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Summary Abstracts and Titles

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General Session Abstracts

1. Role of the E3 ubiquitin ligase SAP5 in plant stress responses

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AtSAP5, one of approximately 14 members of the *Stress Associated Protein* gene family in Arabidopsis, was identified by its expression in response to salinity, osmotic, drought and cold stress. AtSAP5 is similar to OsiSAP1, an A20/AN1-type zinc finger protein implicated in stress tolerance in rice. To evaluate the function of AtSAP5 in the regulation of abiotic stress responses, transgenic Arabidopsis plants that over-express AtSAP5 were characterized, along with wild type and T-DNA knock-down plants. Plants that over-express AtSAP5 showed increased tolerance to environmental challenges including salt stress, osmotic stress and water deficit. Analysis of gene expression patterns under normal conditions and water deficit stress indicated that over-expression of AtSAP5 correlates with up-regulation of drought stress responsive gene expression. Recombinant AtSAP5 has E3 ubiquitin ligase activity *in vitro* and a potential target of AtSAP5 ubiquitination has been identified. These results indicate that AtSAP5 has E3 ligase activity and acts as a positive regulator of stress responses in Arabidopsis.

This work was funded in part by grants from the Oklahoma Center for the Advancement of Science & Technology.

2. Phosphobinding domain in plants

David Chevalier, Dept. of Biological Sciences, Mississippi State University

DAWDLE (*DDL*) is one of the eighteen genes that encode a protein with a Fork-Head Associated (FHA) domain in *Arabidopsis thaliana*. FHA is a phospho-threonine binding domain found in plant and animal proteins that function in DNA repair and cell cycle regulation. DDL also has an arginine-rich N terminal domain that has sequence similarity with proteins involved in RNA processing. The DDL ortholog, SMAD nuclear interacting protein 1, regulates RNA stability of the Cyclin D1 gene and the level of several microRNAs in humans. Similarly, DDL regulates the level microRNA in *Arabidopsis*. Our aim is to understand the function of DDL in RNA metabolism. Two *ddl* T-DNA insertion alleles in the WS-2 ecotype exhibit a strong phenotype and pleiotropic developmental defects including short root and hypocotyl, reduced fertility, and distorted organs. In contrast a *ddl* T-DNA insertion allele in the Columbia ecotype exhibits a weak *ddl* phenotype. To study the structure-function of DDL, twelve point mutations spanning *DDL*, were isolated and the severity of each point mutation allele is being compared to T-DNA alleles. To identify DDL interactors, we have identified several suppressors of *ddl* and a modifier of *ddl* caused by a natural variation between the WS-2 and Columbia ecotypes.

3. Generation of Cotton with Enhanced Resistance to Pathogens - Plastid Transformation Strategies

Caryl Chlan, University if Louisiana at Lafayette

4. Identification of selenium (Se)-responsive genes in a Se-hyperaccumulator *Astragalus racemosus*

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Plants with capacity to accumulate high levels of selenium (Se) are desired for phytoremediation and biofortification despite the fact that Se is not required for plant growth. Plants of genus *Astragalus* accumulate and tolerate high levels of Se, but slow growth, low biomass and non-edible properties limit their use for the above purposes. Genetic engineering may be an alternate and effective way to produce edible and high biomass Se-accumulating plants. A first step towards this goal is to identify genes that are responsible for Se accumulation and tolerance. Later, these genes can be introduced into other edible and high biomass plants. In the present study, we applied fluorescent differential display to analyze transcript profile of *A. racemosus*, a Se-hyperaccumulator, treated with 20 μM K_2SeO_4 to identify Se-responsive genes. A total of 214 differentially expressed bands were identified and 70 of them were studied in detail. Among 125 Se-responsive candidate genes, nine had their expression levels to be either induced or suppressed more than 2-fold by selenate treatment in two independent experiments. Six of them also showed 2-fold or more transcript changes when treated with 20 μM K_2SeO_3. Among identified genes, the *CEJ367* with unknown function was found to be highly induced by both selenate (1920-fold) and selenite (579-fold). Root- or shoot-preferential expression of these genes was also investigated. Further defining the functions of these identified genes will enhance our understanding of how selenium accumulation and tolerance is regulated in this Se-hyperaccumulator, which may allow us to create Se-enriched transgenic plants.

Graduate Student Oral Competition Abstracts

1. Identification of genes associated with cotton fiber development in a chromosome substitution line CS-B25

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Seventeen 17 interspecific chromosome substitution lines (CS-B lines) of upland cotton in *G. hirsutum* TM-1 have been recently developed and released to the public. These substitution lines have TM1 as background and contain either whole chromosomes or chromosome arms of *G. barbadense* (line 3-79) chromosomes. Among them, the CS-B25 line was reported to have superior fiber properties with increased fiber length, strength, and lower micronaire. CS-B25 has chromosome 25 from *G. barbadense* substituted into TM-1. A comparative analysis of CS-B25 and TM-1 will provide an opportunity to identify and study the genes associated with fiber quality traits. An integrated approach of Affymetrix cotton genome arrays and suppression subtraction hybridization (SSH) was used to identify differentially expressed genes in CS-B25. Poly (A) RNAs from 10-DPA (days post synthesis) fiber were used to perform SSH and microarray analysis. A subtracted cDNA library was constructed with CS-B25 as tester and TM1 as driver. Microarray and SSH analyses showed that 23 genes were up-regulated and 9 down-regulated in CS-B25. The majority of up-regulated genes identified were part of the ethylene signal pathway, ubiquitin-proteasome pathway, and cell wall synthesis.

