

Southern Section American Society of Plant Biologists Annual Meeting April 10-12, 2010



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Phylogenetic analysis of GRAS family genes

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The GRAS family of transcription factors, including GIBBERELLIN-INSENSITIVE(GAI), REPRESSOR OF GA1-3(RGA) and SCARECROW(SCR), are a conserved family of genes which govern many diverse aspects of plant development. There is evidence that this family is likely to have originated before the advent of land plants. Thus an analysis of the phylogeny of the GRAS genes might help us to understand how these genes have diversified especially with the radiation of flowering plants and have taken on many more functions.. We observe that most subfamilies of the GRAS family for flowering plants include both monocot and eudicot species indicating considerable diversity before the monocot-eudicot split. We track in particular members of the HAM (HAIRY MERISTEM) clade and observe how the highly conserved miRNA binding site, a sequence of 21 nucleotides, has been precisely retained in some genes and somewhat modified in others. Perhaps some or all of these modifications mean a loss of negative regulation by miRNAs of the affected genes.

Study of Dinoflagellate Phylogeny by Characterization of Protease Activity

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Dinoflagellates are single-celled eukaryotic phytoplankton that, due to their complex genome and lack of major morphologic differences, are difficult to identify. In this study we analyzed the protease profiles of five dinoflagellate species, *Prorocentrum minimum*, *Prorocentrum micans*, *Alexandrium catenella*, *Alexandrium tamarense*, and *Amphidinium operculatum*, to determine whether or not dinoflagellates could be identified and characterized phylogenetically by their proteases. In order to control protease expression, all these species were cultured under identical conditions and were not allowed to feed if they were mixotrophic. We found that although these species had unique protease profiles, the profiles of closely related species were so different from each other and from distantly related species that we were unable to draw any phylogenetic relationships by this method. We also used EDTA and 1,10-phenanthroline to inhibit metalloprotease activity and found that *P. minimum* and *P. micans* had metalloproteases of 135 kD and 85 kD, respectively.

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Non-glandular Trichome Cell Walls are Compositionally Distinct from Atrichoblasts, Glandular Trichomes, and Fiber of Upland Cotton (*Gossypium hirsutum*)

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Although trichomes play critical roles in many plant species, their cell wall composition and organization remains generally unknown and has generally been believed to be similar to that of other cells with primary walls. In this study, Pilose and wild type (DP5690) cotton lines were used to investigate and compare the composition of epidermal and trichome cell walls. Pilose cotton leaves contain an abundance of non-glandular trichomes on the leaf surface, whereas the wild-type line possesses a more glabrous leaf, with non-glandular trichomes much more separated and sparse. In the Pilose lines, the non-glandular stellate trichomes are greatly increased in abundance, virtually cover the leaf surface. In the glandular and non-glandular trichome cell walls, most antibodies used in this study reacted similarly; however, xylans and extensin were found in the non-glandular trichome cell walls as opposed to the lack of reaction in all other leaf tissues, excepting xylem elements. Generally xylans and extensin are confined to heavily lignified woody tissue, indicating that these trichomes may be important in inhibiting herbivory. These data also indicate that trichome cell wall composition can differ significantly from the cell walls of the underlying plant tissue. In the case of the non-glandular trichomes of cotton, they may prove an excellent way to investigate xylan biosynthesis in a tissue that is easily separable from that not undergoing xylan biosynthesis.

A Study of Heat Shock Response Among Diverse Ecotypes of *Arabidopsis thaliana*

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Plants respond to elevated temperatures by eliciting the heat shock response that is characterized by the rapid accumulation of heat shock proteins (HSPs). Underlying this response, however, is considerable species-specific variation that we recently characterized using a comparative transcriptome network approach supported by physiological and biochemical validation. Our results indicated that expansion of Hsp17s, timing of the heat shock response subnetwork and production and scavenging of reactive oxygen species (ROS) varied among the *Arabidopsis*, Poplar and Soybean heat shock responses. In this study, we extend these findings to investigate the adaptive significance of these mechanisms among different *Arabidopsis* ecotypes. Representatives were taken from diverse geographical locations; Germany, United States, France, Spain, Russia, Canada, Sweden, Japan and the Cape Verde Islands representing a wide range of growth temperatures. The seedlings of the fifteen ecotypes selected were exposed to gradually increasing temperature from 25⁰C to 40⁰C followed by a gradual recovery over a period of 8h. The ROS scavenging was analyzed by assaying for key ROS sequestering enzymes, peroxidase and glutathione reductase. In addition, we measured the antioxidant status of these samples by using a novel, oxygen radical absorption capacity (ORAC) assay. The timing of the heat shock response was estimated by gene expression and protein blotting using antibodies raised against HSP101, HSP 70 and HSP17.7. Our preliminary results indicate variation among *Arabidopsis* ecotypes in the timing of heat shock response and total antioxidant capacity. Future reverse genetic and ecological selection studies will be used to determine adaptive significance of these processes in thermotolerance.

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Luminescence Imaging and its application to Biosensor Research

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Bioluminescence resonance energy transfer or BRET is the physical transfer of energy from a donor molecule, such as renilla luciferase, to a receptor molecule for example yellow fluorescent protein. BRET has the ability to provide insight into protein interactions and protein conformational changes in living cells and in real time. Our imaging system consists of an inverted Nikon microscope that is attached to a beam splitter and feeds into a EM-CCD that is cooled to -80° C. The beam splitter allows for the simultaneous acquisition of photons emitted in the two different wavelengths that rLuc and YFP proteins emit, 530nm and 480nm respectively. In addition the EM-CCD allows for the amplification of charge induced photons with minimal background noise. Through our experiments we hope to optimize the temporal and spatial resolutions for BRET imaging using Arabidopsis seedling roots that express renilla luciferase alone (no BRET) and renilla luciferase fused to yellow fluorescent protein (rLuc-YFP; BRET). Our goal is to develop an imaging technique utilizing the advantages of luminescence over strictly fluorescence to detect time resolved biosensor responses over a long period of time and in addition to calculate the ratio of photons emitted at (530nm/480nm). The potential applications of BRET are the imaging of signaling molecules involved in plant development over an extended period of time.

Effective Small RNA Destruction by Short Tandem Target Mimics through the Small RNA Degrading Nucleases in *Arabidopsis thaliana*

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An existing long target mimic (LTM) IPS1 has been found endogenously regulating the miR399 action in *Arabidopsis*¹. However, successful application of IPS1 in the study of other small RNAs has been limited, and the underlying machinery is still not fully understood. Here, we demonstrated the development of novel short tandem target mimics (STTMs) without using the IPS1 backbone. We also successfully applied STTMs in blocking the function of both miRNAs and siRNAs through small RNA destruction in plants. STTMs were constitutively expressed in two copies that were linked together by a short RNA spacer. The destruction of miR165/166 by STTM165/166 resulted in unprecedented phenotypic alterations of the plant development in *Arabidopsis*. The destruction of miR156/157 promoted early leaf phase transition from juvenile to adult stages. Destruction of ARF-tasiRNAs D7(+) and D8(+) phenocopied the leaf features of the mutant *rdr6-15*. Further study revealed that the small RNA destruction by STTMs was through the small RNA degrading nucleases (SDNs). Mutation of SDN1 and SDN2, two of the six SDNs in *Arabidopsis*, dramatically blocked the degradation of miR165/166 triggered by STTM165/166, suggesting that STTM-mediated small RNA degradation was facilitated by the SDN pathway. Furthermore, the level of small RNA destruction showed a reverse correlation with the expression levels of the STTM that are related to the spacer between the two domains. The optimal length of the RNA spacer in STTM for destroying small RNAs is about 48 nt; shorter (24 nt) and longer (88 nt) were less effective and shorter than 8-nt failed completely to block small RNA function. Taken together, we demonstrated a novel approach for the destruction of endogenous small RNAs, thereby providing a powerful tool for functional genomics of small RNA molecules in plants.

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Mutation of a molecular motor, myosin XI-A, leads to reduced fertility in *Arabidopsis thaliana*.

