2013 MEPS Symposium Abstracts

Featured Speakers

**Dr. Luis Herrera-Estrella**

Dr. Luis Herrera Estrella, a pioneer and leading expert on genetic engineering, received a Ph.D. in plant molecular biology from the State University of Ghent, Belgium, where he also conducted postgraduate research.

His current research is focused on the study of the molecular mechanisms that regulate the development of plant roots in response to environmental factors and in the field of functional genomics of several endemic species of Mexico.

He is a Howard Hughes Distinguished Fellow, and a member of US National Academy of Sciences. Dr. Herrera has received the Distinguished Graduate Research Award by the New York Academy of Sciences, Javed Husain award by UNESCO, Gold Medal by WIPO (World Intellectual Property Organization), etc.

Currently, Dr. Herrera is the Director and Full Professor of the National Laboratory of Genomics for Biodiversity.

**Presentation Abstract**

**A novel fertilization and weed control system based on transgenic plants able to metabolize phosphite**

Luis Herrera-Estrella

Laboratorio Nacional de Genómica para la Biodiversidad del Centro de Investigación y de Estudios Avanzados.

Irapuato, Guanajuato, Mexico

Poor soil fertility and aggressive weeds pose major constraints to meeting the increasing demand for global food production. Starting with the green revolution in the 1960s, higher yields have been accompanied by a steady increase in the use of fertilizers and herbicides. Phosphorus (P) is a nutrient that limits crop yield in over 60 percent of the world's arable land. To increase plant productivity in soils with low P availability, several million tons of P fertilizer is applied every year to agricultural soils. However, by some estimates, world resources of inexpensive P may be depleted by 2080. Low Pi availability in the soil is mainly due to its high reactivity with soil components and rapid conversion by soil bacteria into organic forms that are not readily available for plant uptake. Due to both of these factors, as little as 20–30% of the Pi that is applied as fertilizer is actually used by cultivated plants. The inefficient utilization of Pi present in fertilizer is further aggravated by the competition of weeds with crops for soil resources. Because Pi cannot be substituted in plant nutrition, relatively little attention has been given to the use of other chemical forms of phosphorus to formulate effective and potentially less environmentally hazardous fertilizers.

Phosphite, a reduced form of phosphorus, was proposed as a promising alternative fertilizer after the Second World War, owing to its distinct chemical and biochemical properties compared with orthophosphate, including higher solubility, lower reactivity with soil components and the inability of most microorganisms to use it as a phosphorus source. However, plants cannot metabolize phosphite, limiting its use as a fertilizer. In this presentation I will report on the development of a novel fertilization and weed control system by engineering plants to metabolize phosphite. This was achieved by expressing a phosphite oxidoreductase that converts phosphite into Pi, in transgenic plants. When grown in soil that contains native microflora and fertilized with phosphite, engineered plants expressing the phosphite oxidoreductase achieve maximum productivity with 30 to 50% less P than that required to reach the same productivity using Pi as fertilizer. Since non-engineered plants are unable to use phosphite as a P source, when fertilized with phosphite the engineered plants easily outcompete weeds reducing or eliminating the need for herbicides to achieve maximum yield. In contrast to Pi that when released from contaminated rivers into the ocean promotes toxic algal blooms that kill aquatic organisms, phosphite should not cause these severe ecological problems since it cannot be used as a nutrient by algae. Thus these metabolically engineered plants allow the design of a dual fertilization and weed control system with both potentially important economical and ecological benefits.

**Dr. Takeshi Fukao**

Dr. Fukao is Assistant Professor in Department of Crop and Soil Environmental Sciences at Virginia Tech. He received a bachelor’s degree in Plant Breeding from Kyoto Prefectural University, a master’s degree in Agronomy
and Horticultural Sciences from Kyoto University, and a Ph.D. degree in Molecular and Environmental Plant Sciences from Texas A&M University. His postdoctoral training was done at University of California, Riverside.

Dr. Fukao’s research focuses on understanding the regulatory mechanisms underlying submergence and drought tolerance in rice and other crop species. He also has collaborated with agronomists and plant breeders, applying the knowledge obtained from basic research toward enhancement of stress tolerance in rice. He has published his research in Nature, PNAS, The Plant Cell, Plant Physiology, and The Plant Journal.

**Presentation Abstract**

**Waterproof Rice Gene, SUB1A - from genes to farmers’ fields**

Takeshi Fukao  
Department of Crop and Soil Environmental Sciences, Virginia Tech

Submergence and drought increasingly impact agricultural productivity and sustainability throughout the world as a consequence of global climate change. Rice is a semi-aquatic plant that adapts to partially flooded environments. However, complete submergence causes annual losses of over $1 billion in the world. Through QTL analysis and map-based cloning, we identified SUB1A, a single master regulator that dramatically enhances survival of submergence in rice. Detailed molecular analyses have uncovered regulatory mechanisms governing physiological and molecular adaptations through hormonal regulation. Recent functional analysis revealed that SUB1A also coordinates acclimation responses to cellular dehydration caused by de-submergence and drought. Elucidation of the genetic basis underlying submergence tolerance enabled to develop new rice cultivars with enhanced survival of the stress. Currently, more than 3.5 million farmers grow Sub1 rice in India, Nepal, Bangladesh, and the Philippines.

**Dr. Sibum Sung**

Dr. Sibum Sung is an Assistant Professor of Molecular Cell & Developmental Biology at The University of Texas at Austin and a Fellow at UT’s Institute for Cellular and Molecular Biology. He earned his bachelor of science degree in biology (*summa cum laude*) from Seoul National University. He also received his master of science degree in biology from Seoul National University, South Korea. He received his doctoral degree in biochemistry from the University of Wisconsin at Madison for his work on the molecular basis of vernalization in Arabidopsis. In 2007, he was appointed as an assistant professor at The University of Texas at Austin. His research on epigenetic mechanism on vernalization and other developmental processes in plants at UT has been supported by NSF, NIH and USDA.

**Presentation Abstract**

**Vernalization: Coordinated Epigenetic Silencing by Protein and Noncoding RNA Components**

Sibum Sung  
Section of Molecular Cell & Developmental Biology, School of Biological Sciences and Institute for Cellular & Molecular Biology, the University of Texas at Austin

In a model plant species Arabidopsis, one of key determinants for flowering time is a MADS-box floral repressor, FLOWERING LOCUS C (FLC). FLC is silenced after a sufficient period of winter cold has been perceived (known as vernalization response). The lack of FLC expression allows plants to achieve the competence to flower in spring through the activation of floral integrator genes. Previous studies revealed that repression of FLC by vernalization is achieved in part by an evolutionarily conserved chromatin modifying complex, Polycomb repressive complex 2 (PRC2). In Arabidopsis, PRC2 (which contains CLF, an E(z) homolog, a H3K27 methyltransferase) is recruited to FLC chromatin upon vernalizing cold and mediates methylations at Histone H3 Lys 27 (H3K27me3), a repressive histone modification mark. Recent reports identified a plethora of long and/or short noncoding RNAs (ncRNAs), which contribute to the recruitment of PRC2 to its target chromatins. In vernalization, a long noncoding RNA (lncRNA), named as COLDAIR, was shown to mediate FLC silencing by vernalization. COLDAIR lncRNA binds directly to CLF, a PRC2 component, and is necessary for increased enrichment of PRC2 at FLC chromatin by vernalization. Using the FLC regulation as a model system, I will discuss current understandings on epigenetic FLC silencing by protein and noncoding RNA components.

**Dr. Tzung-Fu Hsieh**

Dr. Hsieh grew up in Taiwan and received his Bachelor degree in Chemistry from National Tsing Hua University in Taiwan. He received his PhD degree in Biology from Texas A&M University in College Station, Texas in 1998. In
2001, he joined Dr. Robert Fischer's lab as a postdoctoral fellow in the Plant and Microbial Biology Department at University of California, Berkeley. In August 2012, he joined the Plants for Human Health Institute and the Plant Biology Department at North Carolina State University as an Assistant Professor. He studies epigenetic regulation of plant reproduction, and the roles of epigenetics in regulating plant secondary metabolites biosynthesis.

Presentation Abstract

Active DNA Demethylation during Gametogenesis Regulates Gene Imprinting and Transposon Silencing in Arabidopsis

Tzung-Fu Hsieh1*, Christian A. Ibarra1, Vera K. Schoff2, Robert L. Fischer1, Hisashi Tamaru2, Daniel Zilberman1
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The companion cells of the Arabidopsis thaliana egg and sperm, the central and vegetative cells, undergo active DNA demethylation prior to fertilization. However, its biological significance, extent of conservation, and targeting preferences are not yet clear. We recently showed that localized demethylation of interspersed, small transposable elements is a common feature of A. thaliana companion cells. The DEMETER DNA glycosylase has been shown to encode active DNA demethylase activity and is required for seed production. DME-mediated DNA demethylation in the central cell is required to establish imprinted gene expression in the endosperm, and is considered a master regulator for plant gene imprinting. However, the similarity among DME targets in the central and vegetative cells, despite their different functions and developmental fates, suggests that establishment of genomic imprinting may not be the basal function of DME. Lack of DEMETER in vegetative cells causes reduced methylation of transposons in sperm. This suggests that the basal function of companion cell demethylation is to reinforce transposon silencing in plant gametes.

