P1. Investigation of the Transcription and Splicing of *RPB4* mRNA in Maize and *Arabidopsis*

Maggie Campbell*, *Diane Janick-Buckner, Brent Buckner Department of Biology, Truman State University, Kirksville, MO, USA*

RPB4 is one of twelve subunits that interact to form RNA Polymerase II. In Arabidopsis, plants that are homozygous for a T-DNA-induced exonic mutation within the gene for RPB4 display abnormally small "crinkled" leaves. We have designated this phenotype as the *CRINKLE1*. The orthologous maize mutant displays a phenotype with characteristics that include narrow leaf blades and upward rolling along the leaf margin. Quantitative RT-PCR was used to investigate *RPB4* mRNA accumulation and intron splicing. In maize the accumulation of fully spliced *RPB4* mRNA was reduced in mutant plants. Using primers proximal to the T-DNA insertion, no significant difference between wild-type and *CRINKLE1* Arabidopsis plants was detected in the amount of *RPB4* mRNA produced, however, the *CRINKLE1* mutants accumulate approximately three times as much unspliced *RPB4* mRNA distal to the T-DNA insertion. This suggests that the T-DNA insertion in this gene does not influence transcription initiation but does influence splicing.

P2. Histological Analysis of Rosette Leaves in *Arabidopsis thaliana crinkle* Mutant

Tingting Hua*, *Diane Janick-Buckner, Brent Buckner Department of Biology, Truman State University, Kirksville, MO, USA*

Leaf curling is an important trait exhibited by various plants in response to stress conditions such as dehydration, temperature changes and UV light. Multiple genes controlling cell development modulate this response. We have examined a novel Arabidopsis leaf mutant, *crinkle1*, which develops small leaves and a severe curled leaf phenotype. Environmental SEM and histological analysis of this mutant demonstrate abnormal stomata density and patterning when compared to the wild-type sibling leaves. The trichomes on the *crinkle1* leaves are also morphologically altered, exhibiting an abnormal number of spikes. Analysis of paraffin-embedded sections reveals that mutant leaves are thinner and comprised of cells with abnormal morphology when compared to wild-type. Thus, the *crinkle1* gene is essential for normal leaf development and growth.

P3. Expression of Lonesome Highway Paralogs in Maize Inbred Lines

Sarah Mislan*, *Brent Buckner, Diane Janick-Buckner Department of Biology, Truman State University, Kirksville, MO, USA*

The *lonesome highway* gene in Arabidopsis is involved in regulating root development and controlling cell fate decisions during xylem development. We have identified two paralogs of the *lonesome highway* gene in the maize genome: *ZmLWHA* and *ZmLHWB*. Using quantitative RT-PCR, the expression levels of *ZmLHWA* and *ZmLHWB* paralogs from above-ground plant tissue were examined in nine inbred maize lines. Interestingly, these maize lines showed different ratios of *ZmLWHA* to *ZmLHWB* expression. We measured the expression levels of both paralogs in additional tissues for two of these inbred lines. Expression of the *ZmLWH* paralogs was higher in the shoot and root tip than root tissue; additionally, the paralogs are differentially expressed in these tissues.

P4. Molecular Investigations of the *RPB4* Gene in the *Arabidopsis CRINKLE1* Leaf-Development Mutant

Jordyn Williams*, *Diane Janick-Buckner, Brent Buckner Department of Biology, Truman State University, Kirksville, MO, USA*

RNA Polymerase II (RNA Pol II) is a 12-subunit protein complex that is responsible for mRNA transcription. We performed a reverse genetic analysis in *Arabidopsis thaliana* for the RNA Pol II subunit

4 gene (*RPB4*). A T-DNA insertion in the 5' boundary of the fifth exon of *RPB4* cosegregated with plants that were short in stature and developed small, crinkled leaves (designated *CRINKLE1* or *CNK1*). Analysis of both genomic and mRNA *RPB4* sequences suggests that the insertion is complex and may affect splicing of *RPB4* mRNA. Our analysis of wild type *RPB4* mRNA suggests that unspliced transcripts of this gene normally accumulate in leaves. Interestingly, a mutation in the maize ortholog of *RPB4* produces a narrow, upward-rolling leaf phenotype similar to that of *CNK1*. This suggests that the involvement of the *RPB4* gene in leaf development is evolutionarily conserved.

P5. Unlocking the Secrets of Plant Evolution: A Role for Homeodomain Transcription Factors

Vaithish Velazhahan[1]*, *Charles F. Delwiche[2], Jocelyn K.C. Rose[3], David S. Domozych[4], Kathrin Schrick[1] [1]Division of Biology and Ecological Genomics Institute, Kansas State University [2]Department of Cell Biology and Molecular Genetics, University of Maryland ,[3]Plant Biology Section, School of Integrative Plant Science, Cornell University*

Over 450 million years ago land plants emerged from freshwater green algae of the charophycean lineage. The transition from aquatic to terrestrial environments was aided by class III and class IV homeodomain leucine-zipper (HD-Zip) transcription factors that are master regulators of cell-type differentiation in plants. Class III members are associated with evolution of the shoot apical meristem and development of leaves and other lateral organs. Class IV members are linked to innovations in the epidermis protecting against desiccation and UV light, and targeting of various plant-specific metabolic pathways such as flavonoid biosynthesis. Our bioinformatic analyses of transcriptomes from extant charophycean taxa (Charales, Coleochaetales, Klebsormidiales, Zygnemetales) reveals single genes for both class III and IV HD-Zip transcription factors, in contrast to multi-gene families in land plant genomes. Expressed genes were also discovered for enzymes of polyphenolic secondary metabolite pathways hypothesized to have co-evolved with HD-Zip functions. By cloning and characterizing cDNA sequences using the Arabidopsis and Nicotiana benthamiana expression systems we are currently probing the activity and subcellular localization of representative class III and IV HD-Zip transcription factors from charophytes. Additionally, we are using conditional RNAi knockdown in the emerging charophycean model system, Penium margaritaceum, to investigate the functions of these key regulatory proteins and their transcriptional targets. Studying the ancient roles of HD-Zip transcription factors and their associated metabolic circuits is expected to provide new paradigms in the fields of plant developmental and evolutionary biology and bolster our understanding of the origin of land plants.

P6. Altering Triacylglycerol (TAG) Levels During Cold Stress in Arabidopsis Using *dgat* and *pdat* Mutants

Jennifer A. Myers*[1], *Alexandra Meyer[2], Allison C. Barnes[3], Rebecca Roston3, [1]Agronomy and Horticulture Department, University of Nebraska-Lincoln, [2]University of Saint Francis, Fort Wayne; [3]Biochemistry Department University of Nebraska-Lincoln*

The formation of triacylglycerol (TAG) is essential in developing plants as it is the major storage lipids and are a great source of energy for growing seedlings. Two ways that TAGs can be synthesized are with the enzyme acyl-CoA: diacyglycerol acyltransferase (DGAT) or phospholipid: diacylglycerol acyltransferase (PDAT). In Arabidopsis, mutations in the genes that code for these enzymes cause a disruption in TAG synthesis, and the plant has less TAG as a result. Cold and freezing have been shown to increase TAG levels in leaves, but the enzymes that are responsible have yet to be identified. For this experiment, we are investigating mutations in genes encoding three different DGAT enzymes (DGAT1, DGAT2, and DGAT3) and one mutated PDAT enzyme (PDAT1) and have obtained seed lines for each mutation. Homozygous mutant lines have been currently identified for *dgat1* and *pdat1* using PCR and gel electrophoresis, and homozygous mutant lines for *dgat2* and *dgat3* are currently being tested for

homozygosity. Alongside these seed lines are four lines of insertions in genes encoding membrane-bound o-acyl transferases. The effect of these genes on TAG production is unknown, though they may have TAG-forming activity. All of these lines have been confirmed to be homozygous for the insertion. Once all lines have been identified as homozygous for an insertion, the plants will be grown and placed in a cold room in order to test lipids during cold, and frown to test lipids during freezing. The plants will be tested by extracting their lipids and running them on a thin-layer chromatogram followed by a derivatization to fatty acids. The fatty acids will then be run and analyzed by gas chromatography. Each seed line is predicted to affect the amount of lipids during freezing differently and provide more information about the genes and how they can affect freezing in plants, specifically Arabidopsis.

P7. A Putative E3 RING-H2 Ubiquitin Ligase May Confer Resistance to White Marked Tussock (Orgyia leucostigma) Moth Larvae in Poplar Trees

Erik Carlson*, *Samuel Bandi, Steven Ralph, Department of Biology, University of North Dakota*

Forests are a valuable resource, both environmentally as well as economically. Forest trees face threats including insect infestations, which can potentially defoliate entire forests. A forward genetics approach utilizing activation-tagging (AT) was employed to discover potential insect feeding resistance genes in *Populus tremula x P. alba (Pt x Pa)*. Through insect-feeding bioassays using the larvae of *Orgyia leucostigma,* commonly known as White Marked Tussock Moth (WMTM), an AT mutant (*E8-16*) was identified to have resistance to feeding. Screening determined that the 35S enhancer T-DNA insert was positioned adjacently to the gene *10s12800.* Subsequent quantitative PCR analysis determined that *E8-16* had enhanced expression of *10s12800* (~6.94) compared to wild type. Protein sequence analysis revealed that this gene putatively belongs to the E3 RING-H2 ubiquitin ligase family, which is part of the 26S proteasome pathway involved in protein degradation. Ubiquitin assays are in progress to confirm *10s12800*'s putative function. To genetically confirm the role of *10s12800* in providing resistance to WMTM in *E8-16*, we are producing transgenic *Pt x Pa* lines that over-express the *10s12800* gene. In order to do this, a *10s12800* gene construct with a 35S promoter was inserted into the *Pt x Pa* genome through *Agrobacterium tumafaciens*-mediated transformation. Once moved from tissue culture to greenhouse, these lines will be screened for over-expression of *10s12800* and insect-feeding resistance using quantitative PCR and insect-feeding bioassays respectively. This would establish conclusively that *10s12800* over-expression confers insect-feeding resistance in poplar. If resistance is confirmed, *10s12800* could be a target for traditional tree improvement programs.

P8. Towards More Efficient Nitrogen-fixing: Identifying Early Physiological and Molecular Markers of Cheater Rhizobia in Soybean

Brian Kisely*, *Mason Thorstad, Senthil Subramanian, Department of Biology & Microbiology, South Dakota State University*

Soybean (Glycine max) promotes [potentially] the nodulation of multiple different species of Bradyrhizobium in a single root system. Each of these species consists of multiple strains whose nitrogen-fixation efficiencies vary. Normally, soybean will choke off carbon allocation to less efficient nodules, but some Bradyrhizobium have evolved mechanisms to "cheat" or "trick" soybean into continuing the supply. In both cases, soybean wastes energy and nutrients that, in the long run, affect yield. This study is aimed at identifying early physiological and molecular markers for root nodules colonized by Bradyrhizobium spp. of differing nitrogen-fixation efficiencies.

P9. Determination of Function in New P450s Found in Rice

Benjamin Brown*, *Reuben J. Peters, Department of Biochemistry, Biophysics & Molecular Biology, Iowa State University*

The *Oryza sativa* P450 CYP76M subfamily hydroxylate a range of labdane-related diterpenes (LRD). Previously the CYP76M5-8 enzymes were screened against the range of LRDs found in rice using our metabolic engineering system in *Escherichia coli* (E. coli) to determine their functions. It was found that, even though these P450s all have very similar amino acid sequences, individual enzymes in this family have different specificities e.g., CYP76M8 is a more promiscuous enzyme, hydroxylating a wider range of LRDs than CYP76M7. This study focuses on screening two more recently discovered P450s in this same family, CYP76M14 & 17. CYP76M14 was found to have a high specificity for a few LRD, whereas CYP76M17 was more promiscuous. This poster will present the data comparing the functions of these six enzymes.

P10. Aging in Moss: Lipid Peroxidation by ROS Does Not Limit Longevity in Dry Storage

Sternhagen, Tyler*, *Koster, Karen L., Department of Biology, The University of South Dakota*

Desiccation tolerance generally enables the long-term survival of seeds and other organisms; however, longevity is limited by damage that accumulates in dry tissues, even under optimal conditions. We have begun studying the types of damage incurred by dry tissues using the moss *Physcomitrella patens*, in which desiccation tolerance can be induced by treatment with exogenous abscisic acid (ABA). We determined how the longevity of dried, desiccation-tolerant *Physcomitrella* was affected by relative humidity (RH) at 24°C. We observed that only 50% of the moss survived when stored in darkness at 75% RH for one week, however, the survival did not decrease at 32% RH until after four weeks. Reactive oxygen species (ROS) are reported to cause significant damage to dry tissues and thereby limit storage longevity. ROS do this, in part, by oxidizing lipids to form hydroperoxides, which compromise membrane structure. To assess this possibility, we measured lipid hydroperoxide contents in *Physcomitrella* and determined that levels increased approximately 10-fold during dehydration, but did not increase further during four weeks of dried storage at either 32% or 75% RH in the dark. Hydroperoxide content did not differ between moss samples stored at 32% and 75% RH. Membrane damage was evident in moss that did not survive storage; protoplasts failed to re-expand during rehydration, indicating that their plasma membranes were no longer intact. Collectively, these data suggest that lipid hydroperoxides resulting from ROS attack do not accumulate in dried *Physcomitrella* during storage and are not the primary cause of the loss of viability during storage of the dried moss.

P11. Culturing and Isolation of Actinobacteria from Biofilms in Sanford Underground Research Facility (SURF)

Amanpreet Kaur Brar*, *Dr. Dave Bergmann, School of Natural Sciences, Black Hills State University*

The diversity of cultivable bacteria in nacreous “cave silver” biofilms in a hot, humid area of 4850 level of SURF was examined by diluting samples onto growth media with diluted nutrient broth and cassamino acids (CN) and two gelling agents: agar and gellan gum. The thinness of biofilms prevented an accurate estimate of colony forming units per gram (CFU/g), but CFUs were higher on CN media with gellan gum than media with agar. A high level of fungal contamination was observed on both CN agar and CN gellan gum plates, despite the use of the fungicides (cycloheximide and nystatin) in media. A total of 120 isolates with 30 different bacterial colony morphologies were observed, including many filamentous Actinobacteria. In order to further characterize these isolates, Random Amplified Polymorphic DNA analysis and 16S rDNA sequencing will be performed.