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Cellular organelles in plants are not fixed in cytoplasm but rather mobile structures which are moving in a dynamic but still elusive manner through cells in a process known as cytoplasmic streaming. In pollen tubes, actin filaments are essential components for organelle and vesicle transport to the pollen tip which enables cellular growth. Myosin motor proteins, which move along actin filaments, are likely candidates to provide the motive force for organelle movement. In the model organism *Arabidopsis thaliana* there are 13 different class XI myosin genes. Six of these genes are expressed primarily in pollen: *XI-A* through *XI-E* and *XI-J*. We evaluated the role of each of these 6 pollen myosins in pollen tube growth. We hypothesized that deletion of individual myosin genes would result in decreased pollen tip growth which could result in reduced fertilization efficiency. This would lead to decreased numbers of seeds per silique. The seed number for 50 siliques per mutant line was manually counted and compared to wild-type plants. A significant difference was found for myosin *xi-a* mutants. These findings suggest that myosin XI-A plays a role in pollen tube growth.

Analysis of the methylation states of maize *transgene-reactivated* mutants.

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Epigenetic regulation of gene expression results from changes in chromatin structure, DNA methylation and/or histone modifications. Such modifications allow for tissue specific expression of endogenous genes and transgenes. The silent b1 genomic transgene (BTG-s) can be used as a tool to study epigenetic gene regulation in maize. A forward genetics screen based on reactivation of the silent BTG was conducted using EMS mutagenized maize. Through this screen several mutants, designated *transgene reactivated (tgr)*, were identified. Published work demonstrates that mutations of genes whose proteins are involved in the RNA-mediated DNA methylation pathway cause transgene reactivation. DNA methylation of promoter regions is frequently correlated with low gene expression levels. The hypothesis of this investigation is that differences observed in the expression states of the transgene in these mutants correspond to the level of DNA methylation. To investigate the levels of methylation in the promoter region of the mutants, bisulfite sequencing was performed on silenced and reactivated plants from six different lines of *tgr* mutants. Results show a varying degree of hypomethylation in transgene reactivated plants compared to their respective silent plants from four of the mutant lines. Hypermethylation was observed in plants from one line with an intermediate reactivation phenotype. These results aid in advancing the understanding of epigenetic regulation of transgenes. Further work will ensue to evaluate whether there are differing expression levels of proteins involved in the methylation pathway between reactivated and silenced mutants.

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Mapping of the B1 genomic transgene within the Maize genome.

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The B1 genomic transgene (BTG) was inserted biolistically into one genotype of the Maize (*Zea mays*) genome, to generate stable transgenic lines (referred to as Btg-s).

Transformants were out crossed with a second genotype (B73) to introduce genetic polymorphisms that could be used for genetic mapping experiments; BTG is used as a marker of epigenetic regulation when silenced. This project focuses on using polymorphic DNA markers to identify the exact chromosomal location of the BTG within the maize genome. Initial experiments suggested that the BTG locus is on either chromosome seven or ten. Further analysis suggests that the BTG is most likely inserted into the maize genome within the third bin of chromosome ten. Using polymorphic markers between the two genotypes, PCR is performed using DNA previously isolated from tissues harvested from a mapping population. The PCR product is then described as being like B73, Btg-s, or a heterozygote of the two. Analysis of the data indicates a high incidence of plants like B73, indicating the location of the BTG along chromosome ten within the third bin.

Finding Novel Genes Regulating Early Steps in Development

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Cell differentiation is a fundamental requirement of multicellular organisms. It allows them to develop tissues and organs with specialized functions. In animals most cell differentiation is completed before birth and an animal simply grows in size. Plants, however, develop after germination and cell differentiation is flexible, having the ability to develop in response to the environment. Our lab is interested in understanding the initial decision making process that determines which of the three cell types the leaf protodermal cells differentiate into; pavement cells, trichomes, or guard cells. We are particularly interested in guard cell differentiation. Two kidney-shaped guard cells form a pore or stoma in the epidermis that can be opened and closed for gas exchange and transpiration of water. The number of stomata and the regulation of the opening are important in photosynthesis and drought resistance. In order to better understand the initial decision making process of stomata development, we study genes known to regulate different steps of its differentiation. This pathway involves receptors and their ligands, a cascade of kinases, and transcription factors. To find new components of the stomata development pathway in *Arabidopsis* an EMS screen was carried out. We screened for dwarf mutants that had abnormalities in stomata or epidermal patterning. After analyzing seedlings from over 2300 M2 plants we chose 114 interesting mutants. We checked reappearance and segregation of the original mutation in the M3 generation. Further analysis showed that we identified three lines containing mutations in the ERECTA gene and one line with a mutation in YODA, both known components of the stomata development pathway. Currently we are performing additional analysis of the most interesting mutants with phenotypes that have not been described previously and we are getting ready to positional clone them in order to determine where and what type of gene the mutation resides in.

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Functional Genomic and Physiological Approaches to Improving Cotton Production in Water-Limiting Environments.

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The identification of genes and regulatory pathways that play a role in abiotic stress tolerance could lead to the development of crops with increased yields and improved quality under water-limiting production schemes. In this study, two cotton (*Gossypium hirsutum*) genotypes with contrasting yield response under deficit irrigation were chosen to examine transcript and physiological response to water deficit stress. Cotton cultivars Siokra L-23 (tolerant) and CS50 (susceptible) were grown in greenhouse and field trials. Initial transcript profiling studies were carried out on greenhouse-grown plants (8-leaf stage) exposed to a slow-onset water deficit over 6 days followed by a recovery period 1 day after re-irrigation. After irrigation was stopped the plants were monitored daily for photosynthetic response, leaf water potential, and decline of chlorophyll fluorescence to characterize the physiological state of the plants during the stress. Leaf and root tissue was collected at 0 day (well-watered), 3 days after induced stress, 7 days after induced stress, and 1 day after re-watering to monitor recovery. Expression profiling revealed distinct differences between leaf and root tissues and the timeline in which the magnitude of the stress was reflected in those tissues. Although some differences were identified between the two cultivars, the number of genes identified as unique to a particular genotype was relatively small. Detailed results of this study, including the physiological response to stress and yield trial data will be presented at this meeting.

Linking Carbohydrate Content and Gene Expression for Seasonal Carbohydrate Metabolism in the Xylem (Wood) of *Populus* Species

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The deposition of starch and sugars in the xylem of poplar twigs varies seasonally, with starch being high in the late summer/autumn and low in the winter and during bud break. Starch mobilization to soluble sugars occurs during the winter and early spring. In preliminary work, we found starch to be low in the new growth ring of older stems but high in rings from previous years during June. These seasonal and spatial patterns are consistent with the need to regulate starch storage so that it does not compete with xylem growth and yet can serve as a reserve for bud growth in the spring. We are using these seasonal and spatial patterns to determine whether this regulation involves changes in gene expression for associated enzymes. Total RNA was isolated from xylem of the twigs of 4-year-old *P. balsamifera* and *P. deltoides* and the growth rings of *P. deltoides* saplings grown outside in Lubbock, TX. In the twigs, a decline in starch in spring was associated with a lower transcript level for ADP-glucose pyrophosphorylase, whereas the opposite situation occurred to some extent when starch levels were high. Transcripts for sucrose synthase correlated more with xylem growth than with starch deposition. Low sucrose content was preceded by a low transcript level for sucrose phosphate synthase (SPS), but no clear seasonal pattern was noted in SPS transcript levels. However, the transcript level for β -amylase, an enzyme of starch degradation, followed the seasonal trend in starch and soluble sugars. The growth rings of *P. deltoides* showed high starch levels in the innermost ring and the starch levels decreased towards the outermost ring. Transcripts for the enzymes showed a positive correlation with that of carbohydrate levels. Therefore, some regulation of carbohydrate levels in the xylem of poplar may be associated with the regulation of gene expression for critical enzymes of the pathways involved.

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Identification of telomere length regulating factors in *Zea mays*

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Telomeres are specialized nucleoprotein complexes at the ends of linear chromosomes. They have essential functions in genome stability, meiotic chromosome behavior, and solving the end-replication problem. Mammalian studies have shown that proper regulation of telomere length is important in ageing, cancer and a diverse range of disease states. Quantitative Trait Locus (QTL) mapping is a powerful method for identifying loci that control traits with complex inheritance, that can be precisely measured and exhibit a high degree of heritability. We are utilizing QTL mapping in the well-defined IBM population (302 RILs, >2,000 markers) to identify genes that regulate telomere length. Using terminal repeat fragment (TRF) analysis via Southern blot hybridization, we can determine telomere length phenotypes and will perform QTL analysis to fine map allelic differences affecting telomere length. The physical map regions corresponding to stringent QTL confidence intervals will be used to generate lists of candidate genes. These candidate genes, as well as other *a priori* candidate genes, will then be amplified using RT-PCR in the Maize Diversity Lines in order to determine whether or not these candidate genes have an effect on telomere length. The telomere length data will also be analyzed relative to other phenotypes collected for these lines to search for potentially meaningful trait correlations. The multi-faceted strategy of QTL mapping in the IBM population and investigation of candidate genes in the diversity lines will utilize the genetically-anchored sequenced genome of B73 to identify novel regulators of telomere length homeostasis in maize.