Dr. Terri Long

Dr. Long joined the department of Plant Biology at NCSU as an Assistant Professor in August of 2011. She obtained her undergraduate degree from the University of North Carolina at Chapel Hill, where she worked with Dr. Jeffrey Dangl as a Morehead Scholar to characterize the genetics of plant disease resistance to fungal pathogens. As a National Science Foundation Graduate Research Fellow at the University of Georgia, she then studied a key component of photosynthetic cyclic electron transport in Arabidopsis and Pinus taeda that helps control response to high light and drought under Dr. Sarah Covert. As an NSF Minority Postdoctoral Research Fellow at Duke University, Long worked with Dr. Philip Benfey on generating a high-resolution transcriptional profile of the root of the model plant Arabidopsis thaliana, which revealed two novel regulators of the iron deficiency response in plants. She was then appointed as an assistant professor in the Department of Biology at the University of Illinois at Chicago, where she continued her work on iron homeostasis in plants, before joining the faculty at NCSU. She teaches Plant Physiology (PB421).

Presentation Abstract

Iron Deficiency: Molecular Mechanisms for Sensing and Response in Plants

Devarshi Selote, Jeffrey W. Gillikin, Rozalynne Samira, Anna Stallmann, Terri A. Long*
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Plants respond to iron deficiency with a number of physiological modifications that alter nutrient uptake, translocation, storage, and metabolism. Several transcriptomics and proteomics studies have begun to reveal how various constituents of a plant system coordinate these responses. However, the molecular mechanisms that trigger these dynamic changes await elucidation. Components of this mechanism have been described in the model plant Arabidopsis thaliana. Several bHLH transcriptional regulators, including FIT, bHLH38, bHLH39 and POPEYE (PYE) are involved in responding to low iron. PYE is encoded by a gene that is expressed primarily in the vasculature in response to low iron, while the protein appears to be localized in the nuclei of most root cells. Previously, we have shown that PYE interacts, in vitro, with close PYE homologs (PYHs) and that PYE directly and indirectly regulates the expression of genes that encode proteins involved in iron reduction, storage and translocation. The formation of mobile PYE/PYH heterodimers could play an important role in these regulatory processes. Interplay between PYE and its homologs appears to be controlled at the post-translational level by
interaction with a third protein, BRUTUS, which may bind iron and, thus, sense iron content within roots. Using a number of molecular and biochemical approaches we are determining how and where these proteins interact in planta, and how iron availability triggers this interaction to facilitate response to low iron.

**Dr. Brian Ayre**

Brian Ayre’s research interests revolve around the phloem transport system and how it functions as a whole-plant communication network to enable disparate organs to function as an integrated and complete organism. Within this broad context, a principal line of research is based on the hypothesis that manipulating the sugar content of the phloem can modulate transport patterns and target nutrients and biomass to desired organs (i.e., harvested organs for food, fiber or fuel). A second research area is studying phloem-mobile signals that govern source control of sink development, particularly the function of the CETS/PEBP gene family that includes the flowering factor FT (FLOWERING LOCUS T, encoding florigen) and the vegetative factor TFL1 (TERMINAL FLOWER 1). Ayre received his PhD in Plant Molecular Biology from the University of Alberta, and conducted postdoctoral research at the MRC Laboratory of Molecular Biology in Cambridge, England, and in the Department of Plant Biology at Cornell University, before accepting a faculty position at the University of North Texas where he is now Associate Professor. He recently conducted a sabbatical leave at the Max Planck Institute for Molecular Plant Physiology in Potsdam-Golm, Germany. His current research is funded by NSF IOS, Cotton Incorporated, and the USA-Israel Bi-National Agricultural Research and Development (BARD) Fund.

**Presentation Abstract**

**The phloem network as a whole-plant integrator of developmental signals and nutrient homeostasis**

Brian G. Ayre, Roisin C. McGarry, Kasturi Dasgupta

University of North Texas, Department of Biological Sciences, Denton, TX 76203

The phloem transport system operates as a whole-plant communication network that allows disparate organs to function as an integrated and complete organism. Within this broad context, two projects will be discussed: the role of the phloem in transporting signals from leaves to growing tissues to mediate source control of sink development, and the role of the phloem in coordinating carbon metabolism and nutrient use between source leaves and sink organs. 1) Florigen, encoded by FLOWERING LOCUS T (FT) in Arabidopsis and SINGLE FLOWER TRUSS (SFT) in tomato, was originally described as a flowering hormone, but acts more broadly as a general growth hormone to advance determinate growth. Using a disarmed virus vector as a transient expression system, we delivered FT to both photoperiodic perennial cotton and day-neutral domesticated cotton. Ectopic FT expression in domesticated accessions promoted more synchronized flowering and a compact structure preferred by producers, and ancestral cotton demonstrated photoperiod-independent flowering and precocious determinate growth in branches and leaves. Transient FT expression also facilitated crosses between wild and domesticated accessions, demonstrating an effective mechanism to increase diversity in cultivated cotton. 2) Sucrose/H+ symporters (SUTs) are essential for efficient phloem loading and transport in Arabidopsis and the major crop species. We and others hypothesized that SUT overexpression in companion cells of mature leaves would enhance Suc loading and transport, and possibly increase growth of sink organs and storage reserves while increasing primary productivity by reducing Suc-mediated inhibition of photosynthesis. To achieve enhanced phloem loading, SUT activity was uncoupled from endogenous control by using heterologous phloem-specific promoters and over-expressing genes from diverse clades in the SUT-gene family. Contrary to expectations, both rosette and root growth in Arabidopsis was stunted and photosynthetic rates decreased, despite evidence for more Suc loading and transport. Further analysis revealed that the stunted phenotype resulted from the perception of a phosphate limitation, and could be resolved by increasing phosphate levels in the growth medium. This apparent link between carbohydrate transport and phosphate utilization will be discussed in the context of whether we can improve carbon partitioning and allocation without increasing the need for an essential non-renewable nutrient.

**Dr. Aaron Smith**

Dr. Aaron P. Smith, a native of West Virginia, received his B. S. in Biology from Fairmont State University in 1997. He earned his doctorate in Plant Genetics in 2003 at Purdue University, with Peter Goldsbrough as his advisor. Smith then did his postdoctoral work at the University of Georgia with Rich Meagher. He joined the Department of Biological Sciences at Louisiana State University in 2008, where he currently is an Assistant Professor. Smith’s research investigates the genetic and epigenetic mechanisms that regulate plant responses to environmental stress, such as deficiencies in essential nutrients including phosphorus and iron.
Presentation Abstract

Linking Changes in Chromatin Structure to Phosphorus- and Iron-Deficiency Responses in Rice

Aaron P. Smith, Sara Zahraeifard, Qi Zhang, Maryam Foorozani, Sandra DiTusa, Elena Batista, Niranjan Baisakh, and Maheshi Dassanayake
Louisiana State University

Phosphorus (P) and iron (Fe) are major limiters of crop productivity due to their low availability in most soils. Fe-deficiency is common in aerobic soils, whereas Fe toxicity afflicts rice grown under flooded conditions. Plants modulate complex responses to fluctuating P and Fe levels via global transcriptional regulatory networks. Although chromatin structure plays a substantial role in controlling gene expression, the chromatin-level/epigenetic mechanisms involved in regulating nutrient homeostasis are not understood. We are using genome-wide approaches to identify chromatin-remodeling mechanisms that regulate responses to deficiency of P and/or Fe in rice. Ongoing experiments include 1) identifying P and/or Fe deficiency-dependent changes in nucleosome positioning; 2) examining the role of the H2A.Z histone variant in regulating gene expression; and 3) characterizing the histone modifications present at key rice P and Fe homeostasis genes during deficiency of P and/or Fe. By correlating three aspects of chromatin structure (i.e. nucleosome positioning, histone variant localization, and histone post-translational modifications) with gene expression profiles, the regulatory mechanisms that modulate P and Fe homeostasis genes will be revealed. Identifying these chromatin-level/epigenetic mechanisms will provide opportunities for developing crops with improved nutrient use-efficiency, significantly improving U.S. and global agriculture.