P12. Identifying the Downstream Effectors of miRNA 160 in Soybean Root Nodule Development

Spencer Schreier*[1], *Tao Lin[2], Suvo Chakravarty[2], Sen Subramanian[1], [1]Department of Plant Science, South Dakota State University, [2]Department of Chemistry and Biochemistry, South Dakota State University*

Soybean is an excellent candidate for sustainable agriculture due to its production of nutritious, multi-use beans and the ability to form symbiotic organs called root nodules that allow nitrogen fixation. Understanding how root nodules in soybean are formed may be one way of optimizing nitrogen fixation to sustainably enhance soybean yield and transferring the root nodule formation ability to other plants. microRNA 160 (miR160) has been shown to contribute to proper nodule formation by targeting repressor auxin response factor (ARF) transcription factors for proper auxin sensitivity, but the specific downstream effectors of this interaction remain unknown. This project seeks to resolve these downstream effectors by evaluating the cellular, spatiotemporal, and DNA binding activity of a key target of miR160, ARF 16-2 by confocal microscopy, DamID-Seq, and protein-DNA binding assay. Confocal microscopy of 16-2 promoter:GUS or tdT fusions show that ARF 16-2 promoter is active in dividing and differentiating tissues such as primary root tips, lateral root primordium, and emerging nodules. In addition, 16-2 promoter:GUS or tdT fusions show that this promoter is active in the parenchyma and root stele of mature nodules. Interestingly, 16-2 promoter:16-2 CDS:tdT translational fusions show a reduction of tdT signal in mature nodule tissues, underscoring post-transcriptional regulation of 16-2, potentially by miR 160 during nodule maturation. When genomic binding profile of 16-2 is identified and evaluated, we expect to identify key downstream genes affected by its activity during nodule development.

P13. Optimizing the use of the microalga Monoraphidium sp. Dek19 for phycoremediation of wastewater

Adam Hage*, *Gabriel Holbrook, Department of Biological Sciences, Northern Illinois University*

Microalgae have the potential to become an important additional step for wastewater treatment. The phycoremediation capabilities of microalgae offer an attractive method of dealing with eutrophication. Harvesting of the algae for biodiesel production through lipid extraction and transesterification provides a useful co-product for the spent algal biomass. In order to implement this system into an existing wastewater treatment facility, several aspects of the aquaculture system still need to be addressed. *Monoraphidium* sp. Dek19 is a microalga indigenous to the Midwest USA and capable of growing at low light (30 μmol photons m -2s -1) and low temperatures (10ºC). These conditions are similar to that of a typical outdoor wastewater treatment tank suggesting that *Monoraphidium* may be a suitable species for implementation. The current goals are to 1) Identify the minimum aeration periods needed for sufficient *Monoraphidium* growth in a lab-based wastewater environment and compare those to the aeration capabilities of a common wastewater treatment facility. 2) Determine the consequences of a periodic rather than constant aeration regimen on *Monoraphidium* growth and phycoremediation of pollutants in effluent. Understanding of these aeration parameters is necessary to achieve high algal biomass yields and lower the operating costs for algal treatment steps in a wastewater treatment facility.

P14. Mutagenesis of Genes Associated with Seed Dormancy in Rice (Oryza sativa L.) Using Two CRISPR/Cas9 Multiplex Systems

Alexander Kena*[1]*, Heng Ye[2], Jiuhuan Feng[1], Xingyou Gu[1], [1]Department of Plant Science, South Dakota State University, [2]Division of Plant Sciences, University of Missouri*

Seed dormancy (SD) is an adaptive trait controlled collectively by multiple genes or quantitative trait loci (QTL). The SD QTL *SD7-1*, *SD7-2*, *SD12a*, *SD12b* and *SD12c* were map-based cloned and the QTL, *SD4* was collocated with *Bh4* for black hull color in weedy rice. This research was focused on functional

analysis of six genes associated with SD using CRISPR/Cas9-mediated mutagenesis. Two CRISPR/Cas9 multiplex constructs, which contain 6 or 12 single guide RNAs targeting one site and two sites, respectively, in each of the six target genes, were delivered to the *cv*. Nipponbare using an *Agrobacterium*-mediated transformation system. DNA segments of ~400 bp, encompassing the mutated sites of target genes, were sequenced from T0 plants, and sequences aligned against the Nipponbare genome sequence to decode mutant alleles. Mutation rate for five of the six target genes averaged 79% and 86%, respectively, for the one-target-site and two-target-site multiplex systems. The efficiency for simultaneous editing of target sites for five genes among the T0 plants assayed, was higher in the two-target-site multiplex system (79%) than the one-target-site multiplex system (59%). Of the mutations identified for the one-target-site multiplex system, 61% were deletions, 33% insertions and 5% substitutions, and were classified into the homozygous, heterozygous and biallelic types. These results demonstrate that, the two CRISPR/Cas9 multiplex constructs mutated the target genes, but the efficiency for simultaneous editing of multiple genes could be enhanced by targeting more than one site of target genes. Research is being conducted to evaluate phenotypic effects of the mutants in the genetic background of weedy rice and to develop a transgene-mitigating strategy to reduce the risk of gene flow from genetically modified crops to their wild/weed relatives.

P15. Identification of New Sources of Resistance to Soybean Aphids (*Aphis glycines* Matsumura)

Martha Ibore*[1], *Jessica D. Hohenstein[1], Asheesh K. Singh[1]. Gustavo C. MacIntosh[1], [1]Roy J. Carver Department of Biochemistry, Biophysics and Molecular Biology, [2]Department of Agronomy, Iowa State University, Ames, IA.*

Soybean aphids are phloem feeding insect pests of soybean. Aphids diverge plant assimilates for their nutrition and growth at the expense of host plants, causing yield losses of up to 50% especially in susceptible soybean varieties. One of the management options for soybean aphids is the cultivation of resistant soybean varieties. Aphid resistance in soybean is conferred by Resistance to *Aphis glycines* (*Rag*) genes and in the United States five *Rag* genes (*Rag1* to *Rag5*) have been identified to date. Although host plant resistance is an effective management strategy against aphids, aphid biotypes that can colonize resistant soybean have been discovered. The presence of aphid biotypes that can survive on aphid-resistant soybean indicates the need to identify more new and durable sources of aphid resistance. To identify new sources of aphid resistance for the Midwest, specifically Iowa, 145 soybean accessions in maturity group I were obtained from a diverse USDA gene bank collection and screened for resistance to biotype 1 of soybean aphids using choice tests. There was a significant difference in aphid populations among the 145 soybean lines tested (P value = 0). Aphid numbers and damage symptoms were used to assign respective scores (scale of 1 to 6) to each plant and the means were used to group the lines as resistant, moderately resistant or susceptible. From this panel, 4 soybean lines were resistant and 4 had moderate resistance to biotype 1 of soybean aphids (all had mean scores of $\leq$3.9). Additionally, their plant and aphid phenotypes were similar to the resistant checks. Future studies will involve utilization of the phenotypic data to identify candidate genes for aphid resistance in the resistant soybean lines using genome-wide association studies (GWAS).

P16. Loss of Function of RNS2, a Housekeeping RNase T2 Enzyme, Causes Alterations in Cellular Homeostasis and Growth in *Arabidopsis*

***Stephanie Morriss*[1]**, *Xiaoyi Liu[2], Brice Floyd[2], Diane Bassham[2], Gustavo C. MacIntosh[1], [1]Roy J. Carver Department of Biochemistry, Biophysics and Molecular Biology, [2]Department of Genetics, Cellular and Developmental Biology, Iowa State University, Ames, IA.*

The T2 family of Ribonucleases is a family of ribonucleases highly conserved across Eukaryotes. This family of ribonucleases has been shown to degrade ribosomal RNA in numerous organisms including Arabidopsis, humans, zebrafish and yeast. While the plant Arabidopsis has five RNase T2 family

members, only one, RNS2, has been shown to be responsible for degradation of ribosomal RNA. Mutant plants without RNS2 activity display loss of cellular homeostasis manifested by constitutive autophagy and vacuolar accumulation of ribosomal RNA. Here, we used metabolite and transcriptome (microarray and RNAseq) analyses to determine the metabolic changes that may be responsible for the cellular phenotype. We show that mutation of *RNS2* results in disruption of energy pathways, indicated by differential gene expression of energy related enzymes including an aldolase, transketolase and glyceraldehyde-3-phosphate dehydrogenase, and by differential accumulation of pentose-phosphate pathway metabolites. We also observed differential expression of expansins and glycosyltransferases in the *rns2* mutants concurrent with larger cell size and larger plants as well as higher water content. In addition, measurements of monosaccharides from the plant cell wall reveal differences between the wild type and mutant cell wall. These results suggest that *rns2* mutants modify carbon flux in order to compensate for lack of ribosomal RNA degradation. These changes have an impact on cellular and morphological phenotypes. Our results illustrate the relevance of RNS2 function in Arabidopsis.

P17. Assessment and Management of Hybrid Aspen Stands (*Populus* x*smithii*) in the Niobrara River Valley of Northwest Nebraska

James M. Robertson*, *Mark D. Dixon, Alex Cahlander-Mooers, Catherine C. Beall, Department of Biology, University of South Dakota, Vermillion SD*

The Niobrara River Valley has long been recognized as an area of great ecological diversity in northern Nebraska. It features a unique mix of eastern and western species, which are often far removed from their native ranges. A taxon of particular interest is *Populus* x*smithii*, a hybrid of quaking aspen (*Populus tremuloides)* and bigtooth aspen (*Populus grandidentata)*. Collections of this hybrid have been taken from several stands in Smith Falls State Park and elsewhere along the federally protected Niobrara National Scenic River. Aspens across the western United States are experiencing decline associated with fire suppression, invasive species, and climate change, known as Sudden Aspen Decline (SAD). Managers at Smith Falls have therefore undertaken efforts to promote recruitment and ensure the success of the aspens; by clearing competitive red cedar (*Juniperus viginiana*) from the stands, and fencing-off small areas to protect young aspen stems from browsing by ungulates. This study assessed the size structure and health of the hybrid aspen stands in Smith Falls State Park, and the effectiveness of efforts to protect aspen saplings (suckers) from browsing. During the 2013 growing season I documented the condition of every standing *P.* x*smithii* trunk in Smith Falls State Park and the adjacent Niobrara Valley Preserve (TNC). I also tagged aspen suckers growing in three habitat types (fenced areas, open areas, and woodpiles created by clearing) throughout the park to compare the vitality of suckers in different habitats over the course of the 2013 growing season and the subsequent winter (2013-2014). I found evidence that SAD is affecting the Smith Falls population, and my results confirm the value of protecting habitat for recruitment in this disjunct population.

P18. The octadecanoid pathway is required for nectar secretion independent of COI1 in *Arabidopsis thaliana*

Anthony J. Schmitt*[1]*, Peter M. Klinkenberg[1], Mengyuan Jia[2], Clay J. Carter[1], [1]Department of Plant Biology, University of Minnesota, St. Paul, MN, [2]Department of Plant Science, Pennsylvania State University, State College, PA*

Over 75% of crop species produce nectar and are dependent on pollinators in order to achieve maximum seed set, yet little is known about the mechanisms regulating nectar secretion. The phytohormone jasmonic acid (JA) is recognized to be involved in several plant processes including development and defense. JA was recently shown to positively influence nectar secretion in both floral and extrafloral nectaries. For example, endogenous JA levels peak in flowers just prior to nectar secretion, but the details

of how JA regulates nectar secretion have yet to be elucidated. We have found that the octadecanoid pathway does indeed play a role in the production and regulation of floral nectar in Arabidopsis. Null alleles for several JA biosynthesis and response genes had significantly reduced amounts of nectar, as well as altered expression of genes known to be involved in nectar production. Surprisingly, a knockout mutant for *12-oxophytodienoate reductase* 3 [(an enzyme further down the JA biosynthetic pathway that reduces 12-oxo phytodienoic acid (OPDA)], produced no nectar in newly opened flowers, but did secrete nectar in older flowers. Furthermore, a similar phenotype was observed in *coi1-1*, a mutant for the JA receptor *COI1*. These observations strongly suggest a role for a JA- and COI1-independent pathway in regulating nectar production in Arabidopsis. Additionally, we also have identified crosstalk between the JA and auxin response pathways in nectaries. Allene oxide synthase (AOS) is an enzyme early on in JA biosynthesis. Interestingly, the nectar-less mutant *aos-2* showed no auxin response in nectaries, but both nectar production and the auxin response was restored upon exogenous JA treatment. Conversely, *coi1-1* displayed no auxin response in nectaries under any circumstance, even in older flowers that produce nectar. Cumulatively, our findings indicate an essential role for the octadecanoid and auxin response pathways independent of COI1 in regulating nectar secretion.

P19. Pennycress nectaries and nectar: molecular dissection and evaluation as a nutritional resource for pollinators

Jason B. Thomas*[1], *Carrie Eberle[2], Matt Thom[2], Frank Forcella[2], M. David Marks[1], Clay J. Carter[1], [1]Department of Plant Biology, University of Minnesota Twin Cities, St. Paul, MN, [2]USDA-ARS, Morris, MN.*

Field pennycress (*Thlaspi arvense*) is being developed as a renewable biodiesel feedstock that provides crucial ecosystem services. The seeds can be converted into fuel for both diesel and jet engines. As a winter annual with a short life cycle, pennycress can be intercropped within corn and soybean rotations, utilizing the 16 million hectares of barren soil in the winter. Thus it is a highly marketable "cash" cover crop that will raise farmers' profits while reducing nutrient leaching and erosion. Pennycress may provide yet another important ecosystem function by serving as a nutritional resource for pollinators. Significantly, pennycress flowers in the early spring before many crops are even planted. Both wild pollinator and domesticated honeybee populations are declining and may benefit from this early-season food source. By understanding pennycress nectar production we may increase its usefulness as a renewable energy source while supporting vulnerable pollinators. Toward this end, pollinator visitation to pennycress flowers and nectar secretion dynamics were investigated, with flies and small bees being primary pollinators. Further, we conducted a transcriptomic analysis of gene expression in pennycress nectaries and identified over 20 orthologs to genes from plant species with known roles in nectary development and function. The morphology and ultrastructure of pennycress nectaries was also found to be unique within the Brassicaceae, with nectaries being located inside the base of petals, rather than intrastaminally. Metabolite analyses indicated that pennycress nectar is hexose-rich, while containing little or no sucrose. We are also currently examining the impacts of differential nectar production in wild and mutant populations on pollinator visitation and yield.