Interactions of *N*-Acylethanolamine Metabolism and Abscisic Acid Action in *Arabidopsis thaliana* Seedlings

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N-Acylethanolamines (NAEs) are endogenous plant lipids hydrolyzed by fatty acid amide hydrolase (FAAH). When wildtype *Arabidopsis thaliana* seeds were germinated and grown in exogenous NAE 12:0 (35 μ M and above), growth was severely reduced in a concentration dependent manner. However, when grown in lower levels (up to 20 μ M) of NAE, treated plants showed modest differences in growth compared with untreated plants. Wildtype *A. thaliana* seeds sown on exogenous abscisic acid (ABA) exhibited similar growth reduction to that seen with NAE treatment. Levels of ABA above 0.25 μ M reduced primary root length and cotyledon area, while lower levels had little effect. AtFAAH knockouts grew and developed similarly to WT, but AtFAAH overexpressor lines show markedly enhanced sensitivity to ABA. When low levels of NAE and ABA, which have very little effect on growth alone, were combined, there was a dramatic reduction in seedling growth in all three genotypes, indicating a synergistic interaction between ABA and NAE. Notably, this synergistic arrest of seedling growth was partially reversed in the ABA insensitive (*abi*) mutant *abi3-1*, indicating that a functional ABA signaling pathway is required for the full synergistic effect. This combined NAE and ABA treatment induced a dose-dependent increase in ABI3 transcript levels. The ABA responsive genes AtHVA22B and RD29B also had increased expression in both NAE and ABA treatment. The *abi3-1* mutant showed no expression of ABI3 and AtHVA22B, but RD29B expression remained similar to wildtype seedlings, suggesting an alternate mechanism for NAE and ABA interaction. Taken together, these data suggest that NAE metabolism acts through ABI3-dependent and independent pathways in the negative regulation of seedling development.

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Subcellular Localization of Salicylic Acid Binding Protein 2 in *Nicotiana tabacum*

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Some plants have acquired defense mechanisms to protect themselves against adverse conditions. One of such defense mechanisms is the salicylic acid (SA) mediated defense response triggered in some plants infected by microbial pathogens. Upon microbial infection, the level of SA in the infected plant increases about twenty folds. SA is made in the chloroplast and converted to Methylsalicylate (MeSA) by Salicylic acid Methyl Transferase (SAMT), which then diffuses out of the chloroplast. SABP2 has been shown to catalyze the conversion of MeSA to SA. Increased levels of SA leads to a hypersensitive response resulting in death of the infected cell and immediately surrounding cells. This local resistance (LR) response sends signals to uninfected cells throughout the plant which helps them to prepare for future attack (Systemic acquired resistance-SAR). SABP2 is a 29Kd, extremely low abundance tobacco protein, with methylsterase activity. SABP2 has been hypothesized to be localized in cytoplasm, but its precise localization is not known. Therefore, we used biochemical approaches to study localization of SABP2 in tobacco. Tobacco plants were treated with methyl jasmonate to induce SABP2 expression. Ammonium sulphate was used to precipitate cytoplasm proteins, while intact chloroplasts were isolated using a discontinuous percoll gradient and further fractionated into stroma and membranes. Fractions were separated on a 12% SDS PAGE, and western analysis was done using polyclonal anti-SABP2 antibodies. Results show that SABP2 is associated to the chloroplast membranes. Chloroplast import assay experiments using His-tagged SABP2 shows that SABP2 is imported to the chloroplast. This confirms that SABP2 is localized to the chloroplast and not cytoplasm as previously hypothesized. SABP2 localization studies provides a better understanding of the metabolic pathway involved in SA-mediated defense response. Studies on SA pathway may help to improve plants own natural immunity and could be used to reduce large scale use of pesticides in agriculture, which are environmentally unfriendly.

Constructing A Cytogenetic Map Of Maize In Oat Addition Lines Using Sorghum Bacterial Artificial Chromosomes (BACs) As Fluorescent Probes

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We are developing a pachytene cytogenetic map of the maize (*Zea mays* L.) genome by performing Fluorescence *in situ* Hybridization (FISH) of maize marker-selected sorghum bacterial artificial chromosomes (BACs) as described by Koumbaris & Bass (2003, Plant J., 35:647). Our objectives are to cytogenetically map the core bin markers (CBM) of maize chromosomes 1, 3, 4, 5, 6, & 8 and to FISH map two regions duplicated between chromosome 9 with chromosomes 1 (9-1) and 6 (9-6). Thirty-two maize marker-selected sorghum BACs have already been cytogenetically mapped onto maize chromosome 9 using this technique (Amarillo & Bass, 2007, Genetics, 177:1509). This map established an overall conservation of marker colinearity with linkage maps at 5 cM resolution while uncovering regions of maize genome hyperexpansion relative to sorghum. Preliminary FISH data for chromosomes 1, 4, and 6 is also presented along with data of FISH mapping of loci duplicated between chromosomes 1 and 9 using a single BAC. These results facilitate analysis of the maize and sorghum genomes by using common markers to integrate their physical, linkage, and cytogenetic pachytene chromosome maps. The maize cytogenetic FISH map is described at cytomaize.org and mapping records as well as FISH images are made available at MaizeGDB.

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The Effect of Nitrogen Availability on Photosynthesis of the Invasive Grass *Phalaris arundinacea*

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Phalaris arundinacea (reed canary grass) is an invasive C₃ perennial of temperate/boreal wetland communities. Given that *P. arundinacea* is more abundant in areas with high nitrogen inputs, the main aim of our project is to compare the responses of leaf morphology and photosynthetic parameters to various nitrogen levels for *P. arundinacea* with the responses for a native sedge, *Carex stricta*, that it often displaces. We are determining the appropriate N range for *P. arundinacea* by growing the plants in a course soil medium whose nitrogen content is controlled by varying the nitrate and ammonium in Hoagland's solution. The plants were initially established with 300 mL of full strength Hoagland's solution supplied every two days. After 3 weeks, the N treatments were imposed. Our preliminary research showed that a three-fold increase in the standard nitrogen concentration in Hoagland's solution (33 mM vs. 11 mM) resulted in an 18.3% increase in CO₂ assimilation (*A*) on an area basis. The high N concentration increased specific leaf area (SLA) by nearly 7%, but the effect on chlorophyll content was variable. Growth at 1.1 mM nitrogen reduced *A* and the total chlorophyll content by 13.12% and 8%, respectively, compared to values at 11 mM. The SLA was reduced by 30%. It appears that N level affects total carbon assimilation by affecting both *A* and SLA. We will expand the growth N range for *P. arundinacea* plants to develop leaf N contents that are above and below those contents for plants in typical field conditions before growing *C. stricta* plants with the same range of N.

Functional analysis of NH₃ transport and investigation of transcriptional up-regulation under O₂ deficit conditions for soybean nodulin 26.