Graduate Student Presentations

Regulation of Osmotic Stress Signaling by Arabidopsis C-terminal Domain Phosphatase-like 1 Requires Interaction with a K-homology Domain-containing Protein

In Sil Jeong, Akihito Fukudome, Emre Aksoy and Hisashi Koiwa
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Abstract: Arabidopsis thaliana carboxyl-terminal domain (CTD) phosphatase-like 1 (CPL1) is a protein that regulates plant transcriptional responses to diverse stress signals. Unlike prototypical CTD phosphatases, which contain a phospho-peptide-binding Breast Cancer 1 C-terminal (BRCT) domain, CPL1 contains 2 double-stranded RNA binding motifs (dsRBM) at its C-terminus. While some dsRBMs can bind to dsRNA and/or other protein partners, the function of CPL1 dsRBMs has been obscure. A biochemical approach using tandem-affinity purification followed by mass spectrometry analyses of CPL1 complex identified CPL1-Associating Protein 1 (CAKH1), which contained multiple K homology domains important for binding to single-stranded DNA/RNA. Yeast two-hybrid and luciferase complementation imaging analyses has established that CPL1 and CAKH1 strongly associate in vivo, and dsRBM1 of CPL1 and KH3/KH4 domains of CAKH1 mediate their interaction. Mapping of functional regions in CPL1 indicated important of dsRBM1, as well as catalytic activity and nuclear targeting of CPL1 for its proper in vivo function. These results suggest that tethering CPL1 to CAKH1 via dsRBM1 is required for CPL1 to regulate stress-responsive transcription, and nuclear targeting of CPL1 is likely prerequisite for the interaction. Gene expression profiles of rcf3 alleles of cakh1 mutants overlapped with, but were distinct from that of cpl1. This suggests that CAKH1 regulates only a subset of CPL1-regulated transcripts, which is likely involved in the negative feedback of osmotic stress signaling.

A Novel Role for Extracellular Nucleotides in Early Growth and Development of Ceratopteris Spores

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Abstract: Calcium channels play a critical role in the polarized development of Ceratopteris spores, just as they do in the polarized growth of pollen tubes and root hairs. Recent data indicate that calcium channels in plants can be regulated by extracellular nucleotides and that the concentration of these nucleotides is regulated by ectoapyrases.
Since apyrase activity is needed for the germination and polarized growth of pollen tubes and for the emergence and polarized growth of root hairs, the observed expression of a gene encoding an apyrase-like enzyme in Ceratopteris spores suggested the hypothesis that apyrases and extracellular nucleotides could play a regulatory role in the germination and early polarized development of spores. Our documentation that applied nucleotides can alter polar axis alignment and rhizoid growth in Ceratopteris spores, and that a purinoceptor antagonist can alter the gravity response of rhizoids, is consistent with this hypothesis. Furthermore, our observation that Ceratopteris spores release ATP as they germinate and grow is consistent with the postulate that extracellular ATP could influence calcium transport in the spores. A hypothetical model that could explain how ATP and PPADS could alter the gravity response would include gravity causing stretch-activated channels to open preferentially along the bottom of the spore. In animal and Arabidopsis cells, these channels release ATP. If ATP release was mainly along the bottom of the spore, this ATP could induce the opening of calcium channels preferentially at the pole. The added ATP disrupts this gradient and potentially disrupts the asymmetry of calcium entry. Collectively, these results and hypothetical model describe a novel role for extracellular nucleotides in early growth and development of Ceratopteris spores.

### Differential temperature operation of plant immune responses

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**Abstract:** Microbe and host have co-evolved dynamically in their arms race for fitness and survival. Environmental factors often influence the physiological responses in both sides and have profound impacts on microbial invasion and host evasion. Temperature fluctuates both daily and seasonally, and has long been considered as one of key determinants for disease epidemics. In contrast to animals that maintain a fairly constant body temperature, plant body temperature oscillates on a daily basis modulated by the environment. It remains largely elusive how plants operate inducible defense programs in response to the ambient temperature changes. We report here that ambient temperature changes lead to pronounced shifts of two distinct plant immune responses: pathogen/microbe-associated molecular pattern (P/MAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI). Plants preferentially activate ETI signaling via intracellular nucleotide-binding domain leucine-rich repeat (NLR) immune receptors at relatively lower temperatures (10–23°C). However, the plant defense strategy is switched to PTI signaling mediated by cell surface receptor-like kinase (RLK) receptors at moderately elevated temperatures (23–32°C). Furthermore, the high temperature transcriptional mimicking and phenocopying Arabidopsis mutants, arp6 and hta9hta11 deficient in incorporating histone H2A.Z into nucleosomes exhibit enhanced PTI responses and yet reduced ETI responses compared with wild-type plants. Our findings suggest that plants co-evolved distinct immune strategies under ambient temperature oscillation to counteract the physiological changes of pathogens: multiply vigorously at elevated temperatures (25–30°C), accompanied with increased MAMP production; whereas the secretion of bacterial pathogenic effectors favors low temperatures (16–23°C).

### Pullulanase Activity is Associated with Formation of Vitreous Endosperm in Quality Protein Maize

Wu, Hao; Clay, Kasi; Thompson, Stephanie S.; Love, Sterling; Gibbon, Bryan C.

Baylor University

**Abstract:** The opaque2 (o2) mutation of maize increases lysine and tryptophan content, but the low seed density and soft texture of this type of mutant are undesirable. Lines with modifiers of the soft kernel phenotype (mo2) called “Quality Protein Maize” (QPM) have high lysine and kernel phenotypes similar to normal maize. Prior research indicated that the formation of vitreous endosperm in QPM might involve changes in starch granule structure. Four starch biosynthesis genes, SSIIa, SSIIb, SSIII and Zpu1, have been discovered to have unique alleles in mo2 lines; therefore these genes may play a role in formation of vitreous endosperm. qPCR analysis of recombinant inbred lines (RILs) derived from a cross of QPM and soft o2 lines showed a significant increase in expression of the QPM-derived Zpu1 allele. Quantitative enzyme activity assays showed that QPM lines had higher pullulanase activity than o2 and wild type. Furthermore, pullulanase activity was positively correlated with kernel vitreousness in the RILs. Differential scanning calorimetry showed that the thermal properties of starch from the RILs correlated well with the presence of the QPM-derived allele of Zpu1, which had decreased onset and peak endotherm temperatures while total enthalpy of gelatinization was unchanged. Pullulanase activity was negatively
correlated with the onset and peak endotherm temperatures but was not correlated with enthalpy. Additionally, pullulanase activity was negatively correlated with the sensitivity of starch.

**Metabolic engineering of a green microalga for autotrophic production of isoprenoid-type advanced hydrocarbon fuels.**

Ryan Syrenne, Shangxian Xie, Ugur Uzuner, Susie Y. Dai, and Joshua S. Yuan

1, Department of Plant Pathology and Microbiology, Institute for Plant Genomics and Biotechnology, Texas A&M University, College Station, TX 77843

**Abstract:** The monoterpene limonene could potentially replace jet fuel (JP-8), gasoline and diesel as an alternative ‘drop-in’ liquid hydrocarbon fuel source. Synthetic biology-guided metabolic engineering of microalgae for the direct production of terpenoid-type hydrocarbon could provide the ultimate enablement of third-generation biofuels. Traditional microbial platforms used to produce hydrocarbon molecules similar to petroleum derived fuels rely on tractable heterotrophic hosts, such as E. coli or yeast, and essentially trade sustainability for high product yields. This study demonstrates the heterologous expression of a rice monoterpene synthase gene (OsTPS26) in the eukaryotic green algae Chlamydomonas reinhardtii (cc503) to autotrophically produce the high-volumetric energy dense hydrocarbon limonene. Using a modified nuclear transformation strategy, we engineered nuclear transformants expressing a functional rice limonene synthase localized to the plastid. Although microalgae are not known to poses monoterpene synthases, the precursor substrate geranyl diphsophate (GPP) pool is available as it’s required for downstream sesquiterpenes and other larger terpenoid molecules. Additionally, we show that limonene is emitted from the algal biomass into the headspace region of enclosed photobioreactors – eliminating the requirement of cell harvesting, dewatering, and biomass processing. Chlamydomonas mutant lines were cultured in enclosed photobioreactors enabling a purge-and-trap method of trapped volatile limonene in the culture head-space. Limonene analyte adsorbed on to volatile collection traps were subjected to gas-chromatography/mass-spectrometry (GC/MS) for product identification and quantification analyses. We show limonene production from a single transformant line at ~1 µg/g dry biomass in 72 hrs. In total, ten transformant lines were evaluated for limonene production; mRNA abundance was quantified by qPCR and limonene analyte in the head-space was quantified by GC/MS analysis. Conceptually, determining baseline capability of monoterpene production in algae will pave the way for advanced autotrophic hydrocarbon production.

