. I-SceI has an 18 bp recognition site that, when cut, produces a 3’ overhang of four bases. In the present study, these recognition sites will flank a marker gene and then particle bombardment will be used to introduce the I-SceI gene. Efficiency of marker removal and the structure of the repair sites will be determined by PCR and DNA sequencing.

P33 Characterization of Gossypol Metabolites in Situ in Pigmented Glands of Cotton Embryos using Laser Desorption Ionization (LDI)- and Nanospray Ionization (NSI)-Mass Spectrometry (MS). *Drew Sturtevant, Barbara Walton, Jason Hamilton, Mandi Phelps, Guido Verbeck, Valdimir Shulaev, and Kent Chapman. University of North Texas, Department of Biological Sciences, Denton, TX.*

In the United States, cottonseed industrial products including seed meal and oil generate an excess of 25 billion dollars annually. However, the use of these resources are limited due to the toxic secondary metabolites of gossypol and its derivatives. These complex polyphenolic compounds are found in the pigmented glands of cotton embryos, leaves, and stems and have been

historically difficult to analyze by mass spectrometry due to their high reactivity, stereochemistry, and multiple tautomeric forms. A recent hisotchemical survey of genetically diverse cotton genotypes revealed phenotypic differences in pigmented gossypol glands. Here, we visualized the complex gossypol-related metabolites in these glands by matrix free laser desorption ionization (LDI)-MS. These analyses revealed distinctly different metabolite profiles in embryo sections for each genotype, which also varied widely in gland pigmentation and size. For confirmation and validation of LDI-MS results, we also examined the contents of the glands directly by extraction and nanospray ionization-MS using an on-stage nanomanipulator device. Taken together, these metabolite analyses suggest previously unrecognized features of the *Gossypium* metabolome, and will further our understanding of the complex biochemical pathways leading to the formation of gossypol and related aromatic compounds in cotton germplasm.

P34 Understanding Bacterial and Plant Interactions in *Nicotiana benthamiana* and The Effects That has in Their Normal Physiology and Development. *Andrea Sanchez[1], Derek Lundberg[2], Ye Xia[1] and Seth Debolt[1] 1 Department of Horticulture and Plant Physiology/Biochemestry/Molecular Biology Program, University of Kentucky Lexington, Kentucky; 2 Department of Biology. Curriculum in Genetics and Molecular Biology. University of North Carolina, Chapel Hill.*

Endophyte-plant interactions have been studied for many years in order to have a better understanding on this phenomenon and how this interaction can have positive implications on the use of different plant species in future. Plant growth promoting organisms are considered to be endophytic in nature (most likely facultative); closely studying this dynamic relationship may help shed light on information gap in this area. Our research goal is to look at how the use of certain bacterial strains affects the development of the plant throughout its life cycle and how possible changes in recruitment of bacterial organisms by the plant may shift from treatment to treatment compared to the control. For this, two types of Bacillus strains were utilized to inoculate surface sterilized Nicotiana benthamiana seeds that were transferred to greenhouse environment and monitored during six weeks to evaluate morphological changes. Next generation sequencing, bioinformatics and cell biology approaches have been used herein to help in interpretation of the data. Results to date show that the presence of certain bacterial strains that were over-represented in soil and seeds shift the recruitment of those facultative endophytes that may inhabit the plant during the time periods of study. DR5:GFP auxin reports lines show differences in auxin accumulation among treatments.

P35 Functional analysis of a nodule-specific GRF Zinc Finger transcription factor in the model legume, *Medicago truncatula*. *Lucas Gontijo Silva Maia[1], Michael Udvardi[2], Vagner A. Benedito[1] 1 Genetics and Developmental Biology Program, Plant and Soil Sciences Division, West Virginia University, Morgantown, WV; 2 Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK*.