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Nodulin 26 (Nod26) is one of a number of nodulin proteins, which is specifically expressed during the biogenesis of symbiosome membrane (SM) in soybean nitrogen-fixing nodules, and is the major protein component of the SM comprising 10-15% of the SM protein mass. Nod26 is a multifunctional aquaglyceroporin as a member of the major intrinsic protein (aquaporin channel family). In this study, to investigate ammonia (NH₃) transport properties of Nod26, we purified the Nod26 protein by using *Pichia* expression system and reconstituted it into carboxyfluorescein-loaded liposomes using stopped-flow spectrofluorimetry. Liposomes containing Nod26 showed high NH₃ permeability ($P_{NH_3} = 0.018 \pm 0.0016$ cm/s), a value 2.88 fold higher than that determined with control liposomes. NH₃ transport through Nod26 showed a low activation energy ($E_a = 38.25$ kJ/mol). We have also investigated Nod26 gene expressions in soybean root tissues under oxygen (O₂) deprivation conditions by using quantitative real-time RT-PCR (QRT-PCR). Nod26 transcript increased up to over 10 fold in uninfected root tissues in response to anaerobiosis induced by waterlogging or anaerobic flasks, suggesting that oxygen tension may be among the cues that induce Nod26 expression during nodulation. Nod26 gene expression levels, however, did not increase under anoxic conditions in infected root tissues after inoculation of *Bardyrhizobium japonicum*. Overall, these results indicate that Nod26 is an ammonia transport channel likely playing a role in process of fixed-nitrogen during legume/rhizobia symbioses, and suggest existence of multiple regulators for induction of *Nod26* transcription in addition to hypoxic/anoxic cues after infection event. (Supported by NSF grant: MCB-0618075)

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Role of photoreceptors in R protein-mediated resistance to Turnip Crinkle Virus

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Light harvested by plants is essential for the survival of most life forms. This light-perception ability requires the activities of proteins termed photoreceptors. In addition to various growth and developmental processes, light also plays a role in plant defense against pathogens and is required for activation of several defense genes and regulation of the cell death response. However, the molecular or biochemical basis of light modulated regulation of defense signaling is largely unclear. Previously we have shown that incompatible interaction between Arabidopsis-Turnip Crinkle Virus (TCV) and tobacco-Tobacco Mosaic Virus pathosystems are dependent on light (Chandra-Shekara et al., 2006). Resistance to TCV is dependent on the Resistance (R) protein HRT, which contains coiled coil, nucleotide binding, and leucine-rich-repeat domains. To determine the genetic, molecular and biochemical basis of light-dependent defense pathway, we studied the role of various photoreceptors in HRT-mediated resistance to TCV, HRT protein levels and its localization. Interestingly, mutation in certain photoreceptors led to degradation of HRT via an proteasome-dependent pathway and resulted in susceptibility to TCV. Exogenous application of salicylic acid induced transcription of HRT, which restored HRT levels in some, but not all, mutant backgrounds. These results show that different photoreceptors function distinctly in maintaining post-transcriptional stability of HRT. The current focus is to determine if these photoreceptors undergo direct or indirect interactions with HRT and if they are generally required for other R-mediated defense pathways.

Arsenite induced oxidative stress in *Arabidopsis thaliana*

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Arsenic, a natural and widespread metalloid in the earth's crust is toxic to organisms. Environmental contamination of arsenic is a major public health hazard because of arsenic's carcinogenic potential. Compared to microorganisms, studies on how plants adapt to arsenic stress have not been fully investigated. Arsenic is known to induce oxidative stress by producing Reactive Oxygen Species (ROS). The objectives of this study are to (a) document developmental effects of arsenite [As(III)] on roots, (b) test the hypothesis that exogenous methionine (Met) could protect cells from arsenite toxicity and (c) test whether heatshock transcription factors were involved in arsenite tolerance. Surface-sterilized Arabidopsis (Wild type Columbia) seeds were germinated on Hoagland agar medium with 20 g L⁻¹ sucrose and 3-d old seedlings were transferred to the same medium with 0 μM, 12 μM and 25 μM As(III) as sodium arsenite. In another set of experiments, 3-d old seedlings were pre-treated with 0.5mM Met and 0.5mM Nle (norleucine, an analogue of Met) for 24 h prior to transfer onto the arsenite media. Primary root growth per day and lateral roots were quantified over a 3-d period. Primary root growth was significantly reduced by arsenite (58% and 94% reduction at 12μM and 25μM respectively compared to control with no As (III)). Exogenous supply of L-Met and DL-Nle at 0.5mM concentration for 24 h significantly affected the primary root growth with per cent reductions of 52% and 69% for L-Met and DL-Nle respectively. Though the concentrations of L-Met and DL-Nle were inhibitory, pretreatment with L-Met significantly reduced As(III) sensitivity from 57.8% reduction in primary root growth in control to 42.5% in Met-treated seedlings. However Nle supply had no effect. Despite the severe reduction of primary root growth, initiation of lateral roots were not significantly affected by arsenite. Met pre-treatment increased the number of lateral roots. As(III) treatment inhibited this increase. A double mutant for heatshock transcription factor *hsf1a/1b* was significantly more sensitive to arsenite than the wild-type. Our results showed that arsenite had a differential developmental effects on primary and lateral roots. Role of exogenous L-Met and heatshock transcription factors found here suggest that arsenite tolerance is likely controlled by protein oxidation and heatshock transcription factor-induced genes.

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A novel gating mechanism for the Arabidopsis Nodulin-like intrinsic protein 7;1 boric acid transporter in pollen.

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Functional analysis shows that there are two subfamilies of aquaporin: aquaporin which transport water specifically, and the aquaglyceroporins which can facilitate conduct of glycerol, urea, and other small molecules in addition to water. Structure analysis indicate that all the aquaporin adopt ‘hourglass’ conformation with 6 transmembrane alpha-helices linked by 5 loops. There are two constraints: two highly conserved domains with ‘NPA motifs’ are located on loop B and loop E and a tetrad of amino acid residues that contains aromatic residues and a conserved Arginine referred as Ar/R region. In my research, I focus on the structure and function of one plant MIP: AtNIP7;1. AtNIP7;1 belongs to the second subgroup of plant nodulin-26 like intrinsic protein with AtNIP5;1 and AtNIP6;1. The functions of AtNIP5;1 and AtNIP6;1 have already been elucidated that both of them can conduct boric acid in order to transport boron to the shoot tissue. In order to understand the molecular basis for the unique function of AtNIP7;1, *Xenopus* oocyte system is used for the water or other solutes uptake assay and computational biology improves the understanding of structural complexity and functional diversity. Boric acid uptake assay didn’t show apparent boric acid conductivity in AtNIP7;1. Homology modeling on AtNIP7;1 provides an idea that the Y81 probably is the key residue blocking the hole by observation of up and down conformation of Y81, and mutation of Y81 to C facilitates boric acid transportation by boric acid uptake assay. Molecular dynamics simulation shows another residue---R220, which belongs to Ar/R region, adopts two conformations: up and down which could interact with Y81 by hydrogen bond. These two residues Y81 and R220 could be the very candidates involving in the gating mechanism of AtNIP7;1.

Identification and Characterization of Soybean Cyst Nematode-Induced Genes in Soybean

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Soybean cyst nematode (SCN), *Heterodera glycines*, is one of the most damaging pathogens of soybean (*Glycine max*), causing extensive yield loss throughout the USA. To develop SCN-resistant soybean varieties, it is vital to understand the soybean defense mechanisms against SCN, especially to identify the key genes of the defense pathways. To this end, we compared the transcript abundance of two genetically related soybean lines, one is resistant and the other is susceptible, infected by SCN using the Affymetrix Soybean Genome Array. This analysis revealed a number of genes that were differentially expressed in resistant and susceptible lines infected by SCN infection. Two of the genes encoding enzymes were recognized to be related to activation of plant defense signaling salicylic acid, which has been shown to be important for plant defense response to SCN. The roles of these two genes in soybean defense against SCN are currently evaluated using a transgenic soybean hairy roots system.

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Mechanisms for Cold Tolerance in Flowers of *Helleborus Niger*

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Freeze damage caused by late winter and early spring freezing temperatures affects the commercial values of plants in garden centers and their values in the landscape. Most hellebores have very high cold tolerance. This research was conducted to isolate cDNA fragments in *Helleborus Niger* that allow the flowers of these plants to survive temperature fluctuations. In this study, flower buds from *H. Niger* planted in a Nashville, TN garden were collected in December during some sunny days when leaves were not frozen, in the early morning when the leaves were frozen, and at noon on the same day when the leaves were defrosted and partially covered with snow. These flower buds were compared with some tissues collected when the temperature was above 50F. Total RNA was isolated from all collected tissues and compared using the RNAspectra™ Green (GenHunter Corporation, TN). cDNA population was separated on 6% polyacrylamide gels, bands of interest were isolated and cloned. DNA sequence analysis confirmed that most of the cDNA clones contained unique sequences which could not find any homolog in the database.

Expanding the anaerobic response polypeptide gene family. Role of *AtNIP2;1* and *AtCML38* in plant anaerobic stress.