**Poster Abstracts**

1. *Abscisic Acid Inhibits Leaf Expansion by Limiting Cell Expansion but not Cell Division in Arabidopsis*  
Shinsuke Agehara, Scott A. Finlayson, and Daniel I. Leskovar  
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**Abstract:** Abscisic acid (ABA) accumulation during water stress inhibits leaf expansion to limit plant water loss. When this acclimation is induced by exogeneous ABA, we have previously shown that it is followed by rapid leaf expansion, with leaf area eventually recovering to the control level. We therefore hypothesize that ABA inhibits cell expansion but not cell division, and the maintenance of cell division enables such recovery of leaf expansion after ABA degradation. To test this hypothesis, we treated Arabidopsis (Arabidopsis thaliana) plants with 0 or 1 mM ABA at the rosette stage with 7-8 leaves. During 6 days following the treatment, ABA inhibited expansion of 5th and 7th leaves by 10% and 53%, respectively, while it had no effect on older leaves. Regardless of leaf age, epidermal cell number per leaf was unaffected by ABA, suggesting that ABA inhibits leaf expansion solely by limiting cell expansion. In addition, ABA affected neither number of stomata per leaf nor guard cell length, which regulate the rate of gas exchange and transpiration. These results suggest that ABA-induced inhibition of leaf expansion is a mechanism to conserve water by limiting increases in non-stomatal evaporative area, as opposed to stomatal closure that reduces transpiration. Importantly, this mechanism may not limit plant growth and photosynthetic capacity, as leaves maintain both cell division and stomatal formation.

2. *A novel root-to-shoot cadmium translocation determinant is overexpressed in Arabidopsis cpl1 mutant*  
Emre Aksoy, Hisashi Koiwa  
Molecular & Environmental Plant Sciences, Vegetable & Fruit Improvement Center, Department of Horticultural Sciences, Texas A&M University, College Station, TX-77843
Abstract: Root-to-shoot translocation of non-essential heavy metal cadmium (Cd) is a key determinant of the high Cd tolerance phenotype. Cd ions can enter the plant cells through metal transporters such as IRT1. In Arabidopsis, mutations in RNA polymerase II CTD-phosphatase-like 1 (CPL1) enhance the constitutive expression of IRT1. Moreover, the root growth of cpl1-2 mutant shows higher tolerance to Cd toxicity and the cpl1-2 plants accumulate more Cd in the shoots, suggesting that Cd toxicity in the cpl1-2 roots is circumvented by the transport of excess Cd to the shoots. Unexpectedly, the expression levels of the transporters known to function in root-to-shoot translocation of Cd, such as HMA2/4 and NRT1.5/1.8, were similar between wild-type and cpl1-2 roots. Interestingly, a putative metal transporter termed Cd Determinant 1 (CDM1) gene was expressed 1200 times higher in cpl1-2 roots under Cd toxicity. cdm1 mutant plants accumulate less Cd in the shoots suggesting its role in Cd transport. Here we show data suggesting that the root-to-shoot translocation of Cd in cpl1-2 is primarily mediated by CDM1.

3. Understanding uptake and transport of phosphate and arsenate in Arabidopsis
Elena J. Batista, Sandra F. DiTusa, Naohiro Kato, and Aaron P. Smith
Louisiana State University

Abstract: Currently, agricultural crop production worldwide suffers from phosphorus-deficient and arsenic-toxic soil. Many members of the Pht1 family of plant high-affinity phosphate transporters, which transport phosphate and arsenate, have been described; however, little is known regarding the biochemical properties that contribute to their activities. Split-luciferase complementation assays (SLCA) have been used to test whether Pht1 isoforms from Arabidopsis thaliana form higher-order complexes. Interestingly, initial screenings of multiple combinations of Pht1:1 and Pht1:4 pairs in Arabidopsis protoplasts for reconstituted luciferase activity have resulted in putative hetero- and homo-oligomerization of Pht1:1 and Pht1:4. Currently, another in vivo approach, the yeast mating-based split-ubiquitin system, is being employed to corroborate the interaction results from the SLCA. In addition to testing for Pht1 interactions, we are interested in identifying amino acid residues responsible for Pht1 transport activity of phosphate and arsenate. To identify individual amino acids important in phosphate and arsenate transport, the yeast strain PAM2 (double mutant of Δpho84 and Δpho89) has been complemented with Pht1:1 and Pht1:4 cDNAs via the pYES2 yeast expression vector. The absence of Pho84 and Pho89 phosphate transporter function in PAM2 results in its reduced growth in phosphate-depleted medium. Growth assays of yeast isolates expressing pYES2:Pht1:1 and pYES2:Pht1:4 in low-phosphate and arsenate-containing media confirm the uptake of both phosphate and arsenate by the yeast lines, reaffirming that arsenate uptake is modulated by phosphate transporters. Ongoing experiments are determining whether differences exist in the specificities Pht1:1 and Pht1:4 have for phosphate and/or arsenate. These approaches will lead to a better understanding of the transport functions of Pht1 transporters, which will aid in the development of more efficient crop plants.

4. NITROGEN NEEDS AND EFFICIENCY OF RHIZOBIA STRAINS TO PROVIDE NITROGEN TO CHIPILIN (Crotalaria Longirostrata HOOK. AND ARN.)
Camarillo Castillo, F.1, Mangan, F.2 Autio, W.2, Cox, D.2, and Martinez-Solis, J.3
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Abstract: Chipilin (Crotalaria Longirostrata) is a leguminous plant native to Central America and Southern Mexico and used in the preparation of traditional dishes in this region. Starting in 2009, farmers in Massachusetts have been growing chipilin with a weekly production of 800 kg·ha-1. However, as much as 300 kg·ha-1 of nitrogen has been necessary to apply to the soil in order to obtain a marketable leaf quality. With the goal to determine the nitrogen requirements of chipilin and to quantify the capacity of selected stains to infect and provide nitrogen for this crop, two- field experiments were conducted at the UMass Research farm at Deerfield, Massachusetts, in an occur fine sandy loam soil (coarse-loamy, mixed, mesic Fluventic Dystrudept) soil as a randomized complete block design with five replications. For the field trial in 2011, nitrogen rates were (kg·ha-1): 40, 80, 120, 160, 200 and 240 and 0, 40, 80, 120, 160, 200, 240 and 280 in 2012 in combination with four Rhizobia strains: Bradyrhizobium sp. (Vigna), Rhizobium leguminosarum biovar, Bradyrhizobium USDA 3384 and no Rhizobia were the treatments. Based on the results obtained, nitrogen fertilizer application of 80 kg·ha-1 was economically sufficient for chipilin to
reach optimum yield. However higher nitrogen rates are needed to obtain marketable leaf color and quality. Additionally a greenhouse experiment set up as a factorial experiment with five replications was conducted with seven nitrogen concentrations (mg N·L−1): 0, 26.25, 52.5, 105, 157.5, 210 and 262.5 mg·L−1 and the three Rhizobia strain for the previous experiment plus Bradyrhizobium USDA 2370 as treatments. Results suggest that Bradyrhizobium USDA 3384 is not an efficient strain for chipilin, and Rhizobium leguminosarum biovar potentially may provide the most nitrogen of the strains evaluated. In the greenhouse trial, nodules number per plant decreased with the increase in nitrogen applications, but this was not the case in the field trial in 2012. Nodules were found on the root of chipilin plants in the control. This is suspected to be due to one of the following possibilities: Rhizobia inoculum presence in the seed, Rhizobia in the soil (in the field trial) or contamination during the setup of the experiment.

5. Apyrases and extracellular ATP play a role in Arabidopsis stomatal opening and closing

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Abstract: There is increasing evidence that extracellular ATP can regulate growth in a variety of plant cell and tissue types. In Arabidopsis leaves there is a bi-phasic dose-response to applied nucleotides, lower concentrations (5-15 μM) of the poorly hydrolysable nucleotide ATPγS induces stomatal opening, while higher concentrations (150-200 μM) induces closure. Both ATPγ-induced stomatal opening and closing are blocked by two different mammalian purinoceptor antagonists, pyridoxalphosphate-6-azo-phenyl-2', 4'-disulfonic acid (PPADS), and Reactive Blue 2. These antagonists also partially block the abscisic acid (ABA)-induced stomatal closure and light-induced stomatal opening. Application of ATP instead of ATPγS requires 10-fold more ATP than ATPγS to induce changes in stomatal aperture presumably because applied ATP is hydrolyzed by ecto-apyrases. In Arabidopsis there appears to be two closely related apyrases, AtAPY1 and AtAPY2, which are both expressed in guard cells. Application of different levels of chemical inhibitors of apyrase activity can induce stomatal opening or closing. Treatment with apyrase enzyme blocks ABA-induced stomatal closure and partially blocks light-induced stomatal opening. Taken together these results suggest that the swelling and shrinking of guard cells induced by various stimuli may result in the release of nucleotides that help regulate stomatal apertures.