In legumes, the nodule is a structure developed de novo from root cells upon interaction with rhizobia and is where symbiotic nitrogen fixation occurs. The developmental program leading to nodule organogenesis and function is controlled by an intricate genetic regulatory network, in which transcription factors are essential players. Medtr7g086040.1 codes for a novel GRF zinc finger transcription factor (MtGRF) that is highly expressed and specific to root nodules of *Medicago truncatula*. This gene is expressed since early stages of nodule development (4 days post inoculation) and its expression is sustained in mature nodules. Three insertional mutant lines have been identified in the Tnt1 mutant collection at Noble Foundation. Homozygous plants of an analyzed mutant developed nodules that are capable to grow but remain non-functional. This suggests an important role of this transcription factor in controlling the regulatory gene network associated with symbiotic nitrogen fixation. Furthermore, promoter-GUS analysis is revealing the exact spatial-temporal expression domain of this gene, nuclear localization is being assessed with GFP-gene fusion under confocal microscopy, nitrogenase activity of mutant nodules is being quantified by acetylene reduction assay, anatomical structures of the nodule and bacteroids are being further analyzed by confocal and transmission electronic microscopy, the mutant phenotype is being rescued by genetic complementation using ex vitro hairy root transformation. Tracking of potential MtGRF targets through chromatin immunoprecipitation (ChIP) and assessment of transcriptome in mutant nodules are planned.

P36 Heterologous expression of *Arabidopsis* purple acid phosphatase (AtPAP15) in tobacco for remediation of excess phosphorus from soil. *Jane Bartonjo, Sinilal Bhaskaran, Devesh Shukla, Sneha Murthy and Shivendra Sahi. Department of Biology, Western Kentucky University, Bowling Green, KY.*

Phosphorus is a key macronutrient element that plays vital role in growth and development of the plants. Extensive exposure of livestock manure, phosphorus fertilizers, industrial waste as well as leaching of phosphorus from mining sites has resulted in accumulation of phosphorus in soil. Escape of this excess phosphorus into nearby water bodies with runaway water creates environmental issues like eutrophication. Phytoremediation is considered an efficient, cost effective and environmentally friendly technology for removal of pollutants from soil. Use of transgenic plants overexpressing phosphorus-metabolizing genes like phytase

genes may enhance the uptake and utilization of soil phosphorus. In order to test the feasibility of this strategy, purple acid phosphatase gene from *Arabidopsis thaliana (AtPAP15*) was isolated and cloned to overexpress in *Nicotiana tabacum*. Full length cDNA encoding AtPAP15 was amplified and cloned under the control of CaMV35S promoter in plant transformation vector pBIN19. Transformation of *N. tabacum* was performed using *Agrobacterium tumefaciens* strain EHA105 carrying the recombinant plasmid. Plants regenerated in selection medium were screened by PCR for the presence of *AtPAP15* using gene specific primers. PCR positive plants (F_0 generation) were transferred to greenhouse for production of F_1 generation plants. The expression level of *AtPAP15* gene in different transgenic lines was quantified using Real Time PCR to select the overexpressing lines. F_1 plants will be further screened for *AtPAP15* insert and/or copy number using southern blot. Alternatively, Southern hybridization will be carried out to determine the copy number of the inserted AtPAP15 gene. This will be followed by studies on organic phosphorous uptake using inductively coupled plasma optical emission spectrometry.

P37 A genome-scale resource for the functional characterization of Arabidopsis transcription factors. *Ghislain Breton[2], Jose L. Pruneda-Paz[1], Dawn Nagel[1], Shin-Yong E. Kang[1], Katia Bonaldi[1], Colleen Doherty[3], Stephanie Ravelo[1], Joseph Ecker[4,] and Steve A. Kay[5]. (1) Division of Biology, University of California San Diego, La Jolla, CA; USA; (2) Department of Integrative Biology and Pharmacology, University of Texas Health Sciences Center-Houston (UTHSC), Houston, TX; (3) Department of Molecular & Structural Biochemistry, North Carolina State University, Raleigh, NC; (4) Plant Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA; (5) Dornsife College of Letters, Arts and Sciences,University of Southern California, Los Angeles, CA*.