P20. An Ancient Accord Between Plants and Fungi Spells Prosperity for Bioenergy Crops

***Brandon Monier*[1],** *Jose Gonzalez-Hernandez[2], Heike Bücking[1], [1]Department of Biology and Microbiology, South Dakota State University, [2]Department of Plant Science, South Dakota State University*

The arbuscular mycorrhizal (AM) symbiosis is a mutualistic relationship between fungi of the phylum Glomeromycota and the majority of terrestrial plant species. This interaction is highly beneficial for plants because it improves the nutrient acquisition and resistance against abiotic (e.g. salinity, heavy

metals) and biotic stresses. We investigated the impact of AM fungi on biomass production of *Spartina pectinata*, a prospective bioenergy crop to determine the potential of AM interactions to maximize biomass production on marginal lands. Seven genotypes of *S. pectinata* were analyzed for their traits pertaining to nitrogen and phosphate acquisition, above and below ground biomass, and AM colonization rates. The results demonstrate that there is a high genotypic variability in *S. pectinata* in biomass, nutrient uptake potential, and its response towards the AM fungal species, *Rhizophagus irregularis*. Under nutrient limiting conditions, we observed a significant positive mycorrhizal responsiveness of some genotypes, while other genotypes were not affected by the AM colonization and showed a neutral response. These findings demonstrate that AM fungal species can serve as bio-fertilizers and increase the overall biomass production of *S. pectinata* on marginal lands. Future endeavors of this project will include the identification of the AM community composition on marginal lands by metagenomics, and the analysis of the plant transcriptome to identify markers of mycorrhizal responsiveness for breeding programs.

P21. The Flood of 2011: Effects of a Large Infrequent Disturbance on Riparian Forest Vegetation along the Missouri River

Christopher J. Boever*[1], *Mark D. Dixon[1], W. Carter Johnson[2], Michael L. Scott[3], [1]Department. of Biology, University of South Dakota, [2]Department. Natural Resources Management, South Dakota State University, [3]Department. Watershed Sciences, Utah State University*

In 2011, a large, long-duration flood occurred on the Missouri River following six decades of regulated flow impacts on riparian forests. The purpose of this study was to evaluate the effects of the flood on the riparian forest ecosystem. In 2012, forest vegetation on 168 previously sampled study sites was resampled on five floodplain segments between Montana and Missouri, with 86 sites resampled in 2013-2014. Live stem densities were compared using repeated measures ANOVA to examine (1) the initial effects (pre-flood to 2012) of the flood on stem density of trees and shrubs, (2) post-flood (2012-2014) changes in woody stem density, and (3) species-level responses for cottonwood (*Populus deltoides* W. Bartram ex Marshall), eastern red cedar (*Juniperus virginiana* L.), and Russian olive (*Elaeagnus angustifolia* L.). Live tree density declined 26-47% across forest age classes and 19-49% across segments from pre-flood to 2012, but did not decline significantly from 2012-2014. Shrub density declined 52-89% across segments with 73-78% declines in the two youngest age classes. Live stem density of shrubs and saplings increased by 42% from 2012-2014. Live tree densities of the three focal tree species (cottonwood, red cedar, Russian olive) declined from pre-flood to 2012, but did not change significantly from 2012-2014. Cottonwood and Russian olive shrub/sapling density, however, showed signs of partial post-flood recovery, increasing from 2012-2014, while red cedar did not. While flooding is an important aspect of floodplain health, the unnaturally long duration of the flood of 2011 led to mixed effects in regards to restoration, with significant mortality of native floodplain trees and shrubs and only limited cottonwood recruitment. The decline in invasive woody species, however, suggests that flooding may be an effective management tool.

P22. Defining connectivity between the chloroplast inner envelope membrane and the thylakoid membrane

David R. Logan*, *Rebecca L. Roston, Department of Biochemistry, University of Nebraska-Lincoln*

Photosynthesis is the foundation of nearly all life on Earth and perhaps life on distant planets. Biogenesis of the photosynthetic thylakoid membrane depends upon the effective import of essential membrane lipids such as monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG), and these species are hypothesized the most abundant lipids in the world. Despite the significance and ubiquity of photosynthesis, the molecular mechanisms by which chloroplast envelopes traffic membrane

lipids when building the thylakoid remains mysterious. Our project is using multiple methods to distinguish the chloroplast inner envelope and thylakoid connectivity mechanisms. First, we plan an artificial tether of these membranes to uncover essential components of membrane contacts that have presumably been lethal during genetic screens. In 2009, this strategy was deployed successfully to identify essential components of an ER-mitochondrial tethering complex. Second, we plan a separation and differential centrifugation of the envelope membranes, using known inhibitors of vesicle fission and fusion, in order to detect missing components of a novel and hypothesized vesicle system within the plastid. Finally, a novel split-superfolder green-fluorescent protein (GFP) system is applied as a unique approach to visualize membrane connectivity in real time. By defining the types of contact occurring between the chloroplast inner envelope and thylakoid membranes, we make important progress toward understanding the chloroplast's nature of existence, and we can suggest ways to make photosynthesis more efficient.

P23. Diversity and Evolution of Disease Resistance Genes in Barley (Hordeum vulgare L.)

***Ethan J. Andersen*[1]*, *Shaukat Ali*[2], *R. Neil Reese*[1], *Yang Yen*[1], *Surendra Neupane*[1], *Madhav P. Nepal*[1], *[1]Department of Biology & Microbiology, South Dakota State University, [2]Department of Plant Science, South Dakota State University*

Plant disease resistance genes (R-genes) play a critical role in the defense response to pathogens. Barley is one of the most important cereal crops, having a genome recently made available, for which the diversity and evolution of R-genes are not well-understood. The main objectives of this research were to conduct a genome-wide identification of barley Coiled-coil, Nucleotide Binding Site, and Leucine Rich Repeat (CNL) genes and elucidate their evolutionary history. We employed a Hidden Markov Model using 52 *Arabidopsis thaliana* CNL reference sequences and analyzed for phylogenetic relationships, structural variation, and gene clustering. We identified 175 barley CNL genes nested into three clades, showing a) evidence of an expansion of the CNL-C clade, primarily due to tandem duplications, b) very few members of clade CNL-A and CNL-B, and c) a complete absence of CNL-D clade. Our results also showed that several of previously identified mildew locus A (*MLA*) genes may be allelic variants of two barley CNL genes, MLOC_66581 and MLOC_10425, which respond to powdery mildew. Approximately 23% of the barley CNL genes formed 15 gene clusters located in the extra-pericentromeric regions on six of the seven chromosomes; over half of the clusters were located on chromosomes 1H and 7H. Higher average numbers of exons and multiple splice variants in barley relative to that in *Arabidopsis* and rice may have contributed to a diversification of the CNL-C members. These results will help us understand the evolution of R-genes with potential implications for developing durable resistance in barley cultivars.

P24. Effect of drought on herbivore-induced plant gene expression: Population comparison for range limit inferences

***Gunbharpur Singh Gill*[1], *Riston Haugen*[1], *Steven L. Matzner*[2], *Abdelali Barakat*[3], *David H. Siemens*[1], *[1]Integrative Genomics Program and Department of Biology, Black Hills State University, [2]Department of Biology, Augustana University, [3]Department of Biology, University of South Dakota*

Low elevation "trailing edge" range margin populations typically face increases in both abiotic and biotic stressors that may contribute to range limit development. We hypothesize that selection may act on ABA and JA signaling pathways for more stable expression needed for range expansion, but that antagonistic crosstalk prevents their simultaneous co-option. To test this hypothesis, we compared high and low elevation populations of *Boechera stricta* that have diverged for constitutive levels of glucosinolate defenses and root:shoot ratios; neither population has high levels of both traits. If constraints imposed by antagonistic signaling underlies this divergence, one would predict that high constitutive levels of traits would coincide with lower plasticity. To test this prediction, we compared the

genetically diverged populations in a double challenge drought-herbivory growth chamber experiment. Although a glucosinolate defense response to the generalist insect herbivore *Spodoptera exigua* was attenuated under drought conditions, the plastic defense response did not differ significantly between populations. Similarly, although several potential drought tolerance traits were measured, only stomata aperture behavior, as measured by carbon isotope ratio, was less plastic as predicted in the high elevation population. However, RNAseq results on a small subset of plants indicated differential expression of relevant genes between populations as predicted.

P25. Integrated Organellar and Epigenetic Networks Condition Developmental Reprogramming in the msh1 Mutant

Hardik Kundariya*, *Vikas Shedge, Mon-Ray Shao, Robersy Sanchez, Sally A Mackenzie, Department of Agronomy and Horticulture, University of Nebraska-Lincoln*

MSH1 is a dual targeting protein unique to plants that localizes to mitochondrial and plastid nucleoids and functions in organellar genome stability. A phenotype of developmental reprogramming (MSH1-dr) is observed in a subset of Arabidopsis msh1 mutants. This phenotype is characterized by reduced growth rate and dwarfing, altered leaf morphology, heightened abiotic stress response and delayed flowering. Under short-day conditions, MSH1-dr plants display a perennial growth behavior, with evidence of stem secondary growth, extended juvenility, enhanced branching, aerial rosettes and continuous flowering behavior. Plastid depletion of MSH1 causes heritable, non-genetic changes in development and DNA methylation. While depletion from mitochondria results in 7%–10% of plants altered in leaf morphology, heat tolerance, and mitochondrial genome stability. We investigated the msh1phenotype using hemi-complementation mutants and transgene-null segregants from RNAi suppression lines to sub-compartmentalize MSH1 effects. Crossing these MSH1-dr plants with isogenic wild type produces epi-lines with heritable, enhanced growth vigour. A similarly multifaceted phenotype is produced in other plant species with RNAi suppression of MSH1 expression. We are attempting to learn the genetic networks comprising the organellar versus epigenetic behaviors that underpin the MSH1 effect by both methylome and transcript profile analyses with specialized genetic materials.

P26. Dissecting Plant-Mediated Pest Interactions in Soybean: Systemic Effects of Aphid Infestation

Jessica D. Hohenstein*[1]*, Martha Ibore[1], Michelle A. Graham[2], Asheeh K. Singh[3], Gustavo C. MacIntosh[1], [1]Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, [2]Corn Insects and Crop Genetics Research, USDA-ARS, [3]Department of Agronomy, Iowa State University*

Soybean aphids (SBA) are specialized phloem-feeding insects that cause significant crop damage and yield reduction. Recent studies show that SBA feeding systemically facilitates performance of both intra- and interspecific pests such as root-dwelling parasitic soybean cyst nematodes (SCN). To date, the few molecular studies of SBA infestation focus on locally infested tissues; no molecular data exist that explain aphid-induced signaling between leaves and roots. We hypothesize that foliar SBA feeding produces plant-mediated systemic signaling to roots which in turn, facilitates SCN performance. We used RNA-seq to compare transcriptome changes in leaves and roots during an early (12h) and sustained (7d) foliar SBA infestation in aphid-susceptible plants. Our data indicate a dynamic response across tissue and time; a majority of early response transcripts are repressed in both leaves and roots while the pattern of expression for genes regulated by sustained SBA infestation differs substantially between the two tissues. Early response transcript analysis in roots revealed that several defense-related genes are repressed including cell wall modifying enzymes, salicylic acid signaling-related genes, and multiple negative regulators of ethylene-mediated signaling. *GmERF1*, a transcriptional activator of ethylene-related signaling, is induced in roots suggesting ethylene may play an important role in early aphid-induced

systemic signaling. Sustained foliar SBA feeding repressed some defense-related genes in roots including several 9-Lipoxygenases as well as many disease resistance family proteins that may play a role in defenses against SCN. Our results suggest that SBA produce significant plant-mediated systemic signaling which may facilitate increased SCN performance.

P27. A Potential Non-canonical Route of Fatty Acid Biosynthesis During Nutrient Limitation in Chlorella sorokiniana

Jithesh Vijayan*, *Wayne Riekhof., School of Biological Sciences, University of Nebraska-Lincoln*

Many microbes, including microalgae, accumulate storage compounds like triacylglycerol (TAG), polyhydroxybutyrate or starch upon stress conditions. Most common stress conditions that microalgae experience in nature are nutrient starvation, light and oxidative stress. Much of recent work has focused on nitrogen starvation induced lipid droplet (LD) formation in algae. There are sparse reports on how other nutrient starvation conditions can lead to oil accumulation. Phosphate, being one of the essential macronutrients, is also known to induce TAG accumulation in microalgae upon starvation. We observed that *Pi* starvation in industrial microalgae, *Chlorella sorokiniana*, induces LD formation while chlorophyll content is not reduced to as great an extent as in the case of nitrogen starvation. Chlorophyll loss of cells under nitrogen starvation is due to chloroplast-membrane degradation and sequestration of fatty acid (FA) from the galactolipid of membranes into TAG. Chloroplasts remain intact in *Pi* starved cells while those of nitrogen starved cells are mostly degraded, as shown by transmission electron microscopy. *Pi*-limited cultures also generate more biomass than nitrogen limited ones, when inoculated with equal number of cells. All these observations indicate that studying the mechanism of LD formation in *Pi* starvation condition may provide new insights that can help us engineer microalgal cells to accumulate oil without making a significant compromise in growth. To gain a preliminary gauge of mechanism of TAG synthesis we carried out transcriptomic analysis (RNAseq) of the *Chlorella sorokiniana* cells under *Pi* and nitrogen limited conditions at 9 and 24 hours' time-point. To our surprise, the transcript levels of the FA biosynthesis (FAS complex) genes were significantly downregulated under these conditions. This led us to question if FA in TAG was predominantly derived from membrane remodeling as opposed to de novo synthesis. We carried out 14C-acetate feeding experiment to see if any de novo activity is found in vivo. We found that 14C-acetate was incorporated into FA under these nutrient limitation conditions indicating that fatty acid was synthesized de novo, perhaps indicating the presence of a non-canonical route of FA synthesis in these cells under nutrient limitation conditions. We queried the genome and transcriptome to find if any alternative mechanisms of FA synthesis that is less appreciated is involved. We found three genes, each encoding different size proteins with multiple polyketide synthase (PKS) domains in them. The gene encoding one of these proteins, named PKS-like 3 (PKL3), is upregulated 7.5 and 9.5-fold under N- and P-starvation, respectively. PKL3 has 6 PKS domains, and we hypothesize that this protein is involved in channeling acetyl-CoA to fatty acids under nutrient limited conditions. We plan to further characterize this enzyme function using in vitro assays studying the incorporation of 14C-acetate into FA.