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Abstract: A greenhouse experiment was conducted using alternate and fixed PRD to evaluate leaf gas exchange and WUE of three-year-old split-root potted Mexican Lime trees. Two irrigation trials (daily vs. every three days) included three treatments in each trial: 1) well-watered trees, where both rootzone halves were watered with 50% crop evapotranspiration (ETc; Control); 2) one half of the rootzone received no water while the other half was irrigated with 100% ETc (fixed PRD, FPRD); and 3) one half of the rootzone was allowed to dry while the other half was irrigated with 100% ETc by alternating wet and dry halves every two weeks (alternate PRD, APRD). In the first trial (daily irrigation), FPRD1 plants used 16.3% significantly less water than Control1 plants. Whole plant WUE was significantly higher APRD1 plants than in Control1 plants. Leaf abscisic acid (ABA) content in FPRD1 was higher than in Control1 plants. In the second trial (irrigation every three days), FPRD2 and APRD2 plants used 14.7% and 17.3% less water than Control2 plants, respectively. Leaf ABA content was significantly higher in FPRD 2 and APRD2 plants than in Control2 plants however there were no differences in stomatal conductance among treatments. PRD-treated plants in both trials did not affect leaf gas exchange parameters since all treatments had similar CO2 assimilation (ACO2) and leaf area development. Thus, PRD treatments FPRD1, FPRD2 and APRD2 resulted in water savings without compromising the growth.

7. Identification and characterization of phosphate transporters from the arsenic hyperaccumulating fern, Pteris vittata

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Abstract: Plants frequently encounter soils containing arsenate, which competes with phosphate for uptake through phosphate transporters. Plants have evolved adaptations to arsenate exposure. Most plants exclude arsenate
by suppressing phosphate uptake machinery. In contrast, Pteris vittata, the first arsenic hyperaccumulator identified, has enhanced uptake of arsenate from both increased transporter expression and increased affinity of the phosphate/arsenate uptake system for arsenate. We are reporting on the identification of three Pht1 family phosphate transporters from P. vittata, PvPht1;1, PvPht1;2, and PvPht1;3. All three transporters were shown to localize to the plasma membrane. PvPht1;3 transcripts were shown to be induced in response to Pi deficiency and arsenate exposure. To examine whether the PvPht1 genes encode bona fide phosphate transporters, PvPht1;1, PvPht1;2, and PvPht1;3 cDNAs were each expressed in the PAM2 (Δpho84 Δpho89), Pi uptake-defective Saccharomyces cerevisiae strain under low-phosphate conditions. Expression of PvPht1;3 complemented the PAM2 Pi-uptake defect to a similar extent as cells expressing the native Pho84 phosphate transporter or the Arabidopsis phosphate transporter, AtPht1;5. However PvPht1;3-expressing cells were more sensitive to arsenate than those expressing AtPht1;5. This suggests that PvPht1;3 and AtPht1;5 exhibit similar phosphate, yet different arsenate, uptake characteristics. Uptake experiments using 33Pi (orthophosphate), demonstrated that the PvPht1;3- and AtPht1;5-expressing cells accumulated similar quantities of phosphate. In contrast, preliminary arsenate uptake experiments showed that PvPht1;3-expressing cells accumulate approximately 2 fold more arsenic than cells expressing AtPht1;5. These data confirm that the uptake capacities for AtPht1;5 and PvPht1;3 are the same for phosphate, but different for arsenate. This study reveals that P. vittata contains a phosphate transporter (PvPht1;3) that is upregulated by arsenate exposure and has a higher specificity for arsenate relative to another Pht1 protein from higher plants. Hence, PvPht1;3 contributes to the novel ability of P. vittata to hyperaccumulate arsenate.

8. Characterization of gc2 Mutations in Maize
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Abstract: General control non-derepressible-2 (GCN2), first discovered in yeast as a regulatory protein kinase, plays an important role in cellular responses to amino acid availability. It phosphorylates the alpha subunit of the trimeric eukaryotic translation initiation factor-2 (eIF2), which in turn decreases the general rate of protein synthesis in response to nutrient starvation or stresses. The phosphorylation of eIF2-alpha enhances the translation of the transcription factor GCN4, resulting in increased expression of many amino acid synthesis genes. The GCN2-like kinases are highly conserved among eukaryotes, including fungi, animals and plants. Opaque2 (O2) is a b-zip transcription factor that regulates storage protein accumulation in the maize endosperm and it is the maize homologue of GCN4. We were interested to investigate if O2 is a target of GCN2 regulation in maize endosperm. To date no mutations that affect GCN2 have been reported in maize. Three Mutator insertion mutations of gc2 were identified from the Pioneer TUSC populations and backcrossed to the inbred line B73. Analysis of gene expression by qRT-PCR showed that the gc2-1 transcript is reduced but still present in developing endosperm, although at lower levels than B73. Several O2 regulated genes such as ribosome inactivating protein and alpha-zeins are only slightly down-regulated in gc2-1 homozygous mutants. Additionally, there were no clear differences in the O2 protein accumulation observed on western blots of either genotype. Analysis of tissue specific gene expression indicated that in the gc2-1 mutant the transcript level was much lower than in any other tissue tested. The gc2-1 mutant is more sensitive to herbicides that affect amino acid biosynthesis, which is similar to the phenotype of gccn2 knockouts in Arabidopsis. Furthermore, phosphorylation of eIF2-alpha in response to herbicide treatment is abolished in the gc2-1 mutant plants. Because there are only modest effects on O2-related gene transcription in maize endosperm we are undertaking a bioinformatics approach to identify other genes that are predicted to be subject to translational regulation in response to phosphorylation of eIF2-alpha.

9. Using forward genetics to identify novel Arabidopsis thaliana peroxisomal mutants
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Abstract: Peroxisomes are single-membrane-bound organelles responsible for key metabolic reactions needed for the development of many eukaryotes. In plants, fatty acid β-oxidation and β-oxidation of indole-3-butyric acid (IBA) to the active auxin, indole-3-acetic acid (IAA), are performed by peroxisomal enzymes. Because peroxisomes do not contain their own DNA, these enzymes require peroxisomal import machinery for localization to the peroxisomal matrix. Genetic screens have identified peroxin (PEX) proteins involved in peroxisome biogenesis. However, several expected mutants have not been recovered, suggesting that improved screens for peroxisomal defects will yield novel mutants. We are screening the progeny of mutagenized Arabidopsis thaliana seed for
reduced β-oxidation efficiency. By modifying light conditions and β-oxidation substrates, and by including assessment of matrix protein import in the primary screen, we are improving throughput and isolating more severe alleles. Using these enhancements, we have identified ten new pex14 alleles, three new pex7 alleles, and two new deg15 alleles; positional cloning of novel affected loci is ongoing. These new mutants will increase our understanding of peroxisome biogenesis and function, which may have implications in other plants and other eukaryotes.

10. Gas exchange parameters and leaf water potential in intercropped peanut (Arachis hypogaea L.), watermelon (Citrullus lanatus Thunb.), okra (Abelmoschus esculentus Moench.), cowpea (Vigna unguiculata L.) and hot pepper (Capsicum spp.) under heat stress

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Abstract: Crop rotations during the heat of Texas’ summers are limited due to the poor availability of crops that can withstand the high temperatures. We utilized different combinations of peanut, watermelon, okra, cowpea and pepper in single crop and various intercropping combinations, whereby each crop performed a specific function within the system. Specifically, we investigated the effectiveness of these cropping combinations on alleviating heat stress during the hottest months. Preliminary results from leaf water potential measurements indicate peanut and okra were more water stressed during the daytime when monocropped than in combination with other component crops. Overall, peanut exhibited the most water stress than the other component crops. However, peanut also appeared to rehydrate well overnight as indicated by pre-dawn leaf water potential measurements. These preliminary results suggest there may be a facilitative effect from intercropping utilizing these crops and that this may reduce heat stress on crops during the hottest months. However, it is unclear if this facilitative effect translates into higher photosynthetic activity and increased production. Leaf gas exchange measurements, along with production data, are currently being analyzed in order to assess these relationships. These intercropping combinations may provide small-scale sustainably-minded producers a model system that can be utilized in suboptimal conditions and allow them to reduce inputs while increasing yields.