Extensive transcriptional networks play major roles in cellular and organismal functions. Transcript levels are in part determined by the combinatorial and overlapping functions of dozens of transcription factors (TFs) bound to gene promoters. TF-promoter interactions thus provide the basic molecular wiring of transcriptional regulatory networks. In plants, discovery of TF's functional role is limited by an increased complexity of network circuitry due to a significant expansion of TF families. Here, we present the construction of a comprehensive collection of Arabidopsis TFs developed for TF-centered approaches aimed at uncovering TFs and their biological functions. We leveraged this collection to implement a high throughput DNA binding assay that identified direct regulators of a key clock gene called CCA1. TFs identified here provide molecular links between different plant signaling modules and the circadian clock. The resources introduced in this work will significantly contribute to better understand the transcriptional regulatory landscape of plant genomes.

P38 Peroxidase activity in healthy or *Cercospora kikuchii*-infected soybean seeds. *Bruno Guilherme Torres Licursi Vieira[1], Allan Bruce Downie[1], Roberval Daiton Vieira[2]. 1 - University of Kentucky, Plant Science Building, 1405 Veteran\'s Drive Lexington, Kentucky 40546-0312; 2 - UNESP - Univ Estadual Paulista, Campus of Jaboticabal, Department of Crop Production, Via de acesso Professor Paulo Donato Castellane, s/n, 14884-900, Jaboticabal, SP, Brazil.*

High-temperature at the milky stage of grain-filling affects rice yield and quality primarily by altering the composition of starch and storage proteins of the caryopsis. So far, the majority of high-temperature research has focused on studying the effects of simultaneous increase in day and nighttime temperatures on grain quality. However, recent climate change and associated yield data suggest that elevated nighttime temperature specifically and dramatically reduces head-rice yield (HRY), grain quality in terms of chalkiness and other cooking qualities. In addition, rice cultivars have been reported to show genetic variability for HRY and chalkiness in response to high nighttime temperature. Our study aims at identifying the differentially regulated genes in contrasting rice cultivars, which differ in rice grain quality under high nighttime temperature. Cultivars Cypress and Bengal which show less reduction in HRY in comparison to LaGrue and M204, along with High Yield Rice (HYR, a regulatory gene) overexpression lines with better grain quality under high-temperature and its untransformed control Nipponbare, were exposed to high nighttime temperature of 28°C and control nighttime temperature of 22°C by keeping the day temperature constant at 30°C in a controlled growth chamber. Caryopsis from all six cultivars at R6 stage was harvested and comprehensive transcriptome profiling was performed by RNA-sequencing. The differentially expressed genes between control and high nighttime temperature within a cultivar as well as between good and poor quality cultivars will be presented. The candidate genes which are differentially expressed between good and poor quality cultivars will be used in the molecular breeding programs.

P39 *Rhodobacter capsulatus*: An autotrophic platform for production of triterpene fuels. *S. Eric Nybo[1], Nymul Khan[2], Dr. Alex Rajangam[2], Dr. Wayne R. Curtis[2], Dr. Joseph Chappell[1]. 1 Department of Chemical Engineering, The Pennsylvania State University, University Park, PA; 2 Department of Pharmaceutical Sciences, University of Kentucky, Lexington, KY.*

Rhodobacter capsulatus is a metabolically diverse phototrophic, gram negative bacterium. We have metabolically engineered this microorganism to produce botryococcene and squalene triterpenes by modulating carbon flux through its resident methyl erythritol phosphate (MEP) pathway. We have built several triterpene expression cassettes that strengthen accumulation of the fifteen carbon intermediate, farnesyl pyrophosphate (FPP). This C15 compound is then condensed into botryococcene (e.g. by botryococcene synthase, BS) or squalene (e.g. by squalene synthase, SS) in an NAD(P)H-dependent fashion. Expression of several MEP pathway components, including dxs, idi, and fps resulted in the accumulation of 7 mg L-1 squalene and 2 mg L-1 botryococcene in R. capsulatus engineered lines under heterotrophic growth conditions.