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Plants often get exposed to low oxygen availability termed hypoxia as in case of flooding or the less frequent, total oxygen deprivation termed anoxia. Both of these result in a severe stress and reprogramming of metabolic pathways and internal structural changes. In addition, major changes occur in gene expression, the hallmark being the induction of special genes called “Anaerobic polypeptide genes” (ANPs). These ANPs include genes encoding enzymes of the glycolytic and fermentation pathway, transcription factors, signal transduction proteins and others involved in adaptation to the anaerobic stress. We have found two novel ANP genes, *Arabidopsis thaliana* Nodulin 26-like Intrinsic Protein 2;1 (*AtNIP2;1*) and calmodulin-like gene 38 (*AtCML38*) which show acute upregulation of transcript (>600 fold) in response to hypoxia. *AtNIP2;1* is a member of proteins which are plant-specific water and solute transporters with homology to the soybean nodulin 26. Tissue specific expression and functional analysis show that *AtNIP2;1* is a root specific gene that encodes a lactic acid channel protein. Current studies are focused on investigating the role of *AtNIP2;1* in the adaptive response to anaerobiosis in *Arabidopsis*.

AtCML38 is a member of the calmodulin-like family in *Arabidopsis* that consists of 50 genes.

Remarkably out of all the 50 CaM-like genes, *AtCML38* is the only one showing transcript upregulation in response to hypoxia thus making it a unique calcium sensor involved in linking calcium signaling to oxygen deprivation stress signaling. In addition to being an ANP, *AtCML38* also belongs to another 5-membered gene family in *Arabidopsis* called the rgsCaM-like family. Regulator of gene silencing Calmodulin-like protein (RgsCaM) is a tobacco gene known to play an important role in the post-transcriptional gene silencing process. *AtCML38* being the closest homolog of tobacco rgsCaM may have some role in suppression of gene silencing, which remain to be further investigated.

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Investigating the Roles of Class XI Myosins in Pollen Tube Growth

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All cells have polarity which can usually be visualized by the cellular components being unequally distributed throughout the cell. The exact mechanism of how cells set up and maintain their polarity remains unknown. A pollen tube is an excellent model for studying cell polarity because it only grows at the tip. Pollen tubes also have a characteristic organelle/vesicle organization despite vigorous cytoplasmic streaming. We hypothesize that the organelle/vesicle distribution is attained by differential regulation of myosin motors on different compartments. Class XI myosins are the most likely candidates for moving organelles/vesicles in plants. 6 of the 13 myosin XI genes in *Arabidopsis thaliana* are expressed in pollen. To elucidate the functions of each pollen myosin, we are examining T-DNA knockout mutants. Currently, we are focusing on *XI-A* because two independent *xi-a* knockout lines showed significantly fewer seeds per silique than wild type. We are also comparing pollen tube growth rates and organelle movements between wild type and myosin XI knockout mutants.

Oleate-regulated signaling and plant defense

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Oleic acid (18:1) is one of the major monounsaturated fatty acid (FA) which plays an important regulatory role in animal cells. In plants, changes in the levels of 18:1 results in the alteration of salicylic acid (SA)- and jasmonic acid (JA)-mediated defense responses. This is evident in the *Arabidopsis ssi2/fab2* mutant, which encodes a defective stearyl-acyl carrier protein-desaturase (S-ACP-DES) and consequently accumulates high levels of stearic acid (18:0) and low levels of 18:1. Consequently replenishing 18:1 levels results in restoration of wild-type-like signaling in the *ssi2* mutant. We have identified several genes, which either participate in the prokaryotic fatty acid (FA) or generalized defense pathways and loss-of-function of which restores various phenotypes in *ssi2* plants. A reduction in 18:1 levels induces defense response by upregulating the expression of several R genes in an SA-independent manner (1). More recently, we have shown that 18:1 regulated induction of R proteins is dependent on EDS1 or SA and that EDS1 and SA act in a redundant manner to regulate R protein derived signaling regardless of the structure of R protein. Further characterization has led to the isolation of several 18:1 binding proteins, whose enzymatic activities are regulated by 18:1 levels. Detailed analysis of these proteins will be presented. (1) PLOS Genet (2009), 5(7) e1000545.

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Characterization of Site-Directed Mutants in Cytochrome c550 of Photosystem II

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The Photosystem II (PSII) complex catalyzes the initial reactions of photosynthesis, i.e. oxidation of water and reduction of plastoquinone with concomitant evolution of oxygen to the atmosphere. The intrinsic PSII protein, CP43, appears to interact with components required for oxygen evolution. Mutation of Arg305 to serine (R305S), in CP43 in the cyanobacterium *Synechocystis*, produced a mutant with severely reduced growth and oxygen evolving activity under chloride limiting conditions. However, when grown under normal conditions, the R305S showed no extreme phenotype. It was determined that this specific mutation resulted in loss of binding of an extrinsic PSII protein, cyt-c550, which is involved in concentrating chloride at the PSII active site. Deletion of the *psbV* gene, encoding cyt-550, also resulted in loss of growth and oxygen evolving activity under chloride limiting conditions. The X-ray crystal structure of PS II (PDB: 2AXT) shows a close proximity between cyt-c550 and CP43. Based on the crystal structure, we have mutated a highly conserved asparagine, Asn51, on cyt-c550, which we propose is part of a binding domain with CP43; the Asn residue on cyt-c550 is close to Arg305 on CP43. The Asn51 on cyt-c550 was mutated to an alanine (N51A) and aspartic acid (N51D). We are in the process of characterizing the effects of these mutations on PSII function in *Synechocystis*.

Cytosolic glutamine synthetase binds the C-terminal domain of the nodulin 26 channel on the soybean symbiosome membrane.

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Nodulin 26 (*nod26*) is a major intrinsic protein (MIP) that constitutes the major protein component on the symbiosome membrane (SM) of N_2 -fixing soybean nodules. Functionally, *nod26* forms a low- energy transport pathway for water as well as osmolytes within the symbiosome, and may also mediate the efflux of NH_3 from N_2 fixation. Besides their transport functions, emerging evidence suggests that high concentrations of MIPs on membranes provide interaction and docking targets for various cytosolic proteins. Here it is shown that the C-terminal domain peptide of *nod26* interacts with a 40 kDa protein from soybean nodule extract, which was identified as soybean cytosolic glutamine synthetase $GS_1\beta 1$ by mass spectrometry. Fluorescence spectroscopy assays have shown that recombinant soybean $GS_1\beta 1$ binds the nodulin 26 C-terminal domain with a 1:1 stoichiometry ($K_d = 266$ nM). $GS_1\beta 1$ also binds to isolated SMs, and this binding can be blocked by pre-incubation with C-terminal peptide of *nod26*. *In vivo* experiments using a split ubiquitin yeast two hybrid system, and bimolecular fluorescence complementation have shown that all four cytosolic GS isoforms expressed in soybean nodules interact with full length *nod26*. The binding of GS, the principal ammonia assimilatory enzyme, to the conserved C-terminal domain of *nod26*, a putative transporter of NH_3 , is proposed to promote efficient assimilation of fixed nitrogen, as well as prevent potential ammonia toxicity, by localizing the enzyme to the cytosolic side of the symbiosome membrane.

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Silencing ARFs and SPLs by two-hit microRNAs in Arabidopsis

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AUXIN RESPONSE FACTOR (ARFs) and SQUAMOSA PROMOTER BINDING PROTEIN-LIKE PROTEINS (SPLs) are two important transcription factor families that control growth and development in Arabidopsis. Members of the two families can be divided into two subsets: small RNA regulated and non-regulated members. Emerging evidence demonstrated that small RNA regulated transcription factors are not only down-regulated by the small RNAs at the post-transcriptional level, but these small RNAs and their targeting genes also form a feedback and/or feedforward loop for auto-regulation and fine-tuning. On the other hand, roles of the non-small RNA regulated transcription factors and their interactions with the small RNA-regulated members are currently less understood. Here, we reported the functional dissection of members of the two transcription factor families for their unidentified regulatory roles by silencing them individually as well as in combination with each member being targeted by a pair of artificial microRNAs (amiRNAs). The members of the two transcription families were first clustered to generate a phylogenetic tree for potentially functional redundancy. Members of each sub-cluster were then silenced in combination. Using such a strategy, novel regulatory functions have been identified collectively for a number of ARF transcription factor members whose functions were previously unknown through traditional genetic mutations. Our data demonstrated a powerful reverse genetic approach to plant functional genomics for gene families in plants.