11. CPL4 is a bona fide CTD phosphatase, essential for normal growth and development in Arabidopsis thaliana

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Abstract: Eukaryotic transcription is tightly modulated by phospho-regulation on carboxy-terminal domain (CTD) of RNA-polymersase II (Pol II). A model plant Arabidopsis thaliana has four CTD-phosphatase like family proteins (CPL1, CPL2, CPL3, CPL4). Among them, CPL4 has been proposed to be essential in normal growth and development, because disruption of CPL4 function by RNAi results in severe growth and developmental defect, including slow root growth with less lateral root formation. Microarray analysis on the CPL4 knockdown line revealed that the expression profile is similar to those from Acetolactate synthase (ALS)-inhibitor herbicide treated plants, light-response/photomorphogenesis mutants and plants grown under nitrate depletion condition. Tandem-affinity purification and mass-spectrometry analysis identified Pol II subunits in the CPL4 complex, and CTD-phosphatase activity of CPL4 was confirmed both in vitro and in vivo. Transient co-expression of CPL4 reduced reporter gene expression in tobacco leaf, indicating its effect on transcription. Taken together, CPL4 is an essential and bona-fide CTD phosphatase in Arabidopsis, which may regulate growth and development by modulating nitrogen assimilation, amino acid biosynthesis/metabolism and light-response pathways.

12. Phytochrome interacting factors 4 & 5 mediate plant architectural responses to shade and temperature cues.

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Abstract: Plants actively adjust their growth and morphology in response to changing light conditions. Plasticity in the branching/tillering habit is an important developmental response to fluctuating light. The ratio of red light (R)
to far red light (FR) is highly informative to plants. The reduction in R:FR due to the proximity of nearby plants serves as an early signal of competition. In Arabidopsis thaliana, morphological response to reduced R:FR has been attributed to huge network of signaling components controlled by phytochromes (phyA-E). Among the Arabidopsis phy family, phyB plays a key role in eliciting the shade avoidance responses. Phy mediated light signaling transduction have been integrated with bHLH transcription factors, PHYTOCHROME INTERACTING FACTORS (PIFs) linking phy to downstream events. Remarkably, PIF4 and PIF5 have been implicated in the mechanisms regulating responses to shade and temperature cues. The results from our preliminary studies suggest that PIF4 and PIF5 integrate both light and temperature cues to regulate branching. We speculate that phs and PIFs modulate phytohormone biosynthesis and signaling in the buds to control their outgrowth. Auxin and abscissic acid (ABA) have been implicated in regulating bud outgrowth. Investigations on the expression of genes associated with auxin and ABA biosynthesis and signaling pathways are being carried out. The results of this study will provide insights into how light signaling pathways influence axillary branching by fine-tuning auxin and ABA biosynthesis and/or signaling pathways.

13. First structural characterization of ARC6 and its role in FtsZ anchoring
Min Woo Sung, Rahamthulla Shaik, Emily Brown, Leung K. Tang, Akshaya Ravichandran, Stanislav Vitha and Andreas Holzenburg
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Abstract: Chloroplasts and all plastids have to divide in order to maintain their numbers in proliferating cells. The integral membrane ARC6 is one of the key regulatory proteins for division of plastids in Arabidopsis thaliana. In the absence of ARC6, chloroplast division is completely blocked and cells contain only 1-2 grossly enlarged chloroplasts. The N-terminal portion of ARC6 is in the plastid stroma, where it interacts with the C-terminus of the plastid division protein FtsZ2 and serves to stabilize and anchor FtsZ1-FtsZ2 assemblies. Although a large body of data is available on FtsZ ring formation and chloroplast division no structural data have been reported for ARC6 to date. Here single particle analysis is presented as a first step towards its structural characterization. Pichia X33 strain expressing the truncated form of mature ARC6 (D54-D450 - stromal portion at the N-terminus) was used in this study. Averaged projections suggest at least two classes of ARC6, one representing a dimeric form (stack of parallel densities) and the other being triangular in shape and comprised of three dovetailed protomers forming an equilateral triangle, i.e. constituting a trimeric form of ARC6. In order to investigate the in vivo functional role of ARC6, the mobility of GFP-tagged FtsZ assemblies was assessed by Single Particle Tracking in mutant plants lacking the ARC6 protein. Mean Square Displacement analysis showed that the mobility of FtsZ assemblies is to a large extent characterized by anomalous diffusion behavior (indicative of intermittent binding) and restricted diffusion suggesting that besides ARC6-mediated anchoring, an additional FtsZ-anchoring mechanism is present in chloroplasts.

14. Plastid signaling in the control of leaf size
Sonia Irigoyen, Diana N. Sagiroi, and Wayne K. Versaw
Department of Biology

Abstract: Mature leaf size for a given species is relatively uniform and is a function of the size and number of cells in the organ. Leaf development is a complex process and multiple pathways can independently control cell proliferation and affect final leaf size. Arabidopsis loss-of-function mutants for the root plastid Pi transporter PHT4;2 have rosette leaves that are 40% larger than wild type, but the plants are otherwise morphologically and developmentally indistinguishable from wild type. Increased cell proliferation fully accounts for the greater leaf area and biomass because the size of mature leaf cells is unchanged. The pht4;2 mutants show altered expression of some trehalose metabolism genes, which indicates a possible role for trehalose or its biosynthetic precursor, trehalose-6P, in the pht4;2 phenotype. Trehalose-6P, is a potent signaling molecule in Arabidopsis with roles in sugar sensing, cell cycle and in plastids, starch synthesis. We hypothesize that hyperaccumulation of Pi within root plastids influences trehalose-6P signaling processes that affect cell proliferation in the developing leaf and/or shoot apical meristem. Transgenic experiments to discern potential relationships between plastidic phosphate accumulation, trehalose/trehalose-6P signaling and leaf size will be discussed and results of a kinematic analysis of leaf development to define the spatial and temporal effects of the pht4;2 mutation on cell proliferation will be presented.
15. **Coordinating vernalization response through epigenetic regulatory networks of VIN3 and FLC gene families in Arabidopsis**
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**Abstract:** Developmental fates of cells are determined by innate genetic programs as well as by interactions with their environments. Plants use many systems to sense their environment and to modify their growth and development accordingly. Vernalization is one example of such a system. Vernalization is an environmentally-induced epigenetic switch in which winter cold triggers epigenetic silencing of floral repressors, and thus provides competence to flower in spring. Vernalization triggers the recruitment of chromatin-modifying complexes to a clade of flowering repressors which are epigenetically silenced via chromatin modifications. In Arabidopsis, VIN3 and its related PHD finger proteins act together with Polycomb Repressive Complex 2 to increase repressive histone marks on floral repressors, including FLC and its related genes, by vernalization. Here, we show that members of VIN3 family of proteins function to repress different members of FLC gene family during the course of vernalization. We will discuss regulatory networks of related families of proteins, noncoding RNA components and chromatin modifying complexes, which differentially contribute to establishment and maintenance of vernalization-mediated epigenetic silencing of floral repressors.

16. **Synergistic effect of plant furocoumarin and a protease inhibitor on the cowpea bruchid**
*Callosobruchus maculatus*
Jiaxin Lei, Fengguang Guo, Bhimu Patil, Hisashi Koiwa, and Keyan Zhu-Salzman

*Department of Entomology, Vegetable & Fruit Improvement Center, Texas A&M University, College Station, TX 77843, USA*

**Abstract:** Bergapten, a furanocoumarin compound, is a plant secondary metabolite that has anti-insect function. When incorporated into artificial diet, it retarded cowpea bruchid development, decreased fecundity, and even led to their death. cDNA microarray analysis indicated that cowpea bruchids altered expression of 543 midgut genes in response to dietary bergapten. Among these bergapten-regulated genes, 225 unique genes have known functions, for instance, those encoding proteins related to nutrient transport and metabolism, development, detoxification and various cellular functions. Such differential gene regulation presumably facilitates the bruchids to counter the negative effect of dietary bergapten. Many did not have hits (E-value 10^-6) with known genes in a BLASTX search (206) or had homology only with genes of unknown functions (112). Interestingly, when compared with the transcriptomic profile of cowpea bruchids treated with dietary soybean cysteine protease inhibitor N (scN), 195 out of 200 overlapping midgut genes are oppositely regulated by the two treatment compounds. Simultaneous administration of bergapten and scN resulted in attenuated differential expression of selected, oppositely-regulated genes in response to each of these two compounds, as well as synergistic anti-insect effect. Therefore, targeting insect vulnerable sites that may compromise each other’s counter-defensive response has the potential to increase the efficacy of the anti-insect molecules.