P40 Comprehensive expression profiling of rice grain quality-related genes in contrasting cultivars under high nighttime temperature. *Ramegowda Venkategowda, Subodh Srivastava, Karen Moldenhauer, Paul Counce, Andy Pereira*, 115 Plant Science Building, University of Arkansas, Fayetteville, AR.*

High-temperature at the milky stage of grain-filling affects rice yield and quality primarily by altering the composition of starch and storage proteins of the caryopsis. So far, the majority of high-temperature research has focused on studying the effects of simultaneous increase in day and nighttime temperatures on grain quality. However, recent climate change and associated yield data suggest that elevated nighttime temperature specifically and dramatically reduces head-rice yield (HRY), grain quality in terms of chalkiness and other cooking qualities. In addition, rice cultivars have been reported to show genetic variability for HRY and chalkiness in response to high nighttime temperature. Our study aims at identifying the differentially regulated genes in contrasting rice cultivars, which differ in rice grain quality under high nighttime temperature. Cultivars Cypress and Bengal which show less reduction in HRY in comparison to LaGrue and M204, along with High Yield Rice (HYR, a regulatory gene) overexpression lines with better grain quality under high-temperature and its untransformed control Nipponbare, were exposed to high nighttime temperature of 28°C and control nighttime temperature of 22°C by keeping the day temperature constant at 30°C in a controlled growth chamber. Caryopsis from all six cultivars at R6 stage was harvested and comprehensive transcriptome profiling was performed by RNA-sequencing. The differentially expressed genes between control and high nighttime temperature within a cultivar as well as between good and poor quality cultivars will be presented. The candidate genes which are differentially expressed between good and poor quality cultivars will be used in the molecular breeding programs.

P41 Soybean NDR1-like proteins bind pathogen effectors and regulate resistance signaling. *MB Shine, Devarshi Selote, Guillaume Robin, Aardra Kachroo. Department of Plant Pathology, University of Kentucky, Lexington, KY.*

NON-RACE-SPECIFIC DISEASE RESISTANCE1 (NDR1), a plasma membrane-localized protein, plays an essential role in signaling derived from several resistance (R) proteins. We have identified two NDR1-like sequences (GmNDR1a, b), which regulate resistance derived from three R loci in soybean. Functional characterization of GmNDR1a and b, which share > 93% nucleotide identity was carried out using gene silencing. We find that GmNDR1a and b are required for Rpg3-derived resistance against Pseudomonas syringae (Psg) expressing AvrB2, and also partially regulate Rpg1-b and Rpg4 derived resistance against Psg avrB and Psg avrD1, respectively. Importantly, we show that the GmNDR1 protein interact with the AvrB2 and AvrD1 effectors and regulate their virulence function in the absence of the respective cognate R loci. Thus, Psg avrB2 and Psg avrD1 exhibit increased virulence in GmNDR1-silenced rpg3 rpg4 plants, even though these strains are not normally more virulent on rpg3 rpg4 plants. GmNDR1 proteins also interact with GmRIN4 and contribute to activation of the Rpg1-b protein. The role of GmNDR1 proteins in Rpg1-b activation, their direct interactions with AvrB2/ AvrD1, and a putative role in the virulence activities of Avr effectors, provides the first experimental evidence in transducing extracellular pathogen-derived signals.

P42 Identification of novel regulators of a calcium calmodulin dependent protein kinase (DMI3) controlling plant-microbe symbioses. *Arijit Mukherjee, Jonathan Pennington, Aakash Rana, Shane Radford. Department of Biology, University of Central Arkansas*

Acquisition of nitrogen from the soil is a major issue for sustainable agriculture. This has led to an increasing dependence on nitrogen-based fertilizers. In order to minimize the economic, ecological and health hazards associated with such treatments, we need to take advantage of beneficial plant-microbe interactions like root nodule symbiosis. Plants from the legume family have the ability to form a very efficient nitrogen-fixing symbiosis with soil bacteria, rhizobia. Unfortunately, very few efficient nitrogen-fixing symbioses exist outside of the legume family. Therefore, in order to engineer efficient nitrogen fixation in non-leguminous crops, we first need to better understand the mechanisms underlying the establishment of the rhizobium-legume symbiosis. Genetic studies in model legumes, such as *Medicago truncatula*, allowed the identification of plant genes that are required for the establishment of the