2. The impacts of nitrogen utilization and allocation strategies on photosynthesis for an invasive grass, Phalaris arundinacea, in comparison to the native sedge, Carex stricta.

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Phalaris arundinacea (reed canary grass) is an invasive C3 perennial grass of temperate/boreal wetland communities. It is more abundant in areas with high nitrogen (N) inputs. Our hypothesis is *P. arundinacea* gains a competitive advantage over *Carex stricta*, the native sedge it often displaces, with increased N, because it allocates more N to features that enhance net carbon gain than *C. stricta* does. To test this hypothesis, we are comparing the responses of photosynthetic parameters and leaf morphology to various N levels (0.1 to 33 mM N) for *P. arundinacea* and *C. stricta.* We grow the plants in a course medium whose N content is controlled by varying the nitrate and ammonium in standard Hoagland's solution. With an increase in the N concentration provided, we observed an increase in net CO2 assimilation on an area basis (*A)*, leaf area, and specific leaf area (SLA) for *P. arundinacea*, but the effect on chlorophyll content was not clear. For *C. stricta*, the highest *A, SLA* and the chlorophyll content was determined for plants supplied only 2.75 mM N, suggesting that higher N levels led to the plants becoming pot-bound. It appears that N level affects total carbon gain for *P. arundinacea* by affecting *A*, leaf area, and SLA, allowing it to rapidly attain its full height and shade *C. stricta.* These advantages of high nitrogen had made the *P. arundinacea* become more aggressive and invade the wetlands displacing the *C. stricta.*

3. Development of Molecular Markers in Safflower (*Carthamus tinctorius* L.)

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Safflower *(Carthamus tinctorius* L.) is an oilseed crop considered to be one of the most drought tolerant crops in the world. An extensive rooting system gives safflower the ability to utilize water unavailable to most crops and scavenge nutrients leached down the soil profile. Recently characterized safflower accessions have the ability to be seeded in the fall and harvested in the spring, giving farmers a valuable crop to incorporate into a cropping rotation. A large amount of diversity between safflower accessions offers the potential for improved agricultural traits such as increased oil content and modified fatty acid composition. Traditional plant breeding has made improvements to this crop, but little molecular work has been done to date. Using molecular markers, our goal is to further characterize inter- and intra-safflower accession diversity in hopes of increasing oil content and producing a profitable crop for farmers in the Lower Great Plains and other arid regions across the globe. Recently a mini-core collection of safflower representing the wide range of diversity within the species was characterized using AFLP analysis. Using SSR molecular marker techniques, we plan to further characterize this mini-core collection in addition to several known winter-hardy safflower accessions. We will be specifically looking to identify markers associated with increased oil content, modified fatty acid composition, and winter hardiness. With the aid of such molecular markers, winter planted safflower can be enhanced to give farmers a profitable crop in conditions where other crops would struggle to compete.

4. Direct visualization of lipid molecules in cotton embryos by matrix-assisted laser desorption ionization mass spectrometry

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Cottonseeds store triacylglycerols (TAGs) as a carbon reserve for utilization during seed germination and seedling growth. These lipid molecules are packaged into discrete cytosolic lipid droplets and deposited throughout parenchyma cells in the cotyledons and embryonic axis. Chemical composition information is important for delineating the biosynthetic pathways operating to produce TAGs in oilseeds and lipidomics approaches have been developed for the routine identification and quantification TAGs in crude extracts. Conventional profiling of TAG molecular species involves whole tissue extractions and analysis of lipid mixtures, and consequently, information about the original cellular compartmentation of these TAG species is lost. One approach that has been developed for the analysis of lipid composition in situ is matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS). Here we have coupled the use of a N2 laser (337nm) with MS analysis (LTQ-Orbitrap) to visualize the lipid composition in cottonseed embryos. Both unfixed and fixed embryos were sectioned at -30°C on a cryostat and mounted onto glass slides for matrix deposition and MS imaging. The variability in the distribution of TAG and major phosphatidylcholine (PC) molecular species content as well as acyl chain length and degree of saturation suggests significant spatial regulation that is not as apparent from conventional lipid profiling. Additional profiling of the cotton-specific secondary metabolite, gossypol, restricted to glandular cells of the seed and the presence of TAGs containing one or more cyclic fatty acids localized to the embryonic axis demonstrate the ability to detect, localize and visualize compounds from traditionally inaccessible tissues.

5. Expression and purification of recombinant tung tree diacylglycerol acyltransferase 2

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Diacylglycerol acyltransferases (DGATs) are responsible for the last step of triacylglycerol (TAG) biosynthesis in eukaryotic organisms. Different forms of DGATs have nonredundant functions in TAG biosynthesis in species such as tung tree (*Vernicia fordii*), which contains approximately 80% high-value eleostearic acid in its seed oils. DGATs are integral membrane proteins and difficult to express and purify. The expression of DGAT2 as a full-length or partial protein in *E. coli* was not reported previously. The objective of this study was to develop a procedure for full-length DGAT2 expression and purification in *E. coli*. Expression plasmid was engineered to express tung DGAT2 fused to maltose binding protein and poly-histidine affinity tags. Immunoblotting showed that recombinant DGAT2 was expressed and localized in both soluble and insoluble fractions of *E. coli*. DGAT2 fusion protein from the insoluble fraction was partially solubilized by 7 detergents (Brij 35, CHAPS, NP-40, SDS, Triton X-100, Tween 20 and Tween 80) with SDS being the most effective. Recombinant DGAT2 was purified to near homogeneity by SDS solubilization and Ni-NTA affinity chromatography. This study represents the first description of a procedure for producing full-length DGAT2 fusion protein from any species using a bacterial expression system. Production of recombinant DGAT2 should help to understand plant oil biosynthesis and create new oilseed crops with value-added properties (Supported by USDA-ARS Quality and Utilization of Agricultural Products Research Program.)