P28. Tocopherols, rather than tocotrienols, protect seeds from lipid peroxidation during germination in *Chamaerops humilis* var. *humilis*.

Laura Siles*, *Leonor Alegre, Veronica Tijero, Sergi Munne-Bosch, Department of Vegetal Biology. University of Barcelona*

Chamaerops humilis (L.), the only dwarf palm native of continental Europe that is found in the Iberian Peninsula, accumulates tocotrienols rather than tocopherols in quiescent seeds, as it occurs in other monocots. To unravel the protective role of either tocopherols or tocotrienols against lipid peroxidation during seed germination; seed viability, natural and induced germination capacity, seed water content,

malondialdehyde levels (as an indicator of the extent of lipid peroxidation) and vitamin E levels (including both tocopherols and tocotrienols) were examined at various germination phases in a simulated, natural seed bank. At the very initial stages of germination (operculum removal), malondialdehyde levels increased 2.8-fold, to decrease later up to 74%, thus indicating a transient lipid peroxidation at initial stages of germination. Tocopherol levels were absent in quiescent seeds and did not increase during operculum removal, but increased later dampening malondialdehyde accumulation. Thereafter, tocopherols continued increasing, while lipid peroxidation levels decreased. By contrast, tocotrienols levels remained constant or even decreased as germination progressed, showing no correlation with lipid peroxidation levels. We conclude that despite having a high amount of tocotrienols, seeds synthesize tocopherols to protect from lipid peroxidation when germination takes place, thus indicating that *de novo* synthesis of tocopherols, rather than tocotrienols, protect seeds from lipid peroxidation events during germination. By contrast, it is suggested that tocotrienols may exert an antioxidant role in quiescent seeds.

P29. Soybean Rhizosphere Bacterial Community Structure as Influenced by Root Isoflavonoids

***Laura J. White*[1]**, *Xijin Ge[2], Volker S. Brözel[1,3], Senthil Subramanian[1,4], [1]Department of Biology and Microbiology, South Dakota State University, [2]Department of Mathematics and Statistics, South Dakota State University,[3]Department of Microbiology and Plant Pathology, University of Pretoria,[4]Department of Plant Science, South Dakota State University*

Rhizodeposits play a key role in shaping rhizosphere microbial communities. In soybean, isoflavonoids are a key rhizodeposit component that aid in plant defense and enable symbiotic associations with rhizobia. However, it is uncertain if and how they influence rhizosphere microbial communities. Isoflavonoid biosynthesis was silenced via RNA interference in soybean hairy root composite plants and rhizosphere soil fractions tightly associated with roots were isolated using successive sonication. PCR amplicons from 16S rRNA gene variable regions V1-V3 and V3-V5 from these fractions were sequenced using 454. The resulting data was resolved using MOTHUR and vegan to identify bacterial taxa and evaluate changes in rhizosphere bacterial communities. The soybean rhizosphere was enriched in Proteobacteria and Bacteroidetes, and had relatively lower levels of Actinobacteria and Acidobacteria compared to bulk soil. Isoflavonoids had a small effect on bacterial community structure, and in particular on the abundance of Xanthomonads and Comamonads. The effect of hairy root transformation on rhizosphere bacterial communities was largely similar to untransformed plant roots with ~74% of the bacterial families displaying similar colonization underscoring the suitability of this technique to evaluate the influence of plant roots on rhizosphere bacterial communities. However, hairy root transformation had notable influence on Sphingomonads and Acidobacteria.

P30. Abscisic acid regulates plantlike stress responses in algae

***Maya Khasin*[1]**, *Richard Bekeris[1,2], Kenneth W. Nickerson[1], Wayne R. Riekhof[1], [1]School of Biological Sciences, University of Nebraska - Lincoln,[2]Department of Biochemistry, University of Massachusetts - Amherst*

Abscisic acid (ABA) is a phytohormone that has been extensively characterized in higher plants for its roles in seed and bud dormancy, abscission, and stress response. Though primarily bioinformatics-based studies have identified orthologs for ABA-related genes throughout Viridiplantae, including algae, the role of ABA in algae has not been characterized and the existence of such a role has been the matter of some dispute. In this study, we demonstrate that ABA is involved in regulating algal stress response. Reciprocal BLAST searches indicate that *Chlorella sorokiniana* UTEX 1230 contains orthologs for ABA biosynthesis, sensing, and degradation. RNA sequencing studies reveal that treatment with ABA induces dramatic transcriptomic changes, including to genes involved in DNA replication and repair, a

phenomenon which has been demonstrated in higher plants. Accordingly, pretreatment with ABA exerts a modest protective effect on cell viability in response to a sublethal dose of ionizing radiation. Additionally, *C. sorokiniana* produces and secretes biologically relevant amounts of ABA into the growth medium in response to saline stress. Taken together, these phenomena suggest that ABA signaling evolved as an intercellular stress response signaling molecule in eukaryotic microalgae prior to the evolution of multicellularity.

P31. Expression of CBF-like genes in alfalfa (Medicago sativa L.)

Praveena Kanchupati[1]*, *Md. Rokebul Anower[1], Arvid Boe[2],Yajun Wu[1], [1]Department of Biology and Microbiology, South Dakota State University, [2]Department of Plant Science, South Dakota State University*

Plant growth and development is adversely affected by exposure to freezing temperatures. Identification of germplasm with superior freezing tolerance and understanding the molecular biology of the underlying mechanisms would be key to improving freezing tolerance in plants. As the first step towards the improvement of freezing tolerance in alfalfa, a major forage crop in the United States, we recently discovered a germplasm, River side (RS), that is naturally adapted to the Grand River National Grassland environment in South Dakota and showed greater freezing tolerance compared to some of the known freezing tolerant germplasm. To understand the molecular basis of freezing tolerance in RS, we examined expression of the *C-repeat binding factor-like* (*CBF-like*) genes in alfalfa. Studies in Arabidopsis and other plants have shown that the *CBF3* transcriptional cascade plays an important role in improving freezing tolerance in plants. *CBF3* transcripts in Arabidopsis are rapidly upregulated after exposure of plants to a low temperature and this is one of the key genes involved in the process of cold acclimation. The objectives of the study were to understand how the *CBF-like* genes were regulated in alfalfa and identify the genes that are associated with the improved tolerance in RS. After examining the *Medicago truncatula* (a close relative to alfalfa) genome, we identified 18 CBF-like genes. Phylogenetic analysis grouped them into 5 distinct clusters. Expression profiling of these genes in SD201 revealed diverse induction patterns under cold stress. Detailed studies of eight genes that were induced early under cold stress, showed that they had different diurnal and developmental expression patterns and were regulated differentially in roots and shoots. Only three of the eight genes, however, showed early and greater induction under cold stress in RS compared to non-cold tolerant germplasm, suggesting that these three genes are potentially important to freezing tolerance in RS.

P32. Silence-ome of Soybean Nodules

Sajag Adhikar*[1], *Siwaret Arikit[3], Blake C Meyers[4] and Senthil Subramanian[1,2], [1]Department of Plant Science, South Dakota State University, [2] Department of Biology & Microbiology, South Dakota State University, [3]Kasetsart University, Department of Agronomy, Bangkok, [4]Donald Danforth Plant Science Center, St. Louis, MO*

Plant microRNAs are a class of 21-24nt small RNAs that play a regulatory role during number of developmental processes in plants and animals. We identified microRNAs that play a potential role during soybean root nodule development by high throughput sequencing and analysis of small RNA and degradome/Parallel Analysis of RNA Ends (PARE) libraries. Root segments above and below the nodules were used as a control to identify nodule enriched microRNAs and target cleavage. We identified 455 unique miRNA sequences belonging to 270 miRNA families. Among these 368 miRNAs are potentially novel miRNAs belonging to 78 miRNA families. Among the 455 candidate microRNAs 245 had validated targets identified from the PARE library. Functional annotation of identified target showed transcripts that were related to transcription factors families like TCP, MYB, NAC, GRAS and WRKY. A search for expression pattern of these targets in the transcriptome data showed, 24 of the target had an

inverse relationship with the expression pattern of the miRNA in the nodule vs control tissue, 11 of them were conserved and 13 were novel. Our study suggests microRNAs excluded from nodule tissue and or present in root tissue might regulate the target levels in the corresponding tissue for proper nodule development.

P33. GLAND4: a Putative Plant Transcription Factor Secreted by Cyst Nematodes

Stacey N. Barnes*[1], *Catherine L. Wram[1], Melissa G. Mitchum[2], Thomas J. Baum[1], [1]Department of Plant Pathology & Microbiology, Iowa State University, [2]Division of Plant Sciences, University of Missouri*

Cyst Nematodes (CN) are devastating plant pathogens that infect a wide range of economically important crops. CN successfully parasitize the roots of their hosts through the formation and maintenance of feeding sites, termed syncytia, which are located close to the root vascular tissue. One parasitic mechanism employed by CN is the release of effectors through a hollow protrusible mouth spear referred to as the stylet. To date, we have discovered more than 70 candidate CN effectors. Characterization of a subset of these effectors has revealed that they have the ability to alter host cell gene expression. In this study we have focused on the GLAND4 candidate effector from dorsal gland of the Sugar Beet Cyst Nematode *Heterodera schachtii* (*Hs*GLAND4). Transient expression of a *Hs*GLAND4-GFP fusion protein showed that it localizes to the nucleus once inside plant cells. Bioinformatic predictions provided early indications that *Hs*GLAND4 displays DNA binding properties leading to the hypothesis that *Hs*GLAND4 functions as a plant transcription factor once inside the host nucleus. In-vitro genomic selection and electrophoretic mobility shift assays (EMSA) revealed that *Hs*GLAND4 has DNA-binding properties. Translational fusions of various portions of *Hs*GLAND4 with the Gal4 DNA-binding domain caused an alteration of transcription levels of reporter genes in both yeast and plant systems documenting the ability of *Hs*GLAND4 to alter gene expression. Our recent advances on *Hs*GLAND4 DNA-binding and modulation of host gene expression will be presented.

P34. GmSUR2 expression is crucial for soybean root nodule development

Suresh Damodaran*[1], *Pavel Buravtsov[1], Mary Schreurs[1], Senthil Subramanian[1,2], [1]Department of Plant Science, South Dakota State University, [2]Department of Biology and Microbiology, South Dakota State University*

Leguminous crops such as soybean form symbiotic nitrogen-fixing root nodules in association with soil-borne rhizobia bacteria. The bacteria reside inside the nodules where they convert atmospheric nitrogen into a plant usable form of nitrogen, and henceforth help reduce the need for nitrogen fertilizer. A better understanding of plant mechanisms that regulate nodule formation will enable us to develop biotechnological strategies to optimize nodule formation and nitrogen fixation, or even transfer this trait to non-legume plants. We identified a nodule specific gene cytochrome P450 oxidase enzyme, GmSUR2 based on RNAseq analysis and reciprocal BLAST analysis suggested that this gene is a close ortholog for Arabidopsis SUR2 gene. Tissue specific expression analysis using a promoter: GUS construct revealed that this gene is expressed in root cortex cells in the emerging nodule (EN), and is later confined to nodule parenchyma of mature nodules (MN). Suppression of GmSUR2 using RNA-interference led to a reduction in the number of nodules and resulted in an impaired nodule vasculature branching pattern, suggesting that this gene plays a key role in nodule development. Our primary hypothesis is that potential increase in auxin levels due to reduced GmSUR2 activity resulted in impaired nodule development. Experiments are in progress to validate this and other alternate hypotheses. Understanding the role of GmSUR2 is expected to provide more insights into the role of auxin in nodule development.

P35. A Quantitative PCR Assay to Screen for Disease Resistance in Sunflower

Taylor Olson*[1], *Brian Kontz[1], Anjana Adhikari[1], Laura Marek[2,3,] Febina Mathew[1], [1]Department of Plant Science, South Dakota State University,[2]Department of Agronomy, Iowa State University, [3]USDA-ARS, Ames IA*

Phomopsis stem canker is a disease that's been affecting sunflower production in the United States since 1983. However, it did not gain much attention until the 2010 epidemic in Minnesota, North Dakota and South Dakota, where 80% of the sunflower production is in the United States. This epidemic fueled research efforts, and led to the identification of two pathogens that cause the disease; *Diaporthe helianthi* and *Diaporthe gulyae*. This disease continues to be a problem in Minnesota, North Dakota and South Dakota where sunflower fields have had yield losses of 40%. Symptoms produced by Phomopsis stem canker pathogens are easily confused with other sunflower stem diseases. As a result of this, a quantitative PCR assay was developed for detection and quantification of the two *Diaporthe* species. The qPCR assay has been validated against pure DNA of *Diaporthe helianthi* and *Diaporthe gulyae.* The assay has been applied to diagnose field samples for correct identification of the causal pathogen. The qPCR assay will be used to quantify resistance in sunflower germplasm to the *Diaporthe* spp., and also to test the efficacy of fungicides for management of Phomopsis stem canker. This qPCR assay can benefit diagnosticians to diagnose sunflower samples for Phomopsis stem canker, and by breeders to quantify resistance in sunflower germplasm to the two causal pathogens.