rhizobium-legume symbiosis. Among them, a nuclear calcium and calmodulin-dependent kinase (CCaMK), called DMI3, probably acts as decoder of calcium spiking and is also involved in a negative feedback mechanism. Interestingly, activation of DMI3 is not only required, but also sufficient for root nodule development and formation of infection structures. DMI3 interacts with and phosphorylates IPD3/CYCLOPS that regulates transcription. Mutants in DMI3 and IPD3 are affected in most responses to rhizobial Nod factors and are unable to form nodules or to induce nodulin gene expression (e.g. ENOD11) in presence of rhizobia. DMI3, IPD3 and other genes are also required for the establishment of mycorrhizal symbiosis, which led to the concept of a shared "common symbiotic pathway" between these two major endosymbioses. Interestingly, DMI3 and IPD3 are highly conserved in non-legumes and their presence correlates very well with the ability of plants to develop mycorrhizal symbiosis. Supporting this evolutionary hypothesis, DMI3, IPD3 and other genes of this pathway are required for mycorrhizal symbiosis in rice. The presence of these genes in cereals is exciting since it means that the building blocks for intracellular accommodation of microbes are already present. This makes the prospect of engineering nitrogen fixation in cereals extremely promising. However, our knowledge of the pathway is still fragmentary. Our project focuses on identifying new regulators of DMI3 and understanding the underlying mechanisms of regulation. We intend to identify genetic loci regulating DMI3 using a suppressor screen. This classical but powerful genetic tool has not yet been exploited to dissect the common symbiotic pathway. Thus, this approach will identify pathway components that have been missed in conventional screens and generate new knowledge about the molecular basis of symbiotic relationships that could be applied to other crops.

P43 Elucidating differences in gene expression of *Epichloe coenophiala* endophytic fungus in reproductive vs. vegetative tissues of tall fescue (*Lolium arundinaceum*). *Padmaja Nagabhyru[1], Randy D Dinkins[2], Christopher L Schardl[3] 1.Department of Plant Pathology, University of Kentucky, Lexington, Kentucky 40546-0312; 2.USDA-ARS, Forage-Animal Production Research Unit, Lexington, Kentucky 40546-009; 3.Department of Plant Pathology, University of Kentucky, Lexington, Kentucky.*

The tall fescue – *Epichloe coenophiala* symbiotic system is the most extensively studied of any grass-microbe symbiosis, mainly because of its economic importance worldwide. This is also an extraordinarily stable and mutualistic symbiosis where the endophyte colonizes both vegetative and reproductive tissues of the plant. The symbiosis is constitutive and is maintained in host plant communities by seed transmission from maternal plants to progeny without causing negative effects to the host seed development. So, to elucidate this important seed transmission process and the genes that may be involved in this hereditary symbiosis, we carried out gene expression studies using "mRNA-seq" with a next-generation sequencing system. For the study, we chose three clone pairs of tall fescue with and without symbiotic endophyte, and analyzed the differential expression of both plant and fungal genes in vegetative (pseudostem) and reproductive (ovaries) tissues. The transcriptome data obtained from HiSeq was analyzed using CLCbio Genome Workbench. Transcriptome analysis of endophyte-infected versus endophyte-free tall fescue ovaries showed that expression of plant genes was not significantly different in response to the symbiont compared to the corresponding uninfected plant clones. In contrast, endophyte gene expression was dramatically different in reproductive compared to vegetative plant tissues. We identified 226 genes that were up-regulated in pseudostem tissues compared to the ovaries, largely comprising the alkaloid genes and sugar and amino acid transporter genes. In ovaries compared to pseudostem, 47 genes were up-regulated by more than 5-fold, out of which eight of the up-regulated genes were for heat-shock proteins (7-18-fold higher), suggesting a developmental switch in symbiotic fungus that is similar to heat shock in these reproductive tissues. It was also interesting that, compared to plant mRNA reads, total fungal mRNA counts were about an order of magnitude lower in ovaries than in pseudostems. We are currently testing if this difference in number of expressed genes in these tissues correlates with plant to fungal biomass ratio by using qPCR to quantify the fungus relative to plant genomic DNA. Overall, these results suggest the possibility that tall fescue plants may capture and manipulate beneficial symbionts in their reproductive tissues by reprogramming endophyte gene expression.