6. Neutral lipids accumulate in leaves of Arabidopsis mutants

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The gene designated CGI-58 (comparative gene identification-58) in humans is believed to participate in hydrolysis of stored lipids. A disruption in this gene causes the neutral lipid storage disorder -- Chanarin-Dorfman syndrome. Individuals affected by this disease accumulate intracellular lipid droplets in atypical tissues such as skin and blood cells and overall lipid homeostasis is disrupted. A T-DNA insertional mutant of a CGI-58 homolog in *Arabidopsis thaliana* results in a similar cellular phenotype, where tissues that do not normally accumulate storage lipids (e.g., mature leaves) contain excess lipid droplets. Mature leaves from *cgi-58* knockout plants produced a greater than 10-fold increase in neutral lipids over wild-type plants. Isolated lipid droplets from both wild-type and *cgi-58* contained triacylglycerols with known leaf-type fatty acids. Seeds from *cgi-58* contained normal lipid amounts, and showed no difference in viability when compared to wild-type seeds. Our results suggest that the CGI-58 genes in plants and animals may play a related role in lipid homeostasis. Future work is aimed at understanding the mechanisms by which CGI-58 influences neutral lipid accumulation in vegetative tissues of plants, perhaps facilitating new strategies for oil production in crops for industrial feedstock or bioenergy applications.

7. Identification of effector proteins of histone lysine methylation in plants

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Histones are basic proteins associated with nuclear DNA in chromatin. Histones undergo different post translational modifications. Histone lysine methylation can affect the chromatin structure and function, influences many biological processes involving the chromatin template, like transcriptional regulation, DNA repair and genome integrity. Lysine residues of histone H3 can be methylated at positions 4, 9, 27, 36, and 79. They can be methylated in three different (mono-, di-, and tri-) methylation states. The methylated lysines of histones can serve as binding/ docking sites to recruit nuclear effector proteins in a position and state specific manner. The lysine methylation of histones is highly complex and is associated with both activation and repression of transcription depending on the position and state of methylation. We applied the peptide pull-down assay, an unbiased biochemical approach using biotynlated histone H3 peptides to identify the proteins that bind to methylated lysines of histone H3 in Arabidopsis and rice. Results from the pull down assay indicate that di-methyl modification of lysine 36 reduces the interaction/ binding of histones H2A and H2B with histone H3. The identification of effector proteins of histone methylation would aid in understanding the role an evolutionary importance to histone lysine methylation and how histone methylation regulates gene activation and silencing.

8. Enhancing Cotton Fiber Elongation and Cellulose Synthesis by Manipulating Fructokinase Activity.

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The strength of the economically-important cotton "fiber" depends upon its cellulose content. Cellulose synthesis requires UDP-glucose that is produced from sucrose by sucrose synthase (SuSy). The other product of the SuSy-catalyzed reaction is fructose, which can inhibit SuSy. Our hypothesis was that enhancing the removal of fructose via phosphorylation by fructokinase would reduce SuSy inhibition and improve cellulose synthesis in cotton fiber. To test this hypothesis, we have developed transgenic cotton plants harbouring the fructokinase gene, *LeFRK1*, from tomato under the control of the 35S promoter. Six T1 plants for each of six transgenic lines and a control, null line were grown in a greenhouse to study the effects of enhanced fructokinase activity on fiber development. At least three lines had moderate and three had low expression of *LeFRK1* in leaves and in fibers at the stages of elongation and secondary wall synthesis. However, only one line had enhanced (three fold) extractable fructokinase activity in leaves, potentially due to post transcriptional modifications of *LeFRK1* transcripts or protein. Elongating fibers of four lines exhibited an enhancement in fructokinase activity from two to three fold, but this activity had declined for most lines by the secondary wall stage, whereas the activity for such fibers of null plants had increased. There was no improvement in seed cotton mass for plants over-expressing *LeFRK1*. However, we will be having the fiber tested for length and strength properties. Our next experiment will test the hypothesis that elevated fructokinase activity will improve fiber development under drought conditions.

***9. Arabidopsis thaliana* calcium-dependent lipid-binding protein (AtCLB) negatively regulates abiotic stress response**

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The *Arabidopsis thaliana* calcium-dependent lipid-binding protein (AtCLB) contains a C2 like domain that is known to interact with phospholipids in a Ca^{2+} dependent manner. Ca^{2+} is one of the most important second messengers of plant stress signal transduction pathways and hence the study of functions of plant proteins containing Ca^{2+} binding domains and their potential role in stress signaling will give new insights into novel crop improvement strategies. Here we show the expression of this protein, the lipid binding characteristics as well as its involvement in stress responses. AtCLB binds specifically to the promoter of *Arabidopsis* thalianol synthase gene (AtTHAS1), whose expression is induced by gravity and light. Transcripts of this gene can be detected in all tissues examined including root, leaf, stem, flower and silique of *A. thaliana* with relatively a high level in rosette leaves. Immunofluorescence analysis revealed that AtCLB protein is localized in the nucleus of cells in *Arabidopsis* root tips. Lipid binding characteristics of the purified protein using lipid strips revealed that the protein is specifically interacts with ceramide, a component of the cell membrane that mediate diverse biological processes. Analysis of the T-DNA knockout mutant lines of the gene from *A. thaliana* showed an enhanced salt tolerance and a modified gravitropic response. Furthermore, these mutant lines were able to maintain their leaf relative water contents under prolong water deficit conditions compared to wild type plants suggesting a possible involvement of the gene as a negative regulator in stress response.