P36. Fine Mapping and Genetic Characterization of the Seed Dormancy 8 and Awn Length 8 loci in Rice

Wirat Pipatpongpinyo*, *Heng Ye, Jiuhuan Feng, Gu X.-Y., Department of Plant Science, South Dakota State University*

The seed dormancy and awn (a needle-like appendage extended from the lemma of a floret) traits are both of adaptive significance in grass species as they regulate the timing of germination or seed dispersal. These traits tend to associate with each other in weedy rice (*Oryza sativa* L.) and the association was accounted for by a few clusters of quantitative trait loci (QTL), including *Seed Dormancy 8* and *Awn Length 8* on chromosome 8 (*qSD8/qAL8*), in a primary segregating population. The objectives of this research were: 1) to finely map the *qSD8/qAL8*-containing region to delimit the QTL; 2) to precisely evaluate genic effects of *qSD8* and *qAL8* in an isogenic background; and 3) to determine if *qSD8* or *qAL8* interacts epistatically with the seed dormancy locus *SD1-2* on chromosome 1. *SD1-2* was recently cloned as a gibberellin synthesis gene, with the loss-of-function allele inhibiting germination and plant height. Two chromosomal segments encompassing the *qSD8/qAL8* cluster and *SD1-2*, respectively, were introduced from the awned line SS18-2 (weedy rice) into the background of the awnless line EM93-1 (cultivar rice) by recurrent backcrossing combined with marker-assisted selection. A high-resolution map was developed for the *qSD8/qAL8* region with new DNA markers. Progeny testing for seven recombinants selected from the map allowed delimiting *qSD8/qAL8* to <3 mega base pairs. The narrowed region accounted for <5% of the variances for seed dormancy or awn length in progeny lines where *SD1-2* fixed for a functional allele, or for 20% and 40% of the variances for seed dormancy and awn length, respectively, in progeny lines where *SD1-2* fixed for a loss-of-function allele. In these progeny lines, the allele derived from SS18-2 reduced germination and increased awn length. Both *qSD8* and *qAL8* were also involved in two categories (additive×additive and additive×dominance) of interactions with *SD1-2*, and epistatic effects increased germination by ~10% or reduced awn length by 8.5 mm. These results confirmed the effects of *qSD8/qAL8* on the associated traits and also demonstrated that endogenous GA controlled by *SD1-2* tends to reduce the effects. Research is being conducted to clone *qSD8* and *qAL8* to determine if they are underlain by a pleiotropic gene or linked genes and to identify physiological and molecular mechanisms underlying their interactions with *SD1-2* or the GA hormone.

P37. Characterization of Bacterial Endophyte Communities in Brassica carinata

Vincent Peta*[1], *Branon Monier[1], Winly Mai[2], Jerry Cordero-Sepulveda[3], William Gibbons[1], Heike Bucking[1], [1]Department of Biology & Microbiology, South Dakota State University, [2]Deparment of Biochemistry, Allegheny College, [3]Department of Biotechnology, University of Puerto Rico-Mayaguez*

Plant-associated bacterial endophytes have garnered increased interest in both microbiology and plant science as they have been shown to provide nutritional and disease suppression benefits to a variety of plants. There is an increasing interest in certain members of the *Brassicaceae* family as potential sources of non-edible, long-chain oils for the production of biodiesel and jet fuel. *Brassica carinata* (also known as Ethiopian Mustard) is particularly interesting due to its high productivity also in semi-arid regions and marginal lands. We examined the nitrogen fixing endophyte community of *B. carinata*, because these endophytes could serve as microbial biofertilizers and increase productivity under low input conditions. We isolated bacterial endophytes of *B. carinata*, and examined their capability for N fixation, pathogen resistance, P solubilization, and plant growth hormone production, and studied the endophyte community by metagenomics. *B. carinata* is colonized by several diazotrophic endophytes, e.g. species of *Pseudomonas*, *Bacillus*, and *Xanthomonas*, and the results demonstrate that these endophytes have also other plant growth promoting capabilities (e.g. the suppression of *B. carinata* pathogens). The metagenomic analysis of 16S primers revealed that there is a diverse bacterial community that resides in *B. carinata*, and gives insight as to how this community might function and influence the growth and development of *B. carinata* under low input conditions.

P38. Plants Differentially Allocate Their Resources to Root Symbionts in Tripartite Interactions

Arjun Kafle[1]*, *Xiurong Wang[2], Heike Bücking[1], [1]Department of Biology & Microbiology, South Dakota State University, [2]State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, Root Biology Center, South China Agricultural University*

Legume plants form tripartite interactions and are simultaneously colonized by arbuscular mycorrhizal (AM) fungi and rhizobia bacteria (R). Both interactions provide substantial nutritional benefits for their host plant, and the plant delivers carbon to these symbionts in return. Despite their agronomic significance, tripartite interactions are not well studied, and it is currently unknown how the carbon to nutrient exchange in tripartite interactions is regulated. *Medicago truncatula* seedlings were grown in a split root design, and one root half was colonized with the AM fungus *Rhizophagus irregularis* (+AM) and the other root half with the N fixing diazotroph *Sinorhizobium meliloti (+R).* We tracked the carbon and nutrient transport in these systems after labeling with 13C-CO2. The inoculation with both symbionts significantly increased the root and shoot biomass of the plants. Particularly the root biomass results demonstrate that there are synergistic responses and that *Medicago truncatula* gains more from tripartite interactions than from single interactions with either symbiont. However, we also observed a reduction in the AM colonization of the root system, when the plant was simultaneously colonized by *Sinorhizobium meliloti*. When plants are under high N demand, more 13C was allocated to the nodulated root half. The results suggest that plants benefit more from tripartite interactions, but that in tripartite interactions the colonization of the plant with AM fungi is suppressed by autoregulatory mechanisms. Plants control their C allocation to each symbiont depending on their nutrient demand, and this provides additional evidence that resource exchange in these interactions is controlled by biological market dynamics.

P39. Contribution of Long Chain Bases to the Pattern-Triggered and Effector-Triggered Immunity in Arabidopsis thaliana.

Ariadna González Solís*[1,2], *Felipe Cruz García[1], Edgar B. Cahoon[2], Marina Gavilanes Ruiz[1], [1]Department of Biochemistry, National Autonomous University of Mexico, [2]Center for Plant Science Innovation and Department of Biochemistry, University of Nebraska-Lincoln*

Long chain bases (LCB) participate as signaling molecules during the programmed cell death in defense against pathogens (Saucedo-García *et al.*, 2011 New Phytol. 191:943–957). In the context of mechanisms of plant defense, it is known that there are two types of immunity. One, the Pattern-Triggered Immunity (PTI), is elicited upon the perception of pathogen associated molecular patterns (PAMPs) and leads to a basal defense response. Other is the Effector-Triggered Immunity (ETI) in which the detection of pathogen effectors by resistance proteins of the plant cell elicits very specific and robust defense machinery associated with the Hypersensitive Response (HR) (Morel and Dangl, 1997 Cell Death Differ 4:671-683). The objective of this study was to determine the contribution of LCB as signaling molecules to both types of immunity. In order to address this question, we used Arabidopsis mutants impaired in the expression of transducers of the signaling network mediated by LCB. These mutants were exposed to FB1 or to the avirulent and virulent strain of *P. syringae* or to the PAMPS flagellin/flg22 and xylanase. These treatments were evaluated by the analysis of the phenotypical lesion, determination of bacterial growth, detection of H2O2 and analysis of defense gene expression. Phenotypic analyses and bacterial growth experiments showed that LCB are implicated in the ETI immunity. Moreover, the exposure of the different mutants to the PAMP xylanase revealed a possible role of LCB in modulating a signaling pathway that involves MPK6 and ERO as signaling elements, and therefore, implying LCB with the PTI. The analysis of gene expression suggested that genes associated with the jasmonic/ethylene pathway are involved in the response to LCB accumulation. The work was funded by Facultad de Química (PAIP 4290-02), DGAPA, UNAM (PAPIIT IN210811) and by CONACYT (101521). AGS received a fellowship by DGAPA, UNAM (PAPIIT IN210811).

P40. Wheat Cultivars Respond Differently to Natural and Additive Communities of Arbuscular

Nina Herrera[1]*, *Yaqoob Thurston[2], Heike Bücking[1], Jose L. Gonzalez-Hernandez[2], [1]Department of Biology and Microbiology, South Dakota State University, [2]Department of Plant Science, South Dakota State University*

Arbuscular mycorrhizal (AM) fungi form a mutualistic symbiosis with a variety of land plants, including wheat, and facilitate the nutrient uptake and biotic and abiotic stress resistance of their hosts. In order to examine the potential of AM fungi to improve the environmental sustainability of agricultural wheat production, we tested the mycorrhizal responsiveness of eight different spring wheat genotypes in a field experiment. We suppressed the arbuscular mycorrhizal colonization of the plants by regular additions of the fungicide Topsin M, or changed the AM community composition by the addition of a commercially available fungal inoculum (MycoApply), and compared the effects to control plants that were colonized by the naturally present AM fungal communities in the soil. The AM colonization of the control plants was high and the mycorrhizal additive did not increase the colonization of the plants, but the colonization of the plants was clearly suppressed by the fungicide addition. Although the addition of the AM fungal additive did not increase colonization rates it did increase yield of some genotypes, while in others there was no difference between the controls. When fungicide Topsin M was added yield significantly decreased in most genotypes. Our findings will assist ongoing efforts aimed to understand the potential of AM fungi to increase growth, yield, and nutrient uptake of wheat under low input conditions, and to identify molecular markers for the trait mycorrhizal responsiveness.

P41. Endoplasmic Reticulum Associated Degradation is Differentially Regulated During Temperature Stress in Arabidopsis thaliana

Gaurav Kandoi*[1, 2], *Julie A. Dickerson*[1,2], [1]*Department of Electrical and Computer Engineering, Iowa State University,* [2]*Department of Bioinformatics and Computational Biology, Iowa State University*

Alternative splicing (AS) produces multiple messenger RNAs by combining various regions of the precursor transcript to produce diversity in gene products. Observed rates of AS are as high as 95% in humans and 60% in plants. Differential expression of a gene is another commonly observed phenomena and is sometimes regulated by AS. Plant growth and development are extensively affected by environmental disturbances. In this study, we search for metabolic networks that involve significant Differentially Expressed Genes (DEGs) and Differentially Alternatively Spliced Genes (DASGs). We observe that during the stress conditions, many genes produce transcripts that are otherwise not produced during the normal conditions. When comparing the normal and stress conditions, increased numbers of AS events are seen under stress conditions. In concordance with previous studies in plants, we found intron retention to be the most frequent AS event. Most differentially expressed and spliced genes are nuclear and associated with molecular process such as catalytic activity, binding, and transporter and regulator activity. Many differential genes are also linked with biological processes such as metabolic processes, response to stimulus, organization or biogenesis and biological regulation. Using publicly available RNA-Seq datasets from Arabidopsis thaliana (Col-0) when subjected to heat and cold stress conditions, we extracted the Endoplasmic Reticulum Associated Degradation (ERAD) pathway as the network most associated with temperature stress-response. Recent evidence highlights the importance of ER-stress responses in plants subjected to adverse environmental conditions. Our analysis suggest that the gene expression and exon/intron usage of the transcripts involved in this pathway are significantly changed during extreme temperature conditions. Thus, it shows that searching for molecular networks based on the combination of gene expression and exon/intron usage is an effective strategy. Next, we will like to investigate the effect of changes in AS pattern on the pathway to generate metabolic models with a high-level regulatory framework.

P42. Evaluation of Ochratoxin A Level in Oat Varieties Commonly Grown in South Dakota

B. Dhungana*[1], *M. Caffe-Treml*[1], *S. Ali*[1], *E. Byamukama*[1], *P. Krishnan*[2], *J. Kleinjan*[1], [1]*Department of Plant Science, South Dakota State University,* [2]*Department of Dairy Science, South Dakota State University*

Ochratoxin A (OTA) is a secondary fungal metabolite produced by several species of fungal genera *Penicillium* and *Aspergillus,* although *P. verrucosum* is the major OTA producer in the temperate region. Ochratoxin A (OTA) may cause toxicogenic effect like hepatotoxicity, carcinogenesis, teratotoxicity, neurotoxicity in humans and animals. Oat (*Avena sativa*), like any other cereal grain, can be contaminated with Ochratoxin A when the storage conditions are favorable for the fungal growth. Although it is known that the storage conditions have an impact on the production of OTA in the grain, the oat genotype may also have an effect on the OTA production during storage. If the cultivar significantly influences grain OTA contamination, the use of resistant oat cultivars would be an effective way to limit the incidence of OTA contamination in oat products. Grain samples of 12 oat cultivars grown at 5 locations in South Dakota in 2014 and 2015 will be analyzed for the presence of *P. verrucosum* and contamination with OTA by plating them on Dichloran yeast sucrose 18% Glycerol agar media and performing ELISA test, respectively. Also, to determine the effect of genotype on OTA production during storage, a subset of grain samples from the same 12 cultivars will be inoculated with *P. verrucosum*, incubated at constant water activity for several weeks, and OTA concentration of inoculated oat samples will be determined by Liquid Chromatography-tandem mass spectrometry.

P43. Association Mapping of Bacterial leaf streak in Winter Wheat

Sai Mukund Ramakrishnan*, *Jagdeep Singh, Navjot Kaur, Dr. Shaukat Ali, Dr. Sunish Sehgal*
Department of Plant Science, South Dakota State University

Bacterial leaf streak (BLS) caused by *Xanthomonas campestris pv. translucens* is the major bacterial disease threatening to wheat production in US Northern Great Plains. It is a sporadic but wide-spread disease of wheat that can cause significant loss. Unlike fungal diseases, bacterial diseases can only be countered by developing disease resistance cultivars. None of the current grown cultivar of wheat in the Great Plains are resistant to BLS. Identification and characterization of genomic regions of wheat that confer resistance to BLS can be an effective way to mobilize resistance genes in wheat breeding. Association mapping, a high-resolution method for mapping quantitative traits, hold a great promise in the dissection of complex genetic traits in plants. Here we focus on identifying the genes/QTLs for BLS resistance in an Association Mapping (AM) panel of 300 winter wheat cultivars representing the entire US winter wheat region. The AM panel has already been genotyped using Infinium 90K array under USDA – TCAP. We screened the AM panel for its reaction to BLS. The bacterial inoculum was infiltrated into the plant during its three leaf seedling stage and the percentage in increase of the bacteria was noted. The finding of this study will be presented.