P44 Physiological measures and plant grafting as means to characterize stress in salt-susceptible and salt-tolerant soybeans. *Lacy D. Nelson, Alma G. Laney, Kenneth L. Korth. University of Arkansas, Fayetteville, AR.*

Increases in saline soils are becoming a problem worldwide for agriculture. Soybean, *Glycine max*, is an important agronomic crop and is generally salt sensitive, though the degree of sensitivity varies by genotype. Cultivars that are able to tolerate saline conditions partially exclude chloride from transport to foliar tissues and are termed chloride 'excluders', whereas those that do not are called chloride 'includers'. To characterize biological differences of soybeans subjected to salt stress, physiological responses were measured in excluder and includer cultivars. Photosynthetic rates and phenotypic ratings were measured after daily flooding with either 100 mM NaCl or water. A chloride excluder, cv. Manokin, exhibited significantly higher rates of photosynthesis than the includer, cv. Clark, as measured by CO2 gas exchange prior to visible salt injury. Likewise, the excluder exhibited less visible damage at

later stages. Transcript accumulation, as measured by reverse-transcription PCR, showed that several genes are expressed at high levels in response to salt treatment in roots and/or leaves, and this can vary by cultivar. To assess the role of plant rootstock in soybean salt tolerance, an excluder, cv. Osage, and an includer, cv. Glenn, were reciprocally grafted. Once the graft union had healed, plants were treated as before. As in the previous experiment, visible salt damage was lower and photosynthetic rates were higher in grafted plants that had the chloride excluder as rootstock. Future studies should focus on the mechanisms of salt tolerance in soybean roots.

P45 Efficient excision of FRT-flanked selectable marker gene from the *Oryza sativa* genome by FLPe activity. *Jaimie Underwood, Linh D. Nguyen[1], Soumen Nandy[1], and Vibha Srivastava [1,2]. 1 Department of Crop, Soil, & Environmental Sciences, and 2 Department of Horticulture, University of Arkansas, Fayetteville, AR 72701, USA*

Transgenic clones are isolated with the help of selectable marker genes; however, their presence in transgenic crops is not desirable, emphasizing the need for efficient methods of DNA excision from plant genomes. The excision of the selectable marker genes can be carried out by the use of site-specific recombination systems. Cre-lox recombination system has been the most efficient system to date, whereas FLP-FRT recombination system has been found to be inefficient in marker excision resulting in chimeric plants that lack the marker-free locus in progeny. This poor efficiency is most likely explained by below-optimal activity of FLP recombinase in plant cells. Previous research had found transient FLPe expression, an improved FLP protein, was at least three-fold higher on genomic targets when compared to the original FLP protein, FLPwt. In the present study, the recombination efficiency of FLPe was evaluated on a specific target containing a FRT-flanked marker gene, and plants were crossed with a recombination tester line to analyze marker excision in F1 and F2 progeny. FLPe showed a greater recombinase efficiency for FLP-FRT mediated marker excision in rice when compared to FLPwt.

P46 "**Drying oil production in transgenic plants: optimization of codon usage, epitope tagging, and promoter choice**." *Jay Shockey[1], Edgar Cahoon[2], Catherine Mason[1], and John Dyer[3] - [1]USDA-ARS, Southern Regional Research Center, New Orleans, LA, USA; 2[1]Center for Plant Science Innovation and Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, Nebraska; [3]USDA-ARS, U.S. Arid-Land Agricultural Research Center, Maricopa, AZ, USA*