10. Proteomic analysis of phosphoproteins involved in abscisic acid signaling in *Arabidopsis*

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Abscisic acid (ABA) is an important plant hormone regulating many aspects of plant growth and development, such as stress tolerance, seed dormancy, and germination. ABA signal transduction pathway is complicated, possibly involving many regulators. The phosphorylation and dephosphorylation of regulatory proteins or enzymes play a key role in ABA signal transduction pathway. *Arabidopsis* SnRK2 protein kinases and PP2C-type protein phosphatases are thought to be positive and negative regulators, representatively, in ABA signaling pathway. However, the function mechanism of these kinases and phosphatases in ABA signaling is still unknown. The objective of this project is to identify the target phosphoproteins that interact with SnRK2s or PP2Cs in *Arabidopsis*. We analyzed the total proteins in two *Arabidopsis* mutant: srk2-2 and abi1-1 after ABA treatment using two-dimensional gel electrophoresis coupled with mass spectrometry approaches. Our data shows that some phosphoproteins are involved in *Arabidopsis* ABA signaling pathway.

11. Identification of Genotypes and Traits Associated with Hydrolysis Yield Variability in *Sorghum bicolor*

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In a changing and unpredictable climate, renewable bioenergy will play an increasingly important role worldwide. Cellulosic bioenergy contributes less CO2 to the atmosphere than fossil fuels and is a renewable resource. As a bioenergy crop, *Sorghum bicolor* offers many advantages including drought tolerance and high biomass in the form of seed, lignocellulose, and soluble sugar. In addition, sorghum possesses a small sequenced diploid genome (~730 Mbp) which makes it an ideal model for C4 grasses. Identifying genes and traits related to hydrolysis yield will lay the groundwork for breeding programs that lead to increases in bioenergy yield. By increasing the efficiency of bioenergy production, the amount of land potentially needed for sustainable bioenergy production can be greatly decreased. In an attempt to identify the genes and genotypes associated with high hydrolysis rates in sorghum, a high-throughput screen of hydrolysis rates was conducted on a diversity panel of ~400 field-grown sorghum varieties and validated on >20 randomly selected genotypes from a second grow out in an alternate location. Results showed a diverse range of hydrolysis rates ranging from 0.6-2.7 ug/hr/U *T.viride* cellulase (avg=1.5mg/hr/U). Additionally, a subset of 20 varieties was grown in a second location to investigate the relationships between crystallinity index, pretreatment and hydrolysis rate. We hypothesize that an identifiable genetic component exists that affects the rate of hydrolysis, crystallinity index as well as pretreatment efficiency in *Sorghum bicolor*. Early observations that support this hypothesis will be discussed.

12. Comparative proteomics of recalcitrant seed death in *Spartina alterniflora*

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Spartina alterniflora is a dominant salt marsh species along the US Atlantic and Gulf Coasts, and its establishment is important in reducing coastal erosion in Louisiana. However, long-term preservation of *S. alterniflora* is challenging because the seeds are recalcitrant, losing viability when dried below 45% water content. Comparative proteomics between *S. alterniflora* and orthodox, desiccation-tolerant *S. pectinata* seeds was performed to identify heat-stable (water soluble after 40 min at 950C) proteins that may be associated with desiccation tolerance. The heat-stable proteomes of *S. alterniflora* and *S. pectinata* contained 174 and 312 spots, respectively, as resolved by two-dimensional gel electrophoresis and detected by *in silico* software analysis (Progenesis SameSpots). Ten spots, which were present in orthodox *S. pectinata* but missing in recalcitrant *S. alterniflora*, have been sequenced so far. Several proteins share homologies to known sequences for cystatin (cysteine protease inhibitor), glyceraldehyde-3-phosphate dehydrogenase and a stress-responsive protein; these three proteins are associated with the desiccation-tolerant state in other organisms. Gels stained with PRO-Q Diamond revealed several *ca.* 20-24 kDa phosphorylated proteins that were highly expressed in *S. pectinata* but not in *S. alterniflora*. In addition, at least two glycosylated proteins (*ca.* 20 kDa and 60 kDa detected with PRO-Emerald 300 stain) were uniquely expressed in *S. pectinata*. These data suggest that a modestly-sized suite of proteins and post-translational modifications may confer desiccation tolerance to *S. pectinata* seeds, and demonstrate the utility of comparing related species to understand physiological processes.

13. Over-expression and antisense suppression of a gene encoding a heat shock factor binding protein in *Arabidopsis* affect the photosynthetic tolerance to heat stress

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Plant cells respond to heat stress by synthesizing heat shock proteins (HSPs). The up-regulation of HSP genes is induced by a heat shock transcription factor (HSF). Controlling the level of active HSF includes the use of a small protein that binds to it and prevents it from activating a HSP gene. The level of this heat shock factor binding protein (HSBP) rises with exposure to heat. Our aim has been to determine whether suppressing the production of the HSBP would maintain the plant in a heat-acclimated state and provide enhanced protection for photosynthesis during heat exposure. We compared the rates of CO2 assimilation (*A*) from 20 to 40oC for Arabidopsis plants over-expressing the sense and antisense HSBP gene with *A* for wild-type plants. There were no significant ($P > 0.05$) genotypic differences in *A* at 25 and 30°C. At 35 and 40°C, there were no significant differences between *A* for wild-type plants and the antisense lines, but *A* for the over-expressing lines was significantly ($P < 0.001$) lower than *A* for wild-type plants. The *A* relative to that at 25oC for the antisense lines was only slightly higher than that for wild type at 40oC. Maintaining the plants at 40oC and a high light intensity reduced the extent of photosystem II inactivation to the same extent for wild-type and antisense leaves after dark acclimation. The reduction for over-expressing leaves was greater. Therefore, normal levels of HSBP do not appreciably reduce an initial heat response, but higher levels increase heat susceptibility.