P44. Capturing the Genetic Diversity of Wild Ancestors of Bread Wheat

Jagdeep S. Sidhu*, *Sai Mukund Ramakrishnan, Sunish K. Sehgal, Department of Plant Science, South Dakota State University*

Bread wheat (*Triticum aestivum*. L - AABBDD) evolved from a spontaneous cross between cultivated emmer (*Triticum dicoccon* - AABB) and goat grass (*Aegilops tauschii* - DD) approx. 8000 years ago. This event happened only once or twice and its reproductive isolation lead to a narrow genetic base of hexaploid wheat. With rise in the frequency of abiotic stresses like heat and drought stress and rapidly evolving pathogens, we need to broaden the genetic base of wheat to mitigate these challenges posed by the changing climate. To accomplish this, exploiting desirable variability from wild relatives of wheat is an attractive approach. As a base of this study, 1,890 accessions (NBRP collection) of tetraploid wheat were genotyped using 275 DArT markers and a core set of 172 lines was identified based on their genetic distance. Eventually a minicore set of 60 accessions was identified from 172 lines by diversity analysis using Genotyping – By – Sequencing (GBS). Similarly, a core set of 52 accessions from D genome (2x) species was identified. These lines represent the genetic and geographic diversity of their corresponding species. To exploit this diversity in bread wheat we are developing synthetic hexaploid wheat (SHW) lines by making crosses between these tetraploid and diploid wheat accessions. Eventually these synthetics (6x) will become a permanent germplasm and act as a reservoir of rich genetic diversity that is easily accessible for wheat improvement. Out of the first attempt of 72 cross combinations we recovered 15 new SHW lines. The SHWs will be screened for a number of interesting traits like thousand-grain weight (TGW), seed size and higher protein.

P45. Acquisition of Desiccation Tolerance in the Moss Physcomitrella patens by Intrinsic Mechanisms

N. R. M. Kumudu Nadeeka Rathnayake*, *Karen L. Koster, Department of Biology, University of South Dakota*

Desiccation tolerance allows organisms to survive the loss of almost all cellular water and resume growth upon rehydration. We study desiccation tolerance in the moss Physcomitrella patens. Studies have shown that application of the plant stress hormone abscisic acid (ABA) can induce desiccation tolerance in

Physcomitrella, but acquisition of desiccation tolerance without addition of exogenous hormone is not well-studied in this moss. Slow drying, as occurs in its natural habitat, may induce tolerance in mature Physcomitrella; however, intrinsic mechanisms triggered by slow drying are not known, nor is it known whether younger, protonemal moss also survives slow drying. My goals were to determine whether prolonged drying times could induce desiccation tolerance in protonemata and to establish a system for continued study of the mechanisms of desiccation tolerance in this moss. Moss protonemata were slowly dehydrated in chambers at 91% relative humidity (RH) for 5-10 d prior to transfer to 32% RH. During slow drying at 91% RH, the moss lost 90% of its fresh weight and equilibrated at 0.62 ± 0.02 g H2O/gDW. Additional desiccation at 32% RH dropped the water content to 0.18 ± 0.02 g H2O/gDW. After 3 d at 32% RH, the moss was rehydrated, and chlorophyll fluorescence and survival were assessed. Results show that moss held for fewer than 9 d at 91% RH did not survive additional desiccation, while moss kept for 9-10 d at 91% RH acquired desiccation tolerance and then survived desiccation at 32% RH. Visual estimates of re-growth of protonemata corroborate increased chlorophyll fluorescence ratios that indicate repair of photosynthetic machinery. These results show that desiccation tolerance can be induced by slow, stepwise drying of young moss without exogenous ABA treatment.

P46. Screening Soybean Germplasm for Resistance to Fusarium Root Rot

Paul N. Okello*[1], *Asheesh Singh[2], Febina M. Mathew[1], [1]Department of Plant Science, South Dakota State University, [2]Department of Agronomy, Iowa State University*

Multiple *Fusarium* species are associated with seedling and root rot of soybean (*Glycine max* L.) in the United States. In South Dakota, a total of 2000 soybean samples were collected between R1 (beginning flowering) and R5 (beginning seed) growth stages during a survey of commercial soybean fields in 2014 to achieve the following objectives: 1) characterize the diversity of *Fusarium* species causing root rot of soybean, and 2) determine the pathogenicity of the *Fusarium* species on soybean under greenhouse conditions. Ten *Fusarium* species were identified based on morphology and DNA sequencing. Pathogenicity results showed significant differences in disease severity ($P \leq 0.05$) among the *Fusarium* species with *Fusarium proliferatum* being the most aggressive (disease severity >80%). To identify new sources of resistance to Fusarium root rot caused by *F. proliferatum*, 110 soybean germplasm accessions belonging to maturity groups 0 and I were screened. Preliminary results shows that 9 MG 0 (PI467313; PI468910; PI426930; PI189873; PI068722; PI154196; PI417458; PI567212B; PI13220)7 and 4 MG I (PI 91733; PI 153229; PI 227325; PI 131531) lines had significantly lower disease severity (< 43%) than the susceptible check (43.4%). These new sources of resistance can be used to develop soybean breeding lines and commercial cultivars with resistance to *F. proliferatum*, benefiting soybean farmers by reducing yield losses due to the disease.

P47. Identification of Physiological and Morphological Traits Governing High Water Use Efficiency in Alfalfa

Krishna Ghimire*, *Yajun Wu, Department of Biology and Microbiology, South Dakota State University*

Alfalfa is a deep rooted, N2-fixing and high yielding crop with great economic and ecological benefit. Alfalfa production has decreased worldwide due in part to an increase in drought and a decrease in irrigation water availability. Under limited water supply conditions, enhanced water use efficiency (WUE) can minimize yield loss. WUE is the ratio of biomass production to water use and is considered an important trait that determines yield during drought. Our lab recently identified an alfalfa germplasm, River side (RS), that is naturally adapted to the Grand River National Grassland region in South Dakota and has exhibited higher WUE over several other germplasm. The aim of this study is to identify the traits that contribute to the higher WUE in RS. Under drought stress conditions when irrigation water was supplied to compensate for only 50% of transpirational water loss, RS showed lower stomatal

conductance than other germplasm resulting in a lower rate of water loss in detached leaves. The number of leaf hairs on the lower surface was significantly higher in RS compared to other germplasm. Although the number of stomates was greater in RS, the stomates of leaf epidermal peels from RS were more sensitive to abscisic acid (ABA) application compared to other germplasm. We are determining whether the ABA production during drought may also contribute to a higher WUE in RS.

P48. Developing a Molecular Diagnostic Tool to Detect Phytopthora sojae Causing Root Rot of Soybean

Rawnaq N Chowdhury*[1], Emmanuel Byamukama[2], Sajag Adhikari[1], Senthil Subramanian[1], [1]Department of Plant Science, South Dakota State University, [2]Extension-Plant Pathology, South Dakota State University

Phytophthora root rot caused by *Phytophthora sojae* Kaufmann and Gerdemann, causes severe losses in soybean (*Glycine max* [L.] Merr) yields worldwide including in the United States. In the United States, annual losses have exceeded 1 million ton since 2000. *P. sojae* can infect and kill soybean plants throughout the growing season. Therefore, early and accurate diagnosis is crucial for mitigating yield losses. However, sometimes damping off of soybean seedling might be indistinguishable from *P sojae* and other pathogens such as *Pythium spp*. The different phases of *P. sojae* infection elicit the up-regulation and down-regulation of numerous genes, which may be involved in the pathogenesis. These genes can be used to make quick, accurate and sensitive diagnosis of Phytophthora root rot at the earliest stage of the infection. This tool would aid early detection of *P sojae* in soybean fields so that appropriate management decisions can be made and yield losses can be minimized. Twenty three commercial soybean cultivars with varying level of susceptibility to *P. sojae* were inoculated with two different isolates of *Phytopthora sojae* (PS-02-30 and BR3) using the inoculum layer test method. A set of un-inoculated plants for each cultivar were used as control. After 21 days, leaf samples were harvested from the fully developed trifoliate leaf from each plant and RNA was extracted using the Direct-zol™ RNA MiniPrep kit (Zymo Research Corp). The expression of 11 key marker genes in response to *P. sojae* infection were assayed by qPCR to evaluate their suitability as a diagnostic marker. Changes in gene expression and potential suitability as diagnostic markers will be discussed.

P49. Developing a Molecular Tool to Detect Early Soybean Cyst Nematode (SCN) Infection

Krishna Acharya*[1], *Emmanuel Byamukama[2], Sajag Adhikari[1], Senthil Subramanian1, 1Department of Plant Science, South Dakota State University, 2Extension-Plant Pathology, South Dakota State University*

Soybean cyst nematode (SCN); *Heterodera glycines,* is the top biological yield constraint for soybean production. It can cause significant yield losses without causing above ground symptoms; moreover, the symptoms produced resemble symptoms caused by other biotic and abiotic stresses. The initial step in SCN management is soil testing to determine the presence of SCN and then initiating management program. Soil sampling can be cumbersome and the process of SCN sieving is time consuming. A SCN molecular detection tool can save time and can be more reliable than soil testing. Many genes are responsible for infection and disease development and these are up/down regulated during infection and as the infection takes place. Identification of induced genes allow the detection of SCN early in the growing season. Twenty three commercial soybean cultivars were planted under the greenhouse conditions and inoculated with SCN eggs and juvenile then kept in water bath at 280 C for 35 days. Un-inoculated plants for each cultivar were used as control. Leaf samples (youngest trifoliate) at three time point 3rd, 4th and 5th week after planting were harvested in liquid N2 and stored at -80 0 C until RNA isolation. For preliminary study, six cultivars were selected and grouped as moderately susceptible, susceptible, and highly susceptible, based upon the female index respective to susceptible check. The

expression of ten genes selected based on previous literature was assayed by qPCR to evaluate their suitability as diagnostic marker genes. Consistently induced genes will be used as molecular markers for early detection of SCN infection.

P50. Molecular studies on Pre-harvest Sprouting in Wheat

Pramod Khadka*[1]*, *Aayudh Das[1], Dea-Wook Kim[2], Karl D. Glover[3], Sunish K. Sehgal[3],Jai S. Rohila[1], [1]Department of Biology and Microbiology, South Dakota State University, [2]National Institute of Crop Science, Republic of Korea, [3]Department of Plant Sciences, South Dakota State University*

Pre-harvest sprouting (PHS) of wheat (Triticum aestivum L.) is a phenotype that is characterized by germination of seeds on spikes in field under moist environmental conditions. PHS affects grain quality, seedling vigor, seed viability and thus limits the profits for wheat producers groups. A viable solution to increase the PHS-resistance in wheat is to speed up the plant breeding by employing molecular information, generated from various –omics technologies, in wheat breeding programs. Proteomics and metabolomics technologies offer opportunities to overcome hurdle of polygenic inheritance and identify candidate genes for improving PHS resistance wheat. A comprehensive proteomics and metabolomics analysis of wheat embryos of PHS resistant (Sukang) and PHS susceptible (Baegjoong) wheat cultivar revealed key information about PHS process. Proteomic analysis revealed that 190 differentially expressed proteins might be involved in various cellular functions, such as carbohydrate metabolism, nitrogen metabolism, stress response, redox regulation, ATP synthesis, and protein translation, during this untimely germination of wheat embryo. Expression of stress-related and inhibitors proteins were found to be important in maintaining seed dormancy in resistant germplasm; whereas over-expression of energy metabolism related proteins was observed in PHS-susceptible. Metabolomics analysis revealed key metabolites, which are differentially regulated in various metabolic processes such as lipid and fatty acid metabolism, oxalate metabolism, metabolism involving key plant hormones, raffinose family of oligosaccharides (RFO) metabolism and amino acid metabolism. This study also indicate that production of reactive oxygen species and ABA metabolism could play crucial roles in PHS.

P51. Nodule zone-specific gene expression in soybean

Md Ehsanul Haque*, *Sunita Pathak, Sen Subramanian, Department of Plant Science, South Dakota State University*

Soybean (Glycine max) is one of the most important oil seed crops and a source of animal feed protein in the world. Biological nitrogen fixation in soybean nodules reduces the use of chemical nitrogen fertilizers resulting in cost-savings to producers and minimizes environmental damage due to nitrogen run-off. A better understanding of how nodules form and function is important for selection or generation of soybean genotypes with better nitrogen fixation capacity. Soybean nodules originate from root cortex via de novo cell differentiation. Consequently, two major nodule development zones are formed for instance; the nodule primordium (Npr) in the middle and it is encircled by nodule parenchyma (Npa). At later time point, the Npr gives rise to N-fixation zone and the Npa holds vascular bundles. It is not clear what early signaling pathways drive the conspicuous development of these two nodule zones. Our research is aimed at filling this knowledge gap by identifying key determinants of nodule zone identity. Based on initial evidence obtained by the Subramanian lab, we hypothesize that microRNAs (miRNAs) play important regulatory roles in spatio-temporal expression of their targets during nodule developmental in soybean. For instance, the spatio-temporal regulation of auxin sensitivity by miR160 has been found to be crucial for formation of nodule primordia and vasculature in the parenchyma. Against this backdrop, we will obtain nodule zone-specific transcriptomes and miRNA profiles to identify Npr- and Npa-enriched transcription factors and miRNAs, and subsequently, evaluate the roles of selected candidates in nodule zone identity.

P52. Phenotyping F_2 Grapevine Population for QTL Analysis of Low Temperature Responses.

***Mani Awale*[1]**, *Shanshan Yang[2], Johnathan Fresnedo[2], James Luby[3], Anne Fennell[1], [1]Department of Plant Science, South Dakota State University, [2]Horticulture Section, School of Integrative Plant Science, Cornell University, [3]Department of Horticultural Sciences, University of Minnesota-St. Paul*

Cold acclimation is an important trait enabling grapevines to survive freezing injury in northern continental climates. It is a complex quantitative trait that is influenced by the inherent genetic characteristics of grapevines as well as environmental interactions. To explore the genetic components of freezing tolerance in grapevine, a F2 population was phenotyped for freezing tolerance. The F2 population was developed by selfing a single F1 plant from a cross of *Vitis riparia* (cold hardy, North American species) and a hybrid wine cultivar *Seyval*. Freezing tolerance was phenotyped at two different time points during multiple dormant seasons using differential thermal analysis (DTA) to identify low temperature exotherms (LTE), which result from the heat released when supercooled water freezes. Genotypes that were slow to acclimate exhibited LTE at higher temperatures than genotypes that acclimate early. Increased exposure to low nonfreezing temperatures increased freezing tolerance and with this cold acclimation, the number of genotypes capable of surviving lower temperatures increased. Subsequently, as the temperatures increased in late winter, vines deacclimated and were killed at higher temperatures. QTL analysis will be conducted to identify freezing tolerance loci and provide a greater understanding of the genetic regulation of cold hardiness.