Genetic analysis of *phyA'* – a case of gene silencing associated with exonic methylation

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We isolated a transcriptionally suppressed epi-allele of *Arabidopsis thaliana* Phytochrome A gene termed *phyA'*, which contains methylation only in symmetric CG sites (mCG) resident to exonic regions. These exonic modifications confer a strong *phyA* mutant phenotype, characterized by elongated hypocotyls in seedlings grown under continuous far-red light (FRc). Demethylation of *phyA'* in the DNA methyltransferase I mutant (*met1*) background resulted in restoration of the WT expression level and phenotype, confirming the pivotal role of the mCG in *phyA'* silencing. Genetic analysis revealed that a number of chromatin modification and RNAi genes have no significant role in *phyA'* silencing. This analysis covered DNA methylation genes (CHROMOMETHYL TRANSFERASE and DOMAINS REARRANGED METHYLASE), histone methylation gene (KRYPTONITE), and RNAi genes (RDR2, RDR6, AGO 1, 4 and SGS3). To identify the novel genes involved in keeping *phyA'* suppressed we took the approach of suppressor screening. Seeds of *phyA'* line were mutagenized by EMS and M2 seeds were screened for a reversion to the WT phenotype. Phenotypic screening of M2 populations resulted in identification of suppressor of *phyA'* silencing (*sps-1*). Molecular analysis of *sps-1* revealed that *phyA* locus is reactivated in spite of *phyA'* hypermethylation. Genetic analysis of *sps-1* suggests that it contains a trans-acting mutation. Genetic mapping of *sps-1* locus will identify a chromatin factor involved in CG methylation mediated transcriptional suppression.

Sugar and copper responsive miRNAs and their interplay in copper homeostasis in plants

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MicroRNAs (miRNAs) are single-stranded, non-protein coding RNAs of approximately 21-22 nucleotides in length, which negatively regulate gene expression at the post-transcriptional level. In plants, miRNAs control the growth and development by recognizing, binding, and cleaving their target mRNAs, most of which encode transcription factors. In addition, miRNAs are also the key players in various biotic and abiotic stresses including nutritional stresses such as phosphate (Pi), sulphate (S) and

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copper starvation. Recent studies showed that a subset of biotic and abiotic responsive miRNAs were also responsive to sugar and copper treatment. However, the underlying machinery is unknown. Here, we choose *Arabidopsis*, a model plant with less abiotic stress resistance, and its close relative -salt cress (*Thellungiella halophila*), a plant with high abiotic stress resistance, to screen for sucrose responsive miRNAs with our recently established miRNA array platform. We found that in addition to the reported miR398, miR408 was also responsive to sucrose treatment. Together with the previous discovery that miR398 and miR408 are stress- and copper-responsive miRNAs, our data demonstrated that miR398 and miR408 belong to multiple-factor responsive miRNAs. Furthermore, miR398 and miR408 were not only induced by an increased level of sucrose, but also responsive dynamically to sucrose starvation. Similar experimental results were also observed with slightly different response in extent in salt cress. Assay for the expression of miR398 and miR408 in different tissues demonstrated that both miRNAs were more expressed in the stem tissues in response to a 24-hr treatment of 6% sucrose.

To understand the underlying machinery by which miR398 and miR408 are responsive to both copper and sucrose and the potential link between copper homeostasis and sucrose signaling, we assayed for copper uptake in 21-d *Arabidopsis* plants that were cultured in MS medium supplemented with various levels of sucrose. Our data showed that *Arabidopsis* plants under low level of sucrose accumulated a much higher level of copper than those under high level of sucrose. Moreover, qRT-PCR assay experiments showed that some copper proteins including copper transporters and copper chaperones such as CSD1, CSD2, CCS1, FSD1 and COPT1 were down regulated by exogenous sucrose. In contrast, other copper binding proteins such as ZIP2, ZIP4, LAC12, COX17 and CCH, as well as miR408 targeting genes (i.e. plantacyanin, lac3 and lac13) were up regulated by high sucrose. Only a few copper proteins, such as PAA1, RAN1, and YSL3, were not responsive to sucrose treatment. Taken together, our data suggest that sucrose is a key player in plant copper homeostasis and that miR398 and miR408 play a key role in the modulation of copper homeostasis in response to sucrose.

Analyses of natural variation in gene expression & association genetics studies of stress-related genes in Loblolly pine (*Pinus taeda* L.)

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As part of a collaborative effort to identify alleles contributing to economically useful traits in loblolly pine (*Pinus taeda* L.), our role has been to 1) use quantitative RT-PCR to examine the expression of 201 genes implicated in wood and lignin biosynthesis and in disease-and drought-stress responses in 426 unique loblolly clones, and 2) use association genetics to identify associations between gene expression phenotypes and almost 4,000 SNPs. Differences in gene expression among clones were observed for all genes. In cluster analyses genes previously suggested to have similar biological functions clustered together. The web-based tool MARIMBA was used to model biological pathways based on gene expression. The models revealed potential interactions between transcription factors and down-stream genes. We found 159 significant associations between gene expression and SNPs. We are examining the positions of these SNPs to validate previously detected effects and to identify prospective genes contributing to our traits of interest. Stress responses involve multiple and overlapping pathways. Our association analysis examined constitutive defenses in loblolly pine trees. A smaller experiment was performed to examine induced responses to stress. A subset of 24 unique clones from the original population were subjected to four different treatments—drought, pitch canker disease (*F. circinatum*), drought-pitch canker combined, or no stress control. Gene expression data was collected on the original 91 stress response genes from our association analysis along with 23 additional genes containing SNPs identified by our association studies. This additional study will help us further dissect stress responses and to verify some of the previously identified associations.

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Role of a bHLH transcription factor in controlling flowering time in *Arabidopsis thaliana*

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Flowering in plants is a synchronized process where various cues including age, light, temperature and hormones influence and fine-tune the time of flowering. Plants can sense their environmental setting and produce flowers only under optimum conditions. In *Arabidopsis* flowering time is controlled by four pathways, namely Photoperiodic (daylength), Vernalization (cold), Autonomous and Gibberellic Acid (GA). However, two pathways namely Short day (SD) and GA still needs to be understood. This research identified a key gene called *bHLH93* (basic Helix-Loop-Helix 93) that promotes flowering only under SD in *Arabidopsis* and has very little or no role at all in LD. *bHLH93* positively regulates *SOC1* to promote flowering. *bHLH93* is expressed in the shoot- and root- apical meristem and its expression peaks at 8hours after dawn in Wt under SD. This peak of expression coincides with dark under SD, and bHLH93 protein is stable in dark. As expected being a transcription factor, the bHLH93 protein is localized into the nucleus. GA promotes flowering in SD and exogenous application of GA on *bhlh93* mutants rescued the mutant flowering phenotype.

The salient mutant phenotype of *bhlh93* and its rescue by GA has highlighted a significant molecular connection between SD and GA pathway. Further investigation will reveal the downstream molecular mechanism of flowering time regulation by *bHLH93* and GA. This research is very significant to crop plants like rice, maize, and wheat where flowering time can be manipulated to increase the yield of seeds when the conditions are favorable.

Molecular and Biochemical Studies of SABP2 in Defense Signaling Pathway Induced by Acibenzolar-S-Methyl in Plants

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Salicylic acid binding protein 2 (SABP2) converts inactive methyl salicylic acid into salicylic acid (SA), which is required for the successful development of local and systemic acquired resistance (SAR) in plants against microbial pathogens. A functional analog of SA, Acibenzolar-S-Methyl (ASM) has been developed as the most potent activator of SAR in plants. Studies have confirmed that the functioning of ASM is independent of SA accumulation, however the exact action mechanism of this chemical is still not clearly defined. We therefore investigated the mechanism of ASM mediated SAR signaling pathway in plants. Biochemical studies (TLC and HPLC) were performed to test if SABP2 could catalyze the conversion of ASM (ester) into acibenzolar (acid). To elucidate the role of SABP2 in ASM induced SAR, transgenic tobacco plants silenced in SABP2 expression were used. The expression of defense protein, PR1 was used as a molecular marker to test the induction of SAR. The level of ASM induced SAR response was assessed by measuring the TMV induced lesion sizes on the systemic leaves of ASM treated SABP2-silenced and control plants. Our results show that SABP2 converts ASM into acibenzolar, which induces the expression of PR1 proteins and develops the SAR in ASM treated plants. This study will help to develop better chemical elicitors of SAR, which may utilize SABP2 for their conversion into the active forms.