The tung tree (*Vernicia* sp.) is a dying breed in the American Gulf South. Once a thriving crop, raised for the high-value drying oils found in its seeds, the tung industry has been hit hard by hurricanes and shifting global economics. Our lab strives to engineer tung-like drying oils in other oilseed species with more amenable agronomic characteristics. Many genes from the tung oil biosynthetic pathway have been cloned: the most crucial is FADX, a diverged FAD2-like enzyme that catalyzes the formation of α-eleostearic acid from linoleic acid. Preliminary analysis of transgenic *A. thaliana* plants expressing various *FADX* genes showed that tung *FADX*, driven by the French bean phaseolin promoter produced only about 6-8% eleostearic acid, far below the 80% level found in tung seed oils and the 40% goal for transgenic oils. It is possible that differences in promoter strength and timing during *A. thaliana* seed development may have affected the outcomes. Determination of optimal expression conditions for tung FADX will be essential for a successful engineering strategy to produce tung-like drying oils in transgenic systems. Optimal conditions for FADX expression were determined by comparing a variety of variables, including three different selection methods, three different epitope tags, and five different seed-specific promoters. The results of these studies, and a discussion of other factors that may affect transgenic drying oil production are presented here.

P47 Metabolic engineering of photorespiratory bypass pathways to enhance novel biofuel production in transgenic plants. *Sheba Goklany[1], Youngkyoung Kim[2], Hong Ma[2], Yi Cheng Liu[2], Eiji Takahashi[3], Don Ort[3], Joshua Yuan[2], and Joe Chappell[1] [1]Pharmaceutical Sciences Dept, University of Kentucky, [2]Plant Pathology & Microbiology Dept, University of Texas A&M, [3]Plant Biology Dept, University of Illinois at Urbana-Champaign, IL.*

Industrialization and the global population growth rate have placed a tremendous burden on food, energy, and other natural resources, including land and water. The current world population of ~7.1 billion is expected to increase to 9.6 billion by 2050, while the global energy demand is anticipated to increase more than 3-fold over the same time period. In contrast, global oil production will peak before 2030, leading to diminishing supply and increasing cost. Hence, there is a clear and urgent need to harness the full potential of sustainable energy sources and convert these efficiently to resources required for human and industrial applications. The current project is addressing this worldwide challenge by focusing on the development of plant systems to capture sunlight energy into more direct biofuels rather than sugars or fatty acids requiring extensive downstream processing. Our work is also focused on remediating a longstanding inefficiency in photosynthesis, the process known as photorespiration. About 20% of the time,

photorespiration rather than photosynthesis occurs wherein instead of CO_2 being fixed and converted to precursors for carbohydrate biosynthesis, O_2 is condensed with RuBP to yield glycolate. The glycolate is subsequently decarboxylated in an energy intensive process resulting in the net loss of one CO_2 returned to the atmosphere. While attempts to engineer the oxygenation reaction out of the RuBP Carboxylase/Oxygenase have been unsuccessful, attempts to recycle carbon in the photorespiratory glycolate have shown some promise. We too have pursued the latter strategy as illustrated in Fig. 1 with the intent of recycling the carbon into the production of high-value, triterpene biofuels. Our focus on production of linear, branched-chain hydrocarbon triterpenes is driven by the ease of their catalytic cracking into all class of fuels: gasoline, diesel, and jet fuel. To evaluate various photorespiratory constructs, we first generated transgenic lines engineered for novel triterpene production in the chloroplast (G1 line). These transgenic lines were then re-engineered with 3 different constructs as depicted in Fig. 1, and the regenerated lines evaluated for triterpene accumulation by standard GC-MS analyses and for the operation of the putative bypass pathways by measuring the incorporation of radioactivity from glycolate into triterpenes. pT1 lines containing a partial bypass pathway showed a slight increase (33%) in the glycolate incorporation into triterpenes as well as triterpene accumulation (44%) compared to the G1 control line. These levels were further enhanced in the pT3 lines incorporating a complete bypass pathway where higher levels of glycolate incorporation into triterpenes (500%) correlated with higher triterpene accumulation (97%) compared to the G1 controls. The highest triterpene levels (~2500 µg/g FW) were obtained in the pT5 lines, where glycolate incorporation and triterpene accumulation were enhanced by 1200% and 220%, respectively, compared to G1 controls. Our data provides evidence for engineering known and novel photorespiratory bypass pathways in plants for efficient biofuel production. These metabolic engineering strategies demonstrate the use of sustainable energy to meet the growing need for fuels and renewable resources.