P53. Comparing Augmented Experimental Designs for Oat Preliminary Yield Trail Data Analysis

Sudha Neupane Adhikari* *Jixiang Wu, Melanie Caffe, Department of Plant Science Department, South Dakota State University*

Ineffective control of field variation may lead to biased genetic data analysis and conclusions, which in turn could impact on breeding selection efficiency. In this study, a group of 78 early maturity oat breeding lines were grown at five locations in South Dakota in 2015. Due to the large number of oat lines, only three checks were replicated in each location. With linear mixed model approaches, four models (with and without row and column effects) were used and results among these models were compared regarding estimated variance components and predicted genotypic effects for yield, test weight and heading date. Detailed results will be presented at the conference.

P54. Identifying Differences in the Microbial Communities of Plant Roots

***Morgan McPherson*,** *Peng Wang, Ellen Marsh, Daniel Schachtman, Department of Agronomy and Horticulture, University of Nebraska-Lincoln*

The roots of plants are an essential component of the entire plant system that is involved in nutrient and water uptake. The microbial communities present within the endosphere, rhizosphere, and in the bulk soil surrounding the roots play a vital role in plant growth, development, and overall plant health. This research aims to develop a culture collection and to increase the culturability of soil microorganisms associated with root systems of plants. Samples from the endosphere, rhizosphere, and bulk soil of various species of plants were collected from field sites across Nebraska. These samples were cultured using classic plating techniques with 6 types of media as well as with the iCHIP method to create a diverse culture collection. A database was developed to preserve all the data on the isolates. The goal of utilizing novel culturing techniques, such as the iCHIP method, with a wide range of samples is to maximize diversity of the samples within the culture collection. Thus far, this culturing has resulted in a large culture collection of 3,188 isolates. Sequencing was conducted using the 16s rRNA gene to identify

these isolates. Of the isolates that have been sequenced, there is a wide range of variation within the culturable samples. Unique isolates will be tested on plants to determine whether they impart any beneficial effects. The future use of this culture collection will allow for a deeper understanding of the role of the microbial communities present within the endosphere, rhizosphere, and bulk soil of plants.

P55. Spatial Patterns in Vegetation Composition within a Novel Reservoir Delta on the Missouri River

Catherine C. Beall*, *Mark D. Dixon, Department of Biology, University of South Dakota*

Delta habitats have formed in or adjacent to reservoirs on many regulated rivers, including within the upper and middle Missouri River. Reservoir deltas are dynamic, novel habitats that may occupy large areas on a river system, but their ecological value is mostly unknown. We investigated spatial patterns in vegetation across the delta that has formed at the confluence of the Niobrara River and the Missouri River within Lewis and Clark Reservoir in NE Nebraska and SE South Dakota. In July 2015, we sampled vegetation on nineteen 100-m transects across eight different river-delta cross-sections, from older upstream to younger downstream portions of the delta. Plant species richness ranged from 1-23 species among transects. Species of interest, *Phragmites australis* (both native and invasive) had cover ranging from 0% - 82.5% along transects, and was found in all portions of the delta sites. *Typha angustifolia* was found in the older and newer portions of the delta, being less frequent in the middle aged portion, and had cover along transects of 0% - 37.5%. *Xanthium strumarium* was found to have a range of 0% - 27.7%. The cover was mainly found in middle aged portions of the delta. These are just a few examples from the total of the 107 species found in this study. The species richness tends to increase with age, with younger transects having fewer species. The delta's potential as a highly diverse habitat is still under consideration.

P56. Lipid Changes of Panicoid Grasses in Response to Cold Stress

Samira Mahboub[1]*, *Yang Zhang[2], Daniel W.C. Ngu[2], Jennifer A. Myers[2], James C. Schnable[2], Rebecca L. Roston[1], [1]Center for Plant Science Innovation and Department of Biochemistry, University of Nebraska-Lincoln, [2]Center for Plant Science Innovation and Department of Agronomy and Horticulture, University of Nebraska-Lincoln*

Cold stress has a great effect on restraining the growth of many crops, especially the tropical origin crops, such as the panicoid grasses (maize, sorghum, millets). Cold reduces membrane flexibility and changes membrane lipid production. In model species, membrane lipids are dynamically remodeled in response to cold and freezing, and this process is required for low temperature tolerance. Here, we compare glycero-lipid changes among eight species of panicoid grasses. Of these eight species, five are cold sensitive and three are tolerant of 6°C. Plants were grown in 29°C for 12 days then were moved to 6°C. Samples were collected at day 12, day 13 and day 19.We look at the changes in major classes of membrane lipids: monogalactosyldiacylglycerol, digalactosyldiacylglycerol, sulfoquinovosyldiacylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylglycerol, phosphatidic acid, and triacylglycerol. Lipids were quantified by two-dimensional thin layer chromatography/gas chromatography. Based on the results, lipid changes occur in the cold tolerant species which do not occur in the non-tolerant species.

P57. Characterization of Grape Berry Ripening – Genomics to Sensory

Padmapriya Swaminathan*[1], *Kalley Besler[1], Anne Fennell[1], Somchai Rice[2], Jacek Koziel[2], Murli Dharmadhikari[2], Devin Maurer[2], Emily Del Bel[3], Soon Li Teh[3], Bety Rostandy[3], Adrian Hegeman[3], Jim Luby[3], Jenna Brady[4,5], Zata Vickers[4,5], [1]Department of Plant Science, South Dakota State University, [2]Department of Agricultural and Biosystems Engineering, Iowa State University, [3]Department of Horticultural Science, [4]University of Minnesota, [5]Department of Food Science and Nutrition, University of Minnesota*

Frontenac and Marquette are cold climate cultivars emerging from Vitis vinifera and North American Vitis species. Chemical composition of Vitis vinifera cultivars have been extensively studied but not well understood for these cold hardy cultivars. Titratable acidity (TA), pH and soluble solids indicate grape maturity and help determine harvest time for most growers and winemakers. Characterizing the grape berry ripening profile of Frontenac and Marquette berry pulp and skin through transcriptomic, sensory and flavor analyses is critical to understand and obtain the optimal balance between sugars, acids and flavor to aid in harvest decisions and hence better wine quality. Transcriptomic analysis shows changes in gene expression impacting pathways like Anthocyanins, Terpenoids, and Flavonoid biosynthesis. This helps contribute information towards linking transcriptomic analysis with sensory and aroma analyses.

P58. De novo genome assembly for two Vitis species using Illumina short reads

Sagar Patel*[1], *Padmapriya Swaminathan[1], Anne Fennell[1] and Erliang Zeng[2], [1]Department of Plant Science, South Dakota State University, [2]Department of Biology, University of South Dakota, [3]Department of Computer Science, University of South Dakota*

Vitis vinifera cultivars are widely used for wine, table and raisin production throughout the world. A reference genome for the *V. vinifera* inbred line (PN40024, ~93% homozygous) is available but standard cultivars are highly heterozygous. *V. vinifera* 'Sultanina' reads were downloaded from NCBI (accession no SRP026420) and *V. riparia* (PI588259) reads were generated from our laboratory. *De novo* genome assembly for two *Vitis* species of *Vitis vinifera* and *Vitis riparia* completed using two assemblers ALLPATHS-LG and PLATANUS. All statistics for the two assemblies were obtained using Assemblathon script and assembly quality results were obtained using QUAST tool, which indicated the results of PLATANUS tool were more closely related with reference genome of *V. vinifera* than the results of ALLPATHS-LG. The PLATANUS assembly had a greater number of large contigs, scaffolds and longest scaffold length. The PLATANUS N50 and NG50 were also obtained larger than that of the ALLPATHS-LG. We conclude that PLATANUS provides better *de novo* genome assembly results compared to ALLPATHS-LG for highly heterozygous species.

P59. RhizoDive: A High School Introduction to Plant Tissue Development, and Biodiversity Research Techniques through the Study of Legumes and Their Native Rhizobial Diversity

Carl R. Fellbaum*, ASPB Ambassador, *Senthil Subramanian, Department of Plant Science, South Dakota State University*

Promoting a STEM-educated society is crucial for the U.S. to continue to stay at the fore-front of scientific discoveries and for effective utilization of technology for a sustainable society. We developed "RhizoDive", a statewide training project (funded by an NSF-CAREER award to S.S.) with the educational goal of enhancing youth participation in science and the scientific goal of evaluating rhizobial biodiversity in SD. As part of this training pipeline, we executed a high school laboratory experience which uses soybean and red clover nodules to demonstrate the effect of meristem types on plant tissue

development. Participating classrooms throughout South Dakota collected soybean and red clover specimens. The students read and reproduced a tissue sectioning and staining protocol from a peer reviewed journal article to gain experience in reading/understanding scientific literature, and to appreciate how scientific discoveries are communicated. The students analyzed tissue sections for visual meristem differences in legume specific nodule types and created/compared tissue development models for each species. The first year was completed with five diverse classrooms from across the state including a Second Chance High School for at-risk students. We are currently evaluating the experience which we will publish in an education based journal along with the laboratory workbook. We are looking forward to recruiting additional classrooms for 2016 spring/summer/fall participation, and inspiring and preparing the next generation of plant scientists!

P60. microRNA-mediated stress and meristem regulatory networks underlying paradormancy and endodormancy in grapevine

***Shuchi Smita*[1]**, *Monica Accerbi[2], Pamela J. Green[2], Senthil Subramanian[1], Anne Fennell[1], [1]Department of Plant Science, South Dakota State University,[2]Department of Plant & Soil Sciences and Delaware Biotechnology Institute, University of Delaware*

The environmental regulation of the physiology of growth cycling and bud dormancy in grapevines is well known. However, the molecular regulators of paradormancy and endodormancy induction, maintenance, and release are still unclear. In this study the potential role of microRNAs (miRNAs) in dormancy regulation were explored. We performed an integrative analysis of miRNAs and mRNA expression profiling from age matched bud tissue under paradormancy (long day photoperiod, LD 15h) and endodormancy (short day photoperiod, SD 13h) phase in grapevine (*Vitis riparia*). Differential abundance of miRNAs and mRNA dormancy phases were identified and further relationships predicted through regulatory network analyses. Differential expression identified a total of 139 (92 miR family) and 113 (87 miR family) miRNAs (conserved and known) in *V. riparia* paradormant and endodormant buds respectively. Target genes were predicted for abundant miRNAs to assess the biological significance. Gene ontology (GO) enrichment of targets showed little overlap between the enriched biological pathways in paradormant and endodormant buds. Targets for LD-abundant miRNAs were enriched in stress responsive biological pathways. Whereas, targets for SD-abundant miRNAs were specifically enriched in meristematic, reproductive, flowering related biological pathways. Inverse RNASeq expression patterns of corresponding target genes and miRNAs endorsed potential post-transcriptional regulation of some of these genes by miRNAs. Based on the characterization of dormancy phase-specific miRNAs and their target genes a regulatory model has been proposed for miRNAs-mediated stress and meristem regulatory network in paradormancy and endodormancy, respectively.

P61. Analysis of root microbes in crop plants under the elevated CO_2 and O_3 condition in the field

***Peng Wang*[1]**, *Stephanie Canny[1], Ellen Marsh[1], Lisa Ainsworth2, Andrew Leaky[3], and, Daniel Schachtman[1],[1]Department of Agronomy and Horticulture and Center for Plant Science Innovation, University of Nebraska-Lincoln, [2]USDA/ARS Photosynthesis Research Unit and Department of Plant Biology, University of Illinois Urbana-Champaign, [3]Department of Plant Biology, Institute for Genomic Biology, University of Illinois Urbana-Champaign*

Climate change is caused in part by the increase of greenhouse gases such as CO_2 whereas high levels of O_3 are caused by autos and other industrial processes. While elevated CO_2 directly stimulates photosynthesis in C3 crops, rising tropospheric O_3 negatively impacts photosynthesis leading to decreased growth and yield. High CO_2 and O_3 may also affect root physiology of crops which can lead to alterations in root exudation of carbon and other compounds altering the abundance and diversity of specific

rhizosphere organisms. Using the Free Air Concentration Enrichment (FACE) facilities located in Champaign, Illinois, we harvested root, rhizosphere and soil samples from soybean grown under ambient and elevated CO_2 and corn grown under ambient and elevated ozone and looked for changes in microbial communities associated with roots and soils. The results of 16S rRNA amplicon sequencing points to changes in the relative abundance of microbes involved in nitrogen cycling and fixation under the elevated CO_2 condition. In the elevated O_3 conditions the microbial structure in the rhizosphere of the inbred B73 and the hybrid B73 x MO17 were not substantially changed. However, the microbial structure in the soil of B73 and B73 x MO17 was significantly different in the ambient conditions but showed no significant difference in the high ozone conditions. This may indicate that microbial structure in the soil was influenced by the host genotype. Further work using a temporal series of samples will help to dissect the basis of the changes observed between inbred and hybrid corn.

P62. Phenotypic Plasticity Induced by Variation in Nighttime Evaporative Demand

***Walid Sadok*[1]**, *Elodie Claverie[2], Rémy Schoppach[2], [1]Department of Agronomy and Plant Genetics, University of Minnesota Twin Cities, St. Paul, MN, [2]Earth and Life Institute, Université catholique de Louvain, Croix du Sud 2, L7.05.14, 1348, Louvain-la-Neuve, Belgium.*

Over the past few years, several investigations consistently reported that nocturnal transpiration rates (TRN) are significant in many plant species in drought-prone environments. Recently, we have confirmed on wheat the decade-old suspicion that TRN is not negligible and –more importantly– that short-term (hours) variation in nocturnal atmospheric vapor pressure deficit (VPDN) was a major driver of TRN. In a follow-up investigation, we detected several robust QTL controlling TRN suggesting the existence of a yet-to-be-discovered major eco-physiological significance of plant response to VPDN. Because under natural environments plants can be exposed to VPDN regimes as high as 2.5 kPa over developmental timescales (days/weeks), we hypothesized that plant anatomical and functional traits controlling leaf and root hydraulics could be influenced by long-term exposure to high VPDN. We examined 23 leaf and root traits on 4 wheat genotypes, which were subjected to 2 long-term (30d-long) growth experiments where daytime VPD, watering and daytime/nighttime temperature regimes were kept identical, while imposing VPDN at 2 levels (0.4 and 1.4 kPa). The VPDN treatment did not influence phenology, leaf areas, dry weights, number of tillers or their dry weights, consistently with a temperature independent treatment. In contrast, vein densities, adaxial stomata densities, TRN and cuticular TR, were strongly increased following exposure to high VPDN. Simultaneously, whole-root system xylem sap exudation and seminal root endodermis thickness were decreased, indicating a decrease in root hydraulic conductivity. Overall, these results suggest that plants "sense" and adapt to variations in VPDN conditions over developmental scales by optimizing both leaf and root hydraulics.