17. **Inverse modulation of plant immune and brassinosteroid signaling pathways by a receptor-like cytoplasmic kinase BIK1**
Wenwei Lin, Dongping Lu, Xiquan Gao, Shan Jiang, Xiyu Ma, Zonghua Wang, Tesfaye Mengiste, Ping He, and Libo Shan

*Plant Pathology and Microbiology, TAMU*

**Abstract:** Maintaining active growth and effective immune responses is often costly for a living organism to survive. Fine-tuning the shared cross-regulators is crucial for metazoans and plants to make a trade-off between growth and immunity. The Arabidopsis regulatory receptor-like kinase BAK1 complexes with the receptor kinase FLS2 in bacterial flagellin-triggered immunity and BRI1 in brassinosteroid (BR)-mediated growth. BR homeostasis and signaling unidirectionally modulate FLS2-mediated immune responses at multiple levels. We have shown previously that BIK1, a receptor-like cytoplasmic kinase, is directly phosphorylated by BAK1 and associates with FLS2/BAK1 complex in transducing flagellin signaling. In contrast to its positive role in plant immunity, we report here that BIK1 acts as a negative regulator in BR signaling. The bik1 mutants display various BR hypersensitive phenotypes accompanied with increased accumulation of de-phosphorylated BES1 proteins and regulation of BZR1 and BES1 target genes. BIK1 associates with BRI1, and is released from BRI1 receptor upon BR treatment, which is...
reminiscent of FLS2-BIK1 complex dynamics in flagellin signaling. The ligand-induced release of BIK1 from receptor complexes is associated with BIK1 phosphorylation. However, in contrast to BAK1-dependent FLS2-BIK1 dissociation, BAK1 is dispensable for BRI1-BIK1 dissociation. Consistently, unlike FLS2 signaling which depends on BAK1 to phosphorylate BIK1, BRI1 directly phosphorylates BIK1 to transduce BR signaling. Thus, BIK1 relays the signaling in plant immunity and BR-mediated growth via distinct phosphorylation by BAK1 and BRI1 respectively. Our studies indicate that BIK1 mediates inverse functions in plant immunity and development via dynamic association with specific receptor complexes and differential phosphorylation events.

18. Genetic transformation of tomato (Lycopersicon esculentum cv. Micro-Tom) with a Calcium signal modifier gene (CSM-1).

Cecilia Lott* [1], Z. Viloria[1], E. Louzada[1], D. Henne[2].

Abstract: Tomatoes have many infectious diseases that reduce fruit or productivity. Due to tomato’s short lifecycle and because of its ease to be genetically transformed, we can use Micro-Tom (MT) for agrobacterium mediated genetic transformations. Calcium signal modifier gene (CSM-1) is a citrus gene that has been overexpressed in several citrus species, overexpression seems to increase calcium signals in plants, furthermore preliminary results show that transgenic plants are resistant to a list of diseases. We expect to develop broad spectrum disease resistance by inserting a CSM-1 into MT. The objectives of this study are to develop transgenic tomato plants, study morphological and physiological changes, observe how CSM-1 segregates and screen for resistance to pathogens. If CSM-1 holds true, then this can become a great benefit to tomato growers. Micro-Tom cotyledons were inoculated in agrobacterium culture with an optical density of 0.2-0.6 and selection of transgenic plants was performed by using PCR and 5-bromo-4-chloro-3-indolyl glucuronide, (X-Gluc, GUS) to produce 18 transgenic lines. Some transgenic MT lines produced seedless fruit; other lines produced low to moderate seeds giving a mean of 2.12 seeds per fruit and a 60% germination rate. Pollen viability was conducted on semi-solid medium and observed every 3hrs. Wild type MT pollen germination was 37.94% compared to a 2.19% rate of one of the transgenic lines. The low pollen viability may be the cause of low seed set. Offspring of transgenic MT plants were infected with Zebra Chip (Candidatus Liberibacter solanacearum, ZC) through tomato/potato psyllid (Bactericera cockerelli) feeding. Expected infected plants have exhibit leaf curling, and are positive for the bacterium.

19. Genome Target Sequencing in Loblolly Pine (Pinus taeda L.) using Different Multiplexing Strategies

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Abstract: Genome target sequencing provides efficient and cost-effective methods for re-sequencing particular genome regions and for high-throughput genotyping and SNP discovery. We applied the Agilent SureSelect Target Enrichment method to capture unigene-based targeted genomic sequences in loblolly pine (Pinus taeda L.), which is the most important forest tree for timber and paper production in southeastern US. We used 35,386 out of 35,550 unigenes that were assembled by Dr. Chun Liang (Miami University, Oxford, Ohio) and available on http://bioinfolab.muohio.edu/txid3352v1 to design 647,634 oligonucleotide hybridization probes (baits). To make this approach more affordable for population studies, it is important to be able to use multiple barcoded individuals in a single hybridization reaction. Two single (A and B) and two multiplexed (C and D) DNA libraries were constructed and sequenced to test two multiplexed strategies: A and B were non-multiplexed samples representing DNA of the haploid megagametophyte and embryo from a single seed, respectively, while C and D were multiplexed pools composed of four and eight indexed individual DNA samples of megagametophytes, embryos or needles from two and seven individual trees, respectively. Each library was hybridized to the same number of probes. After capturing the targeted sequences, all samples were sequenced in Illuma Hiseq2000 using paired-end sequencing (2×100 bp).

We obtained 74, 66, 551 and 469 million reads for the A, B, C and D libraries, respectively. High quality reads were mapped to the original unigenes and to the draft loblolly pine reference genome assembly (v0.9, provided by the PineRefSeq project; http://pinegenome.org/pinerefseq) using BWA and SAMtools. With the same mapping parameters, 56% of the reads obtained for non-multiplexed samples were mapped to unigenes, but 40% and 44% for C and D pools, respectively. 1.2% of the total unigene length were uncovered or covered by only one read in
non-multiplexed A and B samples and the four-multiplexed C pool, while 1.4% was uncovered in the eight-multiplexed D pool.

On average, 97% and 93% of the reads in non-multiplexed samples and multiplexed pools were mapped to the draft loblolly pine reference genome assembly, respectively. SNP detection was done with each library using SAMtools. On average, 1,905,814; 8,218,249 and 6,484,905 SNPs were detected in the non-multiplexed samples, four-multiplexed and eight-multiplexed pools, respectively. The mean SNP densities are 0.26 SNPs/kb, 0.61 SNPs/kb, 0.50 SNPs/kb.

The results indicate that multiplexing strategies used in this target sequencing study were efficient and cost-effective. We plan to test more samples for multiplexing, but anticipate that it may adversely affect the capture efficiency.

20. **Development of Solanum pennellii as a novel and sustainable biofuel feedstock**
   Sabyasachi Mandal, Wangming Ji, Chika Okonkwo, Thomas D. McKnight
   Department of Biology, Texas A&M University, College Station, Texas, USA

*Abstract:* Solanum pennellii (Solanaceae), a wild relative of cultivated tomato (S. lycopersicum), is native to arid regions of Peru and is a potential new feedstock for biofuels. Potential advantages of this plant over other biofuel sources are that it is a drought tolerant plant which can be grown easily on marginal lands and it is not a crop. This plant secretes glucolipids (2,3,4 tri-O-acylated glucose esters) through trichomes on its leaf surface, presumably to reduce water loss. Transesterification of the secreted compound yields one molecule of glucose and three molecules of C6 to C12 fatty acid esters. These esters are analogous to biodiesel, but with shorter carbon chains that are in the range of bio-gasoline. This bio-gasoline is oxygenated, very low in sulfur, and predicted to be compatible with current fuel transport and storage technologies and with conventional gasoline engines. The biosynthetic pathway of the glucolipid involves only four or five enzymes, making it a good candidate for transferring into other plants. We have successfully cloned cDNAs encoding the first two enzymes of the pathway (UDP:glucose glucosyltransferase and glucose acyltransferase). We will use comparative transcriptomics between high glucolipid-producing accessions and low glucolipid-producing accessions to identify the remaining two or three genes in the pathway. One future goal is to transform at least the first two genes with their native promoters into tobacco and other plants with large leaf surfaces to improve yield of this novel biofuel to fill the critical niche between ethanol and biodiesel.

21. **Cotton Fleahopper Resistance in Upland Cotton**
   Laura Ann McLoud and Steve Hague
   Texas A&M University

*Abstract:* Cotton fleahopper (Pseudatomoscelis seriatus) (Hemiptera: Miridae) is a piercing-sucking insect that has emerged as a major pest in the Texas cotton industry over the past decade. Cotton fleahopper feeding results in square abscission and damage and subsequently, yield-loss. Previous studies in Gossypium hirsutum indicate that plant trichome density plays an important role in conferring resistance to cotton fleahopper, but the mechanism of resistance remains largely unknown. Three families of potentially resistant, wild-type lines (18 genotypes) and two high-yielding lines were screened for resistance to cotton fleahopper under field infestation levels in College Station and Corpus Christi in 2012. Genotypes within the three families exhibited pubescences ranging from smooth to very hairy; of the high-yielding lines, one was smooth and the other hairy. Square-mapping was used as the primary tool by which to monitor the plants’ responses to cotton fleahopper feeding pressure. Overall, the wild-type lines showed significantly less square loss than either of the high-yielding lines, despite harboring more fleahoppers than the high-yielding lines. Within phenotype groups, the wild-type lines significantly outperformed the smooth and hairy high-yielding lines in terms of square loss.

22. **Live imaging of phosphate dynamics with sub-cellular resolution in plants.**
   Pallavi Mukherjee, Amanda Wheeler, Lyndsay Ratliff, Sonia Irigoyen, Steve W. Lockless, Wayne K. Versaw
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*Abstract:* Inorganic phosphate (Pi) is an essential macronutrient that serves a wide range of structural, energy transfer and regulatory roles. Modest changes in the distribution of Pi throughout the plant, including the concentrations within subcellular compartments, can dramatically affect growth and development. Assessment of such changes requires analytical tools with subcellular resolution. A series of fluorescence resonance energy transfer (FRET)-based Pi biosensors named fluorescent indicator protein for Pi (FLIPPi) were developed previously and
their utility for monitoring changes in cytosolic Pi concentrations of cultured animal cells was demonstrated. Here, we describe efforts to optimize FLIPPi sensors for use in different cell compartments of live plants. An in vitro assay system was developed to mimic cellular conditions and thereby minimize potential differences in sensor responses in planta. Sensitivity, or maximum FRET response, was greatly enhanced by substituting the EYFP portion of FLIPPi with a circular permuted version of Venus. A mutant screen for sensors with altered affinity for Pi yielded multiple candidates with Kd values within the predicted physiological range of the cytosol and chloroplast stroma. In most cases, high specificity for Pi was retained. Subcellular localization of one biosensor engineered with and without an N-terminal transit peptide in the chloroplast and cytosol, respectively, of Arabidopsis protoplasts was confirmed.

23. Grafting Reduces Salinity Tolerance of Citrus Rootstocks
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Abstract: Citrus is a valuable horticultural crop in Texas, adding approximately $75 million dollars to the annual economy. Citrus production is concentrated in the lower Rio Grande Valley (LRGV), which is home to many soil types, agricultural management practices and production concerns. The Rio Grande River currently provides most of the irrigation and domestic water for both the U.S. and Mexico, but proximity to the Gulf of Mexico limits groundwater use for irrigation of certain crops in this area. Increased pressure on water sources may lead to using lower quality water sources for agricultural irrigation or alternative methods of managing salt affected areas. Grafting is a common way to enhance plant yield, disease resistance, promote better quality plants and fruits, and salinity tolerance for certain crops. While citrus are commonly grafted to enhance these qualities, new rootstocks C22 and C146 are replacing the commonly used rootstock, Sour Orange. This research compared these new rootstock varieties to the commonly used Sour Orange rootstock to determine tolerance to salinity. When salinity impacts on grafted and non-grafted plants were analyzed, results showed that grafting had a negative impact on citrus salinity tolerance, possibly due to rootstock-scion interaction. However, the new varieties C22 and C146, performed better under higher salinities than Sour Orange rootstocks. This leads us to believe that future research should focus on finding optimal rootstock-scion combinations in areas prone to salinity problems.

24. First structural characterization of ARC6 and its role in FtsZ anchoring
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Abstract: Chloroplasts and all plastids have to divide in order to maintain their numbers in proliferating cells. The integral membrane ARC6 is one of the key regulatory proteins for division of plastids in Arabidopsis thaliana. In the absence of ARC6, chloroplast division is completely blocked and cells contain only 1-2 grossly enlarged chloroplasts. The N-terminal portion of ARC6 is in Chloroplasts and all plastids have to divide in order to maintain their numbers in proliferating cells. The integral membrane ARC6 is one of the key regulatory proteins for division of plastids in Arabidopsis thaliana. In the absence of ARC6, chloroplast division is completely blocked and cells contain only 1-2 grossly enlarged chloroplasts. The N-terminal portion of ARC6 is in the plastid stroma, where it interacts with the C-terminus of the plastid division protein FtsZ2 and serves to stabilize and anchor FtsZ1-FtsZ2 assemblies. Although a large body of data is available on FtsZ ring formation and chloroplast division no structural data have been reported for ARC6 to date. Here single particle analysis is presented as a first step towards its structural characterization. Pichia X33 strain expressing the truncated form of mature ARC6 (D54-450—stromal portion at the N-terminus) was used in this study. Averaged projections suggest at least two classes of ARC6, one representing a dimeric form (stack of parallel densities) and the other being triangular in shape and comprised of three dovetailed protomers forming an equilateral triangle, i.e. constituting a trimeric form of ARC6. In order to investigate the in vivo functional role of ARC6, the mobility of GFP-tagged FtsZ assemblies was assessed by Single Particle Tracking in mutant plants lacking the ARC6 protein. Mean Square Displacement analysis showed that the mobility of FtsZ assemblies is to a large extent characterized by anomalous diffusion behavior (indicative of intermittent binding) and restricted diffusion suggesting that besides ARC6-mediated anchoring, an additional FtsZ-anchoring mechanism is present in chloroplasts.
Abstract: Sorghum is an important source of food, feed, and fodder worldwide, and more recently, it is also being utilized as a biofuel feedstock. In view of its diverse utility, sorghum grain offers a large number of target traits that could be improved to meet the required applications. As in the case of other cereal crops, genetic engineering offers an attractive means to enhance sorghum seed-quality to satisfy the nutritional needs of humans and animals, or to fulfill the specific demands of the biofuel industry. Thus, promoters that provide regulated transgene expression in a seed-specific manner are of critical importance to obtain the desired quality grain. Whether a promoter is homologous or heterologous, it must first be evaluated in the target plant species. In this study, we examined the temporal and spatial transcription pattern of a rice glutelin promoter, GluA-2, in transgenic sorghum. A 1,832 bp long sequence of GluA-2 promoter was ligated at the 5’-end of the gusA reporter gene and this construct was introduced into sorghum by Agrobacterium-mediated transformation of immature embryos. Quantitative GUS analysis of homozygous T2 seeds from three independent transgenic lines showed detectable expression of the transgene at 14 days post anthesis (dpa), which increased as the seed matured. Furthermore, histochemical GUS assay confirmed this pattern of activity and also revealed that the GluA-2 promoter directs GUS expression in the inner starchy endosperm. In addition, the absence of detectable GUS expression in tissues such as embryo, leaf, stem, and root, showed that the activity of this promoter is specific to the endosperm portion of the seed. Our results show the efficacy of the rice GluA-2 promoter as an endosperm-specific promoter in sorghum and suggest that it can serve as a valuable tool in improving the nutritional quality of this important cereal.

26. Structure Dynamics Guided Enzyme Improvement of Endo Beta-1,4-Xylanase 1 From Trichoderma reesei

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Abstract: Enzyme structure dynamics has recently been revealed to be essential for structure-function relationship. Among various structure dynamics analysis platforms, hydrogen deuterium exchange mass spectrometry stands as an efficient and powerful way to analyze protein dynamics upon ligand binding, protein folding, and enzyme catalysis. HDX-MS can be used to study the regional dynamics of proteins based on the m/z value or percentage of deuterium incorporation for the digested peptides in the HDX experiments. Various software packages have been developed to analyze HDX-MS data. However, for the accurate, enhanced, and explicit statistical analysis of HDX-MS data, statistical analysis of software was developed as HDXanalyzer. The capability of HDX-MS analysis for the identification of enzyme structure dynamics was tested by using model catalysis endoxylanase A (XYN I) from Trichoderma reesei. The

XYN I protein. The high level stabilization of XYN I protein was gathered and the two highly active and moderately thermostable XYN I recombinants were developed based on the HDX-MS data which further confirmed the efficiency of the current strategy for the rational designs of catalytic proteins. In summary, the integration of the structure dynamics knowledge to current biochemical and biophysical data of catalysts may provide novel insights to further enzyme improvement applications. Enzyme structure dynamics has recently been revealed to be essential for structure-function relationship. Among various structure dynamics analysis platforms, hydrogen deuterium exchange mass spectrometry stands as an efficient and powerful way to analyze protein dynamics upon ligand binding, protein folding, and enzyme catalysis. HDX-MS can be used to study the regional dynamics of proteins based on the m/z value or percentage of deuterium incorporation for the digested peptides in the HDX experiments. Various software packages have been developed to analyze HDX-MS data. However, for the accurate, enhanced, and explicit statistical analysis of HDX-MS data, statistical analysis of software was developed as HDXanalyzer.
HDX data of XYN I revealed a highly dynamic personality of XYN I through the interaction with two substrates. The dynamic data which certainly restricts the targeted regions for the protein engineering efforts provided useful knowledge about the essential structural modifications for the catalysis of XYN I. The obtained knowledge was then employed for the engineering studies in order to improve the certain characteristics of XYN I protein. The high level stabilization of XYN I protein