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Function of Ekip1:1 - an E3 ubiquitin ligase in Arabidopsis development

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In the plant life cycle, organs are formed by continual activity of apical meristems. While the meristem determines the size, number and identity of organ primordia, later processes in the primordia define the final shape and size of organs. In order to understand how organ growth is controlled, we study the ERECTA family receptor-like kinases. When the entire ERECTA gene family is lost, the shoot apical meristem produces normal organ primordia, but they develop into dwarf organs due to reduced cell proliferation in all tissues and premature cell differentiation in the epidermis. Here we focus on the early steps of this signal transduction pathway in an attempt to find and characterize direct downstream targets of ERECTA.

Using a yeast two-hybrid assay, we identified a protein interacting with the functional kinase domain of ERECTA and named it Ekip1:1 (for Erecta Kinase Interacting Protein 1:1). Ekip1:1 belongs to a family of 4 proteins, all of which contain an N-terminal RING domain followed by a von Willebrand factor type A (vWFA) domain. The C-termini of all four proteins interact with the ERECTA kinase domain in a yeast two hybrid assay. An ubiquitination assay determined that Ekip1:1 has E3 ubiquitin ligase activity. An analysis of promoter activity by a β -glucuronidase reporter system suggests that Ekip1 family genes are expressed in a pattern overlapping with ERECTA family genes including expression in stomatal lineage cells. Analysis of Ekip1 function in planta and its role in ERECTA signaling is ongoing. This work was supported by a National Science Foundation (NSF) Graduate Research Fellowship to Rebecca Wilson, by NSF IOS-0843340 to Elena Shpak, and by American Society of Plant Biology Undergraduate Research Fellowships to Lihan Deng.

Cuticle plays an important role in basal as well as induced defense against bacterial and fungal pathogens

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Systemic acquired resistance (SAR) is a well-known phenomenon in plants that confers protective immunity in the distal tissues towards secondary infections by related or unrelated pathogens. SAR involves the generation of mobile signal (s) at the site of primary infection, which then translocate to, and activates defense responses in the distal tissues. Although several signals have been implicated to play a role in SAR, the signaling events leading to activation of SAR still remains unclear. Recently, we showed that an intact cuticle is required for decoding of the mobile signal in the distal tissues. Genetic mutations leading to abnormal cuticle or physical damage of cuticle on the distal leaves compromised SAR. Interestingly, a requirement for intact cuticle was only relevant within the time frame of mobile signal generation and translocation to the distal tissues. Since most mutations affecting cuticle development also impair fatty acid (FA) and/or lipid biosynthesis, we studied a role for these in SAR. Our results show that impaired biosynthesis of FAs or lipids do not contribute to SAR. Furthermore, we have uncovered several mutations that specifically alter cuticle without influencing FA or lipid biosynthesis and these mutants were impaired in SAR. Besides SAR, most mutants with abnormal cuticle showed enhanced susceptibility to necrotrophic fungal pathogens and this phenotype did not correlate with cuticular permeability. Together, these studies demonstrate an important role for cuticle in induced as well as basal defense responses.

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Functional studies of AtAPYs 3-7 in *Arabidopsis thaliana*

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Apyrases (ATP-diphosphohydrolases) are a family of enzymes that catalyze the hydrolysis of phosphoanhydride bonds of nucleoside tri- and di- phosphates in the presence of divalent cations. In *Arabidopsis*, AtAPY1 and AtAPY2 function in part as ectoapyrases and have been shown to play important roles in controlling the concentration of extracellular nucleotides, which, in turn, can regulate pollen germination and growth, and cell expansion in diverse plant tissues. We used a NCBI nucleotide blast keyed to Apyrase Conserved Regions (ACRs) to identify five other AtAPYs (3-7). Their subcellular localization is as yet unknown, but we have characterized the tissue specificity of these five apyrases, using pAPY:GUS transgenic lines, and carried out qRT PCR assays to confirm the pAPY:GUS analyses. As judged by GUS staining, AtAPY3 and AtAPY4 are primarily expressed in roots but not in rosette leaves. AtAPY5 is expressed primarily in rosette leaves but not in roots. AtAPY6 and AtAPY7, however, are expressed in many different tissues, including roots, leaves, pistils and mature pollen. AtAPY5 and AtAPY7 are upregulated when the rosette leaves are wounded or exposed to drought stress. To assess the biological function of each of these five AtAPY genes, the phenotypes of their T-DNA insertion mutants have been analyzed. So far, we have not observed any obvious phenotypes for the T-DNA insertion knockout of any of these genes. Current experiments include the generation of double mutants between Apy5,6,7 and analysis of their phenotypes. Supported by an NSF grant (IOS-0718890) to SJR.

Expressed Sequence Tag analysis of switchgrass seeds

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Switchgrass (*Panicum virgatum L.*) is a warm season C4 perennial grass widely grown in North America. It has been recognized as a promising feedstock for bioenergy industry by the U.S government. Seed dormancy is one of the major problems that potentially hinder the large-scale production of switchgrass. Genomic resources for analyzing the molecular basis of dormancy in switchgrass are lacking. To facilitate this process we are conducting Expressed Sequence Tag (EST) analysis of dormant and sprouting switchgrass seeds. In this study we isolated messenger RNA from dormant seeds and sprouting seeds of switchgrass. The mRNAs were converted into double stranded cDNAs and following size selection, were cloned into the pSPORT1 vector. We have collected approximately 4000 clones from these two EST libraries. We are in the process of sequencing these seed ESTs. Preliminary analysis of the seed EST sequence data and their eventual use in constructing switchgrass cDNA-based microarrays will be discussed.

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Talks

Translation factors and plant development

Fujun Zhou, Bijoyita Roy, John R Dunlap, Justin N Vaughn, Byung-Hoon Kim and **Albrecht G von Arnim***

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Myosin motor proteins require dimerization for efficient binding of their tails to organelles during cytoplasmic streaming

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Identification and Biochemical Analysis of Secondary Product Glucosyltransferases of *Citrus paradisi*

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Selenium tolerance in *Arabidopsis* requires the major isoform of adenosine 5'-phosphosulphate reductase (APR)

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Posters

Expression of *Arabidopsis* fatty acid amide hydrolase (FAAH) in transgenic cotton (*Gossypium hirsutum* L., cv. Coker 312)

Bikash Adhikari*¹, Shanmukh S. Salimath¹, Elison B. Blancaflor², and Kent D. Chapman¹

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Sequencing and characterization of microRNAs of a monocot halophyte *Spartina alterniflora* toward understanding regulation of its salinity adaptation

Niranjan Baisakh*¹, Julio Solis Samiento¹, Mangu Venkata RamanaRao¹, Andy Pereira²

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Towards development of arsenic sensor ferns: Isolation of 5'UTRs from arsenic/metal transporter genes and current efforts on transformation of *Pteris vittata*.

Muthukumar Balasubramaniam*¹, Blake L. Joyce¹ and C. Neal Stewart Jr. ¹Authors have equally contributed.