P63. Advancing Field Pennycress as a New Oilseed Biodiesel Feedstock that does not Require New Land Commitments-Focus on New Mutants

***M. David Marks*[1]**, *Kevin Dorn[2], John Sedbrook[3], Win Phippen[4], Don Wyse[5], [1]Department of Plant Biology, University of Minnesota, [2]Department of Plant Pathology, Kanas State University, [3]School of Biological Sciences, Illinois State University, [4]School of Agriculture, Western Illinois University, [5]Department of Agronomy and Plant Genetics, University of Minnesota*

This is a collaborative project between researchers in MN and IL to convert the weed Field Pennycress (*Thlaspi arvense* L.; pennycress) into a new winter annual oilseed/meal/cover crop. Field trials with current isolates have demonstrated that pennycress can be seeded in upper Midwest cornfields in the late summer and fall, and then produce mature seed in the spring that can be harvested without disrupting soybean planting. 2,200 kg/hectare seeds can be produced by wild pennycress varieties containing 33% oil by dry weight. Wild pennycress varieties are hampered by inconsistent germination and stand establishment, un-optimized maturity for a given growth zone, suboptimal oils quality for biodiesel

production, high seed glucosinolate content, and harvest loss due to pod shatter. We are using a mutation-based approach to identify improved pennycress germplasm. Our goals include identifying lines with improved traits such as more uniform germination, early flowering, early maturation, increased yield, larger seeds, more oil, reduced seed glucosinolates and so on. Our screens of mutagenized populations are facilitated by the diploid nature of pennycress and by the lack of recent genome duplication events that can result in excess gene redundancy. In addition, pennycress is closely related to *Arabidopsis thaliana* so we can use the vast store of information on this plant to aid in our mutant search. To take advantage of this information we have generated the first transcriptomes and draft genomes of pennycress. These analyses have identified most of the gene space in pennycress and comparative analyses between Arabidopsis and pennycress have allowed us to identify gene candidates controlling important agronomic traits. In forward screens we have identified the first generation (M2s) of pennycress mutants with improved agronomic traits from early flowering to reduced dormancy to reduced pod shatter as well as highly desirable semi-dwarf mutants exhibiting reduced lodging and higher seed yields. This presentation will focus on the preliminary characterization of these mutants.

P64. Local Adaptation in Narrow-leaved Purple Coneflower

Amy B. Dykstra*, *Department of Biological Sciences, Bethel University*

Restoration guidelines often call for locally sourced seed, to reduce the likelihood of introducing maladapted genotypes and to avoid outcrossing depression. However, few empirical studies focus on the degree of local adaptation of native plants. To evaluate local adaptation in narrow-leaved purple coneflower (*Echinacea angustifolia*), a native perennial commonly used in prairie restorations, seeds were collected at three sites along a 500-km transect from northwestern South Dakota to west-central Minnesota. Collecting from locations at the same latitude allowed sampling of populations along a moisture gradient, while controlling for daylength and mean annual temperature. The seeds were reciprocally sown into prairie restorations located near each source population. Seedling emergence was lowest in the western South Dakota plot and highest in the Minnesota plot for all three source populations, corresponding to expectations given the moisture gradient. Seedling emergence was higher for the western South Dakota and Minnesota seeds than for the central South Dakota seeds in all three experimental plots. Survival of the seedlings was tracked for seven years, and their overall fitness was estimated using aster models. Although there was no evidence of local adaptation in seedling emergence, local recruits have higher survival than foreign recruits in both western South Dakota and Minnesota plots. These results suggest that restoration ecologists should use caution when introducing seed not locally sourced.

P65. The "Cottonwood Conundrum" and the 2011 Flood on the Missouri River

Mark D. Dixon*[1], *Christopher J. Boever[1], Victoria L. Danzeisen[1], Christopher L. Merkord[2], Michael L. Scott[3], W. Carter Johnson[4], [1]Department of Biology, University of South Dakota, [2]Geospatial Sciences Center of Excellence, South Dakota State University, [3]Department of Watershed Sciences, Utah State University,[4]Department of Natural Resources Management, South Dakota State University*

Decades of flow regulation by dams have led to declines in cottonwood (*Populus deltoides*) forests along many rivers in western North America. On the middle and upper Missouri, riparian forest area has declined by approximately 70% since the 1890s and remaining forests are dominated by older age classes, with 67% of forests >55 years old and limited recruitment over the last 30+ years. These remaining forests are threatened by invasive species, disease and emerging insect pests, and the chronic effects of long-term flow regulation. A large flood in summer 2011, however, had significant impacts on the Missouri River channel and its floodplain, with potential positive and negative effects on cottonwood forests. We investigated effects of the flood in terms of pre- to post-flood changes in forest structure, land

cover change, and cottonwood seedling recruitment. Woody stem densities, particularly in shrubs and saplings and in younger forest age classes, declined from pre- to post-flood. Sandbar area increased sharply and the area of young forests (e.g., <15 years old) decreased. Cottonwood recruitment was widespread following the flood, but occurred primarily on lower sandbar surfaces and was dominated by 2012 cohort seedlings, except for our study segment below Fort Peck Dam in Montana, where more natural flood recession patterns occurred in 2011. All in all, long-term potential for cottonwood forest expansion are limited by the geomorphic legacies of decades of flow regulation, unnatural flow patterns, and conflicting management goals. Ecosystem restoration efforts must address these constraints or look for opportunities in novel habitats (e.g., deltas) on the Missouri River system.

P66. Dynamics in Arbuscular Mycorrhizal Communities and their Impact on the Nutrient Allocation in Common Mycorrhizal Networks

Heike Bücking*, *Jerry A. Mensah, Carl R. Fellbaum, Department of Biology and Microbiology, South Dakota State University*

Arbuscular mycorrhizal (AM) interactions are many-to-many interactions; each fungal partner is able to colonize multiple host plants and to connect plants via a common mycorrhizal network, and each host plant is simultaneously colonized by multiple fungal species that differ in the benefit that they provide for their host plant. Host plant benefits are dependent on this AM community composition, but the mechanisms that control this composition and the root colonization by competing fungal symbionts are currently unknown. We studied whether the host plant demand and the access of competing AM fungal symbionts to specific nutrients plays a role for the AM community composition and examined how nutrients are allocated between plants that share a CMN. We changed the access of nutrients for the target plant and for both AM fungi in multicompartment-systems, and followed the nutrient transport by stable isotope labeling and examined the AM community composition of the target plant by q-PCR. The results demonstrate that fungal species differ in the benefit that they provide for their host plant; the colonization with a high quality fungus led to higher mycorrhizal growth responses and an improved phosphate and nitrogen nutrition. However, nutrient demand and the fungal access to specific nutrients have an impact on the AM community composition and a low quality fungus will be more successful in the fungal competition for the host plant, when the fungus has access to a nutrient that the host plant is in demand of. We will discuss the mechanisms that control the nutrient allocation within CMNs and will correlate the findings to biological market dynamics and the regulation of resource exchange in the arbuscular mycorrhizal symbiosis.

P67. Systems Analysis of the Physiological and Molecular Mechanisms of Sorghum Nitrogen Use Efficiency, Water Use Efficiency and Interactions with the Soil Microbiome

Daniel P. Schachtman*[1]*, Asaph Cousins[2], Jeffrey Dangl[3], Maria Harrison[4], Stephen Kresovich[5], Peng Liu[6], Jessica Prenni[7], Susannah Tringe[8], Ismail Dweikat[9], Arthur Zygielbaum[10], Rebecca Bart[11], Thomas Brutnell[11], Daniel Chitwood[11], Andrea Eveland[11], Todd Mockler[11], [1]Department of Agronomy and Horticulture and Center for Plant Science Innovation, University of Nebraska-Lincoln, [2]School of Biological Sciences, Washington State University, [3]Department of Biology, The University of North Carolina at Chapel Hill, [4]Boyce Thompson Institute for Plant Research, Ithaca, New York, [5]Genetics and Biochemistry, Clemson University, [6]Department of Statistics, Iowa State University, [7]Department of Biochemistry and Molecular Biology, Colorado State University, [8]The Department of Energy Joint Genome Institute Walnut Creek, CA, [9]Department of Agronomy and Horticulture, University of Nebraska, Lincoln, [10]School of Natural Resources, University of Nebraska-Lincoln, [11]Donald Danforth Plant Science Center, St. Louis MO*

The overall project objective is to establish a foundational, systems-level understanding of plant, microbial, and environmental interactions that will lead to strategies for enhancing growth and sustainability of sorghum through genetic and microbial adaptations to water and nitrogen limited environments. To compete in the biofuel energy market, cellulosic feedstocks will need to be high yielding and carbon neutral or negative while requiring low inputs. To avoid competition with existing food production systems, these crops will also need to be grown on marginal lands. This will require the introduction of novel traits to increase abiotic stresses tolerance associated with marginal soils. This project will utilize multiple interdisciplinary approaches in varied settings – including the laboratory, controlled environments, and the field – to identify plant genes and sorghum associated microbes that will enhance the sustainable production of sorghum as a biofuel feedstock. Basic knowledge about physiological and genetic mechanisms involved in nitrogen use efficiency (NUE) and water use efficiency (WUE) and potential mechanisms involved in microbe interaction will be generated. A range of methods will be used, including: classical whole plant physiology, stable isotope detection, phenomics, transcript profiling, metabolic profiling, 16S amplicon sequencing, metagenomics, microbial genome sequencing, comparative genomics, microbiology, genetics, and a range of computational methods for data analysis, integration and storage. To conduct these comprehensive studies, we have assembled a multi-institutional, interdisciplinary team with a wide range of expertise in these areas. This research will increase our knowledge about the genetic and physiological mechanisms involved in WUE and NUE, which will be used to create sustainable biofuel feedstock systems on marginal land. Identification of microbial community membership and testing of culturable microbes, as well as genetic dissection of sorghum genotype X microbe interactions, will result in new strategies for the development of microbial solutions to increase abiotic stress tolerance and sustainable sorghum systems. Two major resources will be created: a sorghum microbe collection and a multi-dimensional relational database to house and access the biological materials and data generated in this project.

P68. Deciphering Grapevine Genotype and Phenotype

***Anne Fennell*[1]**, *Shanshan Yang[2], Jonathan Fresnedo[3], Kalley Besler[1], Mani Awale[1], Gavin Sacks[4], David Manns[4], Jason Londo[5], Bruce Reisch[2], Lance Cadle-Davidson[6], James Luby[7], [1]Department of Plant Science, South Dakota State University, [2]Horticulture Section, Cornell University, [3]Bioinformatics Facility, Cornell University, [4]Department of Food Science, Cornell University, [5]USDA-ARS GGRU, Geneva, NY, [6]USDA-ARS GGRU, Geneva, N, [7]Department of Horticultural Science, University of Minnesota-St. Paul*

Sequencing technology has removed organismal barriers thus expanding the diversity that can be used to study and model environmental and pathological interactions. Increased sequencing efficiency continues to drive an exponential increase of genomic datasets; however, a bottleneck exists in processing the data into functional biological knowledge. Contributing significantly to this bottleneck is limited high throughput technology needed for identification and characterization of phenomics (capture of chemical, morphological, and developmental or organismal interactions) at the subcellular, cellular, tissue and organismal level. In contrast to the relative simple nucleotide structure of genomic data, phenomic data has significant acquisition and processing challenges that intensify the need for biological, technological and computational infrastructure development. Phenotype measures are diverse and need to be made at microscopic to landscape scale, in response to variable environmental cues and record molecular, biochemical, developmental, physiological and systems responses through time. Therefore development and use of such datasets in a perennial plant system requires multiple approaches and years to identify growth, architectural, fruit quality and sustainability traits. A combined computational, genetic and phenotype analyses have identified QTL and underlying candidate genes for low temperature response and fruit quality in grapevine.

P69. Ectopic Expression of a WRKY Gene Resulted in More Root Hair Formation in Arabidopsis

Yafang Wang*, *Department of Biology and Microbiology, South Dakota State University, Department of Grassland Science, Northwest A&F University, Yangling, Shananxi, China*

WRKY proteins are a large group of regulatory proteins that play diverse roles in plant growth/development and response to biotic and abiotic stress. One of the WRKY genes in Arabidopsis showed higher transcript levels under an array of biotic and abiotic stress conditions. When the gene was overexpressed by using a constitutive promoter from an actin gene, the transgenic plants exhibited denser root hairs. Overexpression did not significantly alter cell length and elongation rate in roots, indicating that the denser root hair was not due to smaller and thus more cells in unit root length. Closer observation of root cells revealed an abnormal initiation of root hairs in transgenic plants. The gene was expressed in various tissues including roots. However, the expression was not observed in root hairs based on GUS staining. A knockout mutant of the WRKY gene did not exhibit any alteration in root hair density. Thus, the denser root hairs in the overexpression transgenic plants may be a result of non-specific ectopic expression of the gene.

P70. Effects of Altered Fatty Acid Content on Germination of Transgenic Camelina sativa

Theresa F. Barnes,* ***Karen L. Koster****, Department of Biology, University of South Dakota*

Camelina sativa (L.) Crantz, a member of the mustard family, is an agricultural oilseed crop. Camelina is known to tolerate saline soils and low temperatures compared to many other crops, making Camelina a good contender for growing in northern North American and European semiarid soils. Camelina can be genetically modified, and recently, several lines have been generated with altered fatty acid profiles to facilitate use of the oil in a range of products. We tested two of these transgenic lines to see whether changing the fatty acid composition would affect the rate and percentage of seed germination in comparison to non-transgenic controls. Germination of the three lines was assessed during a two-week period at temperatures from 5°C to 30°C and in salt solutions ranging from 0 to 250 mM NaCl. We found that the percent germination of all three lines was not greatly affected by temperature, although all lines germinated more slowly at temperatures below 15°C. Seed germination was inhibited by NaCl concentrations of 150 mM and higher for all three lines with only the non-transgenic controls achieving greater than 40% germination in 200 mM NaCl. Seeds from all three lines germinated significantly more slowly in saline conditions. Although germination of the transgenic lines appeared to be somewhat more impacted by low temperature and salt than the non-transgenic controls, the differences were largely non-significant, suggesting that the genetic modification of these lines should not limit their use as crops in marginal soils or cold climates.