14. Photosynthetic response of invasive grass, *Phalaris arundinacea*, and native sedge *Carex stricta* that it replaces to climate change and nitrogen availability

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Increasing temperature with climate change in combination with increased inputs of nitrogen from agricultural processes will increase the risk of invasion by exotics in already sensitive freshwater wetlands. Wetlands have high intrinsic value due to their ability to support high biodiversity, making efforts to prevent exotic invasion in wetlands paramount. The invasion of *Phalaris arundinacea* in wetlands inhabited by the native sedge, *Carex stricta*, is an ideal model system to study the effects of climate change and eutrophication on invasion from a physiological standpoint. This system can be easily studied under controlled environmental conditions to examine how the two species may differentially respond to alterations in present temperatures in the presence of enhanced N input. We have determined that *P. arundinacea* exhibits higher rates of photosynthesis, higher specific leaf areas, and great net carbon gain across a broad range of temperatures compared to *C. stricta*. Present research is addressing the following questions: (a) How do seasonal changes affect photosynthetic and respiratory potentials of each species under the current climatic conditions?; (b) How will increased temperatures affect leaf photosynthesis and respiration under different N treatments for each species?; (c) How will increased temperature affect the ecological process of invasion?; (d) What are the physiological bases for the responses to increased temperature and N? Answers to these questions can lead to better targeting of management practices for all invasive species who have an advantage in net carbon gain over native, less-aggressive species.

Undergraduate Student Oral Competition Abstracts

***1. Medicago truncatula NIP* affects lignin and callose deposition and regulation of genes important for secondary metabolism and fungal defense**

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Symbiotic nitrogen fixation in legume root nodules provides a route for conversion of atmospheric N2 to ammonia. *NIP* (Numerous Infections and Polyphenolics), a gene with a predicted transmembrane topology, was discovered through a forward EMS screen for *Medicago truncatula* nodulation mutants (Veereshlingam et al. 2004 Plant Physiol 136: 3692; Yendrek et al. 2010 Plant J 62: 100). *nip* mutants accumulate brown pigments and have abnormal non-fixing nodules. We sought to determine the identity of the phenolic compounds accumulating in *nip* nodules and to find causes for the developmental failure of *nip* nodules. To accomplish this, we used histochemical stains with *nip* and wild-type A17 nodules, including stains for lignin and callose, compounds associated with defense responses. We found that the nodule endodermis is not properly lignified in *nip*, and that some cells are either preferentially lignified or filled with pigments. Callose-deposition appears to be greatly reduced in *nip* nodules, and may suggest particular defects in nodulation. Previous researchers utilized Affymetrix 60k genome arrays to query gene expression in *nip*. Through transcriptomics, we found that the *nip* mutant is mis-regulated in many genes known to be important for symbiosis. These genes include biosynthesis genes of the phenylpropanoid and terpenoid pathways, as well as fungal defense-genes --correlating with the histochemical analysis.

Titles & Abstracts for Posters (* indicates undergraduate competition entry)

Identification of N-acylethanolamine Resistant(NRA) Mutants in *Arabidopsis thaliana*

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Toward the identification and functional characterization of a second fatty acid amide hydrolase (FAAH) in Arabidopsis

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N-Acylethanolamines (NAEs) are fatty acid derivates which are linked via an amide bond with an ethanolamine group. NAEs can be characterized as lipid mediators in plant and animal systems due to their potent and diverse bioactivities at low concentrations. The biological effects of NAEs are terminated through their hydrolysis into ethanolamine and free fatty acid by a membrane enzyme known as the fatty acid amide hydrolase (FAAH). Therefore FAAH enzymes represent an important target to better understand the functions of NAE metabolism in many cellular processes. By sequence homology with a previously characterized FAAH in Arabidopsis thaliana (FAAH1), we have identified a gene candidate that may encode a second FAAH enzyme in Arabidopsis. This candidate FAAH2 gene locus, At5g03760, gives rise to two splice variants encoding two isoforms (FAAH2-1 and 2-2) with a slight difference of 7 amino acids, MVPFAIG, within the characteristic amidase signature domain. Detectable NAE hydrolase activity was recovered in extracts of faah1 T-DNA knockout plants, but no FAAH enzyme activity was detected in faah1/faah2 double knockouts. FAAH2 overexpressing transgenic plants showed some tolerance to the effects of NAE (albeit not as dramatic as FAAH1 overexpression) suggesting that this enzyme indeed functions as an authentic FAAH in planta. According to public databases, transcript levels for FAAH2 are highest in maturing and dry seeds. Interestingly, after artificial, accelerating aging, the faah1/faah2 double knockout seeds showed a marked reduction in viability, while the FAAH2 overexpressing seeds were much more tolerant to aging in comparison to wild-type seeds. The disruption of both FAAH genes resulted in seeds with large concentrations of NAEs, especially after the accelerated aging experiments. Future experiments are aimed at assessing the biochemical differences of the FAAH enzymes and their corresponding physiological importance in plant tissues.

Functional genomics of oxygenic photosynthesis in the model green microalga Chlamydomonas reinhardtii*

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We will be presenting our research about functional genomics of photosynthesis using insertional mutagenesis of the green microalga Chlamydomonas reinhardtii. Chlamydomonas can be grown autotrophically as well as heterotrophically (can use acetate as a carbon source, with no requirement for light) in a laboratory setting. It can be easily genetically manipulated and its genome has been sequenced. For these reasons, Chlamydomonas is an ideal model organism for studying eukaryotic oxygenic photosynthesis.

For our generation of insertional mutants, we have selected the cell walled strain, $4A^+$ with the 137c genetic background, as a parental strain. After comparative growth studies of $4A^+$ with three other wild-type strains under dark conditions, we selected $4A^+$ as our candidate strain because of its ability to grow well and remain green in the dark. The linearized bacterial plasmid pBC1, containing paromomycin resistance, was used to transform the strain $4A^+$ to produce a random insertional mutant library. Even though, mutants incapable of photoautotrophic growth can be isolated and maintained as acetate-requiring mutants in the light, this approach does not allow the recovery of light sensitive photosynthetic mutants which are defective in the CO_2 fixation reactions of photosynthesis. Therefore, in order to isolate light sensitive and non-photosynthetic mutants, transformants were selected in the dark on acetate-containing medium. This insertional mutant library is currently being subjected to multiple primary and secondary phenotypic screenings to identify photosynthesis-related mutants, which are deficient of pigment, light sensitive, non-photosynthetic, or hypersensitive to reactive oxygen species.

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Metabolism and Action of Polyunsaturated *N*-Acylethanolamines (NAEs)

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N-Acylethanolamines are endogenous fatty acid derivatives with potent bioactivities in a wide range of eukaryotic organisms. In *Arabidopsis thaliana*, the majority of NAEs are polyunsatured species—acylethanolamines of linoleic and linolenic acids (NAE18:2 and NAE18:3). Several lines of evidence indicate that the lipoxygenase (LOX) pathway and a hydrolysis pathway (by fatty acid amide hydrolase, FAAH) cooperate in the depletion of endogenous polyunsaturated NAEs during seedling establishment. This may be relevant to seedling growth since both NAE18:2 and NAE18:3 inhibit seedling growth when supplied exogenously to growth medium. This seedling growth reduction is more profound in *faah1* knockouts than in wild type, and not observed in FAAH1 over-expressors (increased capacity for NAE turnover). NAE 18:3-treated seedlings showed a dose-dependent disruption of normal growth that was more severe than with NAE 18:2. *faah1* knockout seedlings treated with NAE18:3 showed not only a marked reduction in seedling growth after 3 days of treatment, but also a profound bleaching of cotyledons (much more rapidly than wild-type seedlings) suggesting that these effects were attributed to a reduced capacity for NAE hydrolysis. This bleaching effect was observed only with NAE18:3, and not with NAE18:2 or 18:3 free fatty acid. When seedlings were removed to fresh media without NAE18:3, cotyledons resumed green color and seedlings resumed normal growth. NAE18:3-dependent disassembly/reassembly of chloroplasts in cotyledons was documented by chlorophyll autofluorescence under confocal laser scanning microscopy and by a loss of extractable pigments. The resumption of growth after removal of NAE18:3 suggested that this metabolite(s) is (are) not detrimental to seedlings and that seedlings have a mechanism to reverse its accumulation when the exogenous source of NAE18:3 is removed. The precise active metabolite(s) of NAE18:3 remains to be identified but 9- and 13- hydroxy derivatives of NAE 18:3 were detected as endogenous constituents in seedling extracts. These results suggest that NAE oxidation may generate novel bioactive oxylipins that may be especially relevant to chloroplast biogenesis in seedlings.

Smaller higher-yielding binary Ti Vectors pLSU with co-directional replicons for *Agrobacterium tumeficeint*-mediated transformation of higher plants

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We constructed smaller higher-yielding binary Ti vectors of *Agrobacterium tumefaciens* to increase the cloning efficiency and plasmid yield in *Escherichia coli* and *A. tumefaciens*, and to improve the transformation efficiency of higher plants. We modified all four components of vectors, i. e. ColE1 replicon (715 bp) for *E. coli* and VS1 STA/REP replicon (2,659 bp) for *A. tumefaciens*, a bacterial kanamycin resistance gene (999 bp), and the T-DNA region (170 bp). We reduced the size of this kanamycinbased vector to 4,543 bp and introduced a number of mutations to increase the copy number and other functionality. The transcriptional direction of STA/REP replicon can bethe same as that of ColE1 replicon (co-directional transcription), or opposite (head-on transcription) as in the case of widely used vectors (pPZP or pCambia). New binary vectors with co-directional transcription yielded in *E. coli* up to four-fold higher transformation frequency than those with the head-on transcription. In *A. tumefaciens* the effect of co-directional transcription is still positive in 1.2 to 1.7-fold higher transformation frequency than that of head-on transcription. Transformation frequencies of new vectors are over ten-fold higher than those of pCambia vector in *A. tumefaciens*. DNA yields of new vectors were three to five-fold greater than pCambia vector in *E. coli*. The proper functions of the new T-DNA borders and new plant selection marker genes were confirmed after *A. tumefaciens*-mediated transformation of tobacco leaf discs. Higher number of leaf discs induced calli and the total fresh weight of leaf discs were larger than positive controls. Genetic analysis of kanamycin resistance trait among T1 progeny showed that the kanamycin resistance and sensitive traits were segregated into the 3:1 ratio, indicating that the kanamycin resistance genes were integrated stably into a locus or closely linked loci of the nuclear chromosomal DNA of the primary transgenic tobacco plants and inherited to the second generation.

A Comparison of Nitrogen Allocation Strategies for the Invasive Wetland Grass, *Phalaris arundinacea*, and the Native Sedge, *Carex stricta

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Phalaris arundinacea (*Pa*, reed canary grass) is a C3 perennial grass invasive in temperate/boreal sedge meadows, often displacing the native sedge, *Carex stricta* (*Cs*). Its abundance is linked to a high soil nitrogen content. In an effort to learn why *Pa* is a better competitor than *Cs* under high nitrogen conditions, we grew both species in a coarse, non-soil medium supplied with Hoagland's solution containing either 33 mM or 0 mM nitrogen for 4-5 months. Since nitrogen can be mobilized and made available to growing areas when nitrogen availability is low, we examined young and old leaves of both species. Although rates of CO2 assimilation (*A*) for *Pa* grown without nitrogen were 60% of the *A* for *Pa* grown with nitrogen, the rates did not vary between young and old leaves. Differences in chlorophyll concentration were not correlated with the treatment differences in *A*. For *Cs* plants growing with no added nitrogen, *A* was 55% of *A* for plants growing with 33 mM nitrogen. Although nitrogen treatment did not affect the chlorophyll content for old leaves, young leaves of plants without added nitrogen had higher chlorophyll content than young leaves with added nitrogen. Leaf area and specific leaf area increased with added nitrogen for *Pa*, but they remained unchanged or decreased for young leaves of *Cs*. Increasing nitrogen has a positive effect on factors affecting carbon gain for *Pa* but less of an effect or even a negative effect on these factors for *Cs*.

Using canopy temperature to predict variation in cotton development

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Immunohistochemical Investigation of Cotton Carpel Tissue Exposed to Xylanolytic Hydrolases of Aspergillus flavus

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Identifying Proteins that Interact with DCD1, a PP2A Phosphatase Regulatory Subunit Needed for Cell Division Plane Orientation in Maize

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During plant cell division, a cortical ring of cytoskeletal filaments called the prepophase band (PPB) establishes the plane of division. The PPB disappears as the mitotic spindle forms. As it disappears, its zone of localization becomes the cortical division site (CDS). The phragmoplast is a structure specific to plant cells that builds the new cell wall during cytokinesis. The phragmoplast expands towards the CDS and separates the two daughter cells. DCD1 and ADD1, closely related B" regulatory subunits of the PP2A phosphatase complex in maize, play a role in the PPB establishment. Plants that lack DCD1 and ADD1 fail to make PPBs, therefore, all cell divisions are disrupted. It has been shown that ADD1 and DCD1 co-localize with the PPB and CDS during metaphase, but not during telophase/cytokinesis. Thus, DCD1 and ADD1 are involved in PPB formation and CDS establishment. Our goal is to find the proteins that interact with DCD1.

To identify DCD1 interacting proteins, a Matchmaker gold yeast two-hybrid screen was performed by fusing DCD1 to the GAL4 transcription factor binding domain (BD) and screening it against a library of maize proteins fused to the GAL4 activation domain (AD). In a yeast two-hybrid screen when the bait and library fusion proteins interact, the DNA-BD and AD are brought into proximity to activate transcription of four independent reporter genes. Any positive interaction will be re-tested, isolated and sequenced to determine the identity of the interacting protein. Then, the interaction will be confirmed via in vivo interaction.

Understanding division plane orientation using the maize *dcd* mutants
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In plants, cell wall placement at cytokinesis is determined by the position of the preprophase band (PPB) and the subsequent expansion of the phragmoplast, which deposits the new cell wall, to the cortical division site delineated by the PPB. New cell walls are often incorrectly orientated during asymmetric cell divisions in the leaf epidermis of the *discordia* (*dcd1*, *dcd2*, and *dcd3*) maize mutants. Cloning *dcd1* showed that it encodes an orthologue of the *Arabidopsis fass/ton2* gene, a putative B'' regulatory subunit that targets the serine/threonine phosphatase PP2A to appropriate substrates. An antibody that recognizes DCD1 and a closely related protein, ADD1, localizes these proteins to PPBs and, more surprisingly, the cortical division site that remains after PPB breakdown. Considered all together, these experiments suggest that phosphatase activity regulated by DCD1/ADD1 is needed for PPB formation and cortical division site establishment. To identify additional proteins needed for division plane orientation in plants and to expand our knowledge of DCD1 function, we are performing a yeast two-hybrid screen to find proteins that interact with DCD1. We are also using a map-based cloning approach to determine the molecular identities of the genes mutated in the *dcd2* and *dcd3* mutants. Progress on each of these projects will be reported.

Is calcium a key player in interaction of FASS/TONNEAU2 and its other proteins in *Arabidopsis thaliana*?
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Arabidopsis thaliana FASS/TONNEAU2 is required for preprophase band (PPB) formation, and has been shown to interact with an Arabidopsis type A subunit of the PP2A phosphatase in the yeast two-hybrid system. In Arabidopsis *fass* mutants, abnormalities of the cortical microtubule cytoskeleton, such as disorganization of the interphase microtubule array and lack of PPB formation before mitosis markedly affects cell shape and arrangement as well as overall plant morphology. Loss of dcd1/add1, the maize *fass* homologues gives rise to a similar phenotype in Zea mays. FASS has a calmodulin (CaM) and two calcium-binding sites, which raises the question we are trying to address: "Does calcium binding contribute to the localization and function of FASS at the PPB?" The FASS gene will be fused with YFP to allow for its visualization, and the Ca2+ binding sites will be mutated. These constructs will be transformed into the *fass* mutant. The localization of FASS and rescued/not-rescued phenotype will be observed, and results analyzed to determine if Ca2+ is a key player in FASS/TONNEAU2 localization and functioning.

Vicilin Derived Plant Defense Proteins
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Effects of salinity and pathogen attack on the epidermal cell walls of seagrass *Thalassia testudinum*
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Engineering and expression of a lipid-inducible, self-regulating lipase in *Saccharomyces cerevisiae*. Implications for bioconversion of commodity vegetable oils to novel value-added products.

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The *Yarrowia lipolytica* lipase 2 gene (*LIP2*) was cloned into *Saccharomyces cerevisiae* expression vectors and used to generate baker's yeast strains that secrete active Lip2p enzyme activity. The *LIP2* gene was expressed behind the *Saccharomyces cerevisiae PEX11* promoter, which maintains basal transgene expression levels in the presence of sugars in the culture medium, but is rapidly upregulated by fatty acids. Northern blotting, lipase enzyme activity assays, and gas chromatographic measurements of cellular fatty acid composition after lipid feeding all confirmed that the *PEX11 promoter-LIP2* construct was responsive to lipids in the media. Cells expressing *LIP2* responded rapidly to either fatty acids or triacylglycerols and accumulated high levels of the corresponding fatty acids in intracellular lipids. These data provided evidence of the creation of a self-regulating positive control feedback loop that allows the cells to upregulate Lip2p production only when lipids are present in the media. Regulated, autonomous production of extracellular lipase is a necessary step towards the generation of yeast strains that can serve as biocatalysts for conversion of low-value lipids to value-added triacylglycerols and other novel lipid products.

Stress Tolerance and Galactinol Synthase in *Verbascum phoeniceum***

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Raffinose family oligosaccharides (RFOs) are responsible for several important functions in plants. RFOs are ubiquitous in angiosperms, as they are essential for seed development. In some flowering plant groups, RFOs are produced in response to stressful situations, including low temperatures and drought. Additionally, RFOs are required for phloem loading in certain vascular plants. The initial step of RFO formation is synthesis of galactinol, which is catalyzed by the gene product galactinol synthase (GAS). In *Verbascum phoeniceum* there are two known *GAS* genes (Vp*GAS1* and Vp*GAS2*), both of which are known to function in phloem loading. However, it is unknown whether either gene is expressed in response to stress. To determine if either gene has a role in stress tolerance , the promoters for Vp*GAS1* and Vp*GAS2* will be fused to the GUS reporter gene and transgenic plants will be created. Transgenic plants will then be subjected to various stressors and analyzed for GUS reporter gene activity indicating potential roles of each *GAS* gene in tolerance to adverse conditions.

Low co-cultivation temperature at 20oC improved *Agrobacterium tumefaciens*mediated transformation of tobacco leaf disks

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The importance of controlled temperature during the four-days co-cultivation period was evaluated under the most physiologically relevant conditions for *Agrobacterium tumefaciens*-mediated transformation of tobacco (*Nicotiana tabacum* L.cv. Xanthi (nn, Smith) leaf disks. We compared the effect of temperatures ranging from15, 18, 20, 22 to 25oC on the stable expression of β-glucuronidase (GUS) activity of 14 days old hygromycin-selected leaf disks, and on the increase in the fresh weight yield of 28 days old kanamycin-selected calli. The highest average of GUS activity was obtained at 20°C among the five temperatures tested although the differences among the 18, 20, and 22°C treatment were not statistically significant. The lowest average of GUS activity was observed at 25°C. The GUS activity in 15 °C treatment was an intermediate between the highest and lowest averages, and was not statistically significantly different. We concluded that cocultivation at 20°C is the most effective condition for the stable expression of maximum GUS activity after transformation of tobacco leaf disks. The highest increase in the plate average of fresh weight yields was obtained at 20oC among the five temperature tested. The 20oC treatment was statistically significantly better than the 15 and 18oC treatments. The 20oC co-cultivation treatment resulted in the higher FW yield than 22 and 25oC even though the differences were not statistically significant. We concluded that low co-cultivation temperature at 20 ºC under the most physiologically relevant conditions is the most critical determinant for the reproducible maximum increase in both the fresh weight yield and stable expression of GUS activity after transformation of tobacco leaf disks.

Phenotypical and genetic characterization of tomato lines expressing *Arabidopsis* truncated ERECTA gene (Δ kinase)

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The ERECTA gene encodes a leucine-rich repeat receptor-like kinase (LRR-RLK) and has been shown to affect plant growth rates, stomatal patterning and transpiration efficiency, inflorescence and floral organ size and shape. In Arabidopsis, the ERECTA gene affects inflorescence development, and controls organ growth by promoting cell proliferation. ERECTA is associated with growth enhancement. The ERECTA genes may find utility in controlling the size of the whole plants, or specific organs in plants. In this project we are studying the changes of plant architecture and growth rates manipulating the signaling pathway in tomato (cv. Micro-Tom). The strategy applied is a pathway downregulation of tomato by integration of a dominant negative version of ERECTA (At::Kinase) from Arabidopsis that encodes a truncated version of leucine-rich receptor-like kinase. Notably, tomato plants transformed with At::Kinase under control of 35S promoter showed a strong dwarf phenotype and produced fruits instead of developing vegetative tissues, whereas tomato plants transformed with At::Kinase under control of a native Arabidopsis promoter (ER) developed normal structures, but showing a reduction of 36% in plant height and significant reduction of leaf size during the first stages of development. Additionally At::Kinase transgenic tomato plants exhibited a delay in development of the floral organ.