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Domain requirements for the ETR1 ethylene receptor

Elizabeth Helmbrecht¹, Heejung Kim¹, M. Blaine Stalans¹, Christina Schmitt¹, Neesha Patel¹, Wuyi Wang² and **Brad Binder***¹

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Adaptors of myosin motor proteins on organelle surfaces

Krzysztof Bobik* and Andreas Nebenführ

University of Tennessee, Knoxville, Biochemistry, Cellular and Molecular Biology, Walters Life Sciences, M407 1414 Cumberland Ave, Knoxville, TN 37996-0840

Identifying Novel MicroRNAs in *Arabidopsis thaliana* Roots

Natalie Breakfield* and Philip Benfey

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Putative ASA3 Gene Product and Function in Arabidopsis

Ricky D. Wright, Morgan D. Kurz, David W. Hayes, and **Jeffrey E. Brotherton***

Department of Chemistry, North Greenville University, PO Box 1892, Tigerville, SC 29688

Antimicrobial activity of Yerba Mate (*Ilex paraguariensis*) against plant pathogens

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Advances in high-resolution chemical characterization of plant neutral lipid compartments

Patrick Horn*¹, Supriyo Ghosh², Xenia Tombokan², William Hoffman¹, Guido Verbeck¹ and Kent Chapman¹

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Over-expression of the transcription factor gene AtA20.5 in cotton improves the tolerance to rapidly-developing water deficit

Moh'd Hozain*¹, Yuanhua Wang¹, Mohamed Fokar¹, Hajaj Abdel-Mageed², Randy Allen², and A. Scott Holaday¹

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Imaging Lipid Droplets in Arabidopsis Mutants

Christopher N. James*¹, Patrick J. Horn¹, Charlene Case Richardson¹, Satinder K. Gidda², Daiyuan Zhang³, Robert T. Mullen², John M. Dyer³, Richard G. W. Anderson⁴, and Kent D. Chapman¹

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Evolutionary Lineages and Functional Diversification of Plant Hexokinases.

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Lauroylethanolamide (NAE 12:0) is a potent competitive inhibitor of lipoxygenase activity

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Regulation of plant growth in tomato by expression of ERECTA genes from *Arabidopsis thaliana*

Hector Villagarcia¹, Kanishka de Silva¹, Elena Shpak², **Mariya Khodakovskaya***¹
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Polysome microarray analysis for translomics

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De novo motif discovery from soybean cyst nematode-induced genes in soybean

Wusheng Liu*, Murali R. Rao, Mitra Mazarei, and C. Neal Stewart, Jr.
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In vivo analysis of synthetic promoters by agroinfiltration of tobacco leaves for pathogen phytosensing

Wusheng Liu*, Mary R. Rudis, Virginia R. Sykes, Mitra Mazarei, and C. Neal Stewart, Jr.
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Accessory Proteins Expressed by *Aspergillus flavus* in a Xylanolytic Environment

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Suppressors of a rhizobial lipopolysaccharide defect restore symbiosis on alfalfa

Hajeewaka C. Mendis*, Brian K. Washburn and Kathryn M. Jones
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Interactions of nitrogen and carbon metabolism in native and non-native submerged aquatic vegetation

Molly Mintz*, Timothy Sherman, Kelly Major and C.S. Major
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Characterization of de novo transcriptome for a non-model plant, horseweed (*Conyza canadensis*), using GS-FLX 454 pyrosequencing

Yanhui Peng*¹, Laura L.G. Abercrombie¹, Joshua S. Yuan^{2,3}, R. Douglas Sammons⁴, Patrick J. Tranel⁵ and C. Neal Stewart, Jr.¹
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Regulation of Carotenoid Metabolism in Orange-Fleshed Sweet Potato (*Ipomoea batatas* (L.) Lam)

María Quirico* and J. Brad Murphy

University of Arkansas 316 Plant Sciences Building, Fayetteville, AR 72701

FLP/FRT recombination from yeast in transgenic plants for enhancing sensitivity of reporter genes in a phytosensing system

Murali R. Rao*, Hong S. Moon, C. Neal Stewart Jr., Mitra Mazarei

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Green light reverses blue light-induced chloroplast movement

Lauren Johnson and **Judy Schmalstig***

Biology Department, Rollins College, 1000 Holt Ave, Winter Park, FL 32789

Role of bHLH93 in controlling flowering time in *Arabidopsis thaliana*

Nidhi Sharma* and Enamul Huq

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Regulation of translation by *Arabidopsis* eIF3

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TN 37996-0840

Selenium tolerance in *Arabidopsis* requires the major isoform of adenosine 5'-phosphosulphate reductase (APR)

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Biology Department, 1878 Fort Collins, CO 80523

Ballistic seed dispersal in bittercress

Kevin C. Vaughn* and Andrew J. Bowling

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Over-expression of the transcription factor gene *AtA20.5* in cotton improves the tolerance to rapidly-developing water deficit

Moh'd Hozain¹, **Yuanhua Wang***¹, Randy Allen², and A. Scott Holaday¹

¹Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409-3131 and ²Oklahoma State University and the Noble Foundation, Ardmore.

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Abstracts, Kriton-Hatzios Symposium “Regulatory Small RNAs in Plants”

Genomical and Functional Analysis of the Small RNA and mRNA transcriptome and Degradome of *Miscanthus X giganteus*

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It is now known that sRNA play key roles in the post-transcriptional regulation of genes involved in many plant biological processes. As well as direct gene silencing, they are important in development, responses to stress, and genome evolution. To discover the sRNA networks and their role in regulating biomass deposition and perennality-related traits in the allo-triploid *Miscanthus X giganteus* (Mxg): a top candidate for next generation bioenergy crops (NGBC), we are deploying the high-throughput sequencing technology to sequence and profile-by-sequence the sRNA and mRNA transcriptome and degradome in various developmental tissues and organs in Mxg. In addition to identifying 61 annotated miRNA families, we discovered 65 novel miRNA families. Many of the novel and annotated miRNA were differentially expressed between inflorescence, rhizome, leaf, stem, stem-apex, stem-subapex, and young-stem. The expression profiles of predicted targets of the novel and annotated miRNA were negatively correlated in many tissues. Moreover, the miRNA guided AGO-cleaved mRNA transcripts were sequenced for many of the targets of both novel and annotated miRNA families. Comparative expression analysis of both miRNA and mRNA across various tissues and organs revealed perennality- and biomass-related clusters of miRNA/mRNA. Relative to the expression in leaf, the perennality-related miRNA clusters had synchronized expression patterns in inflorescence and rhizome, whereas the biomass-related miRNA clusters were up- and down-regulated in the main biomass tissues, mainly apex, subapex, young stem, mature stem. Our results pave the ground for unveiling the role of sRNA networks in controlling the gene expression that defines the quality and quantity of biomass as well as perennality related traits such as flowering time, overwintering, and nutrient sequestration in the fuelstock grasses.

Gene Regulation by Small RNAs and the Technological Application in Plants

Guiliang Tang

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Small RNAs, including miRNAs and siRNAs, play extensive roles in growth and development, epigenetics, genome integrity, defense against viral infection, and responses to environmental changes in plants. miRNAs are especially important in controlling plant development by negatively regulating many transcription factor genes at the post-transcriptional level. siRNAs play a predominant role in RNA interference (RNAi) and in transcriptional gene silencing through DNA and histone methylation. To date, hundreds of miRNAs and hundreds of thousands of endogenous siRNAs have been identified from dozens of plant species. On one hand, functional genomics of small RNAs needs effective tools to block the functions of these small RNAs individually or in combination. On the other hand, these small RNAs can be artificially generated in plants to be used as effective triggers to silence any protein-coding genes of interest. Here, I first review the major pathways in production of these small RNAs in plants. Then, I introduce a technology, termed short tandem target mimics (STTMs), to destroy the small RNAs in vivo in plants for small RNA functions. Finally, I will present a modified technology, using two-hit artificial miRNAs, to effectively silence the protein-coding genes. Examples in detail will be given for these small RNA technologies.

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Explore the Small RNA World using Plant Model Systems

Zhixin Xie

Department of Biological Sciences, Texas Tech University

Small RNAs of 21- ~24-nucleotide (nt) in size have emerged as an important regulatory component expressed by most eukaryotic genomes. Studies over the past decade have firmly established the important role of small RNAs in the control of gene expression, epigenetic modification of the genome, and defense against viruses. In general, these small RNA molecules arise from transcripts that form either bimolecular or intra-molecular double-stranded RNA (dsRNA) precursors. Processing of dsRNA precursors by the evolutionarily conserved RNA silencing machinery gives rise to mature small RNAs that function in diverse cellular processes. The core components of RNA silencing machinery involve several evolutionarily conserved protein families, including DICER (DCR) or DICER-LIKE (DCL), ARGONAUTE (AGO), and RNA-DEPENDENT RNA POLYMERASE (RDR). Genetic analyses have revealed multiple small RNA pathways in plants each requires a distinct set of RNA silencing factors. Plants, therefore, provide a unique system to study the genetic and functional diversification of small RNA-mediated regulatory mechanisms. My presentation will cover recent advances in our understanding of biogenesis and function of virus-derived small RNAs, as well as miRNA-mediated gene regulatory networks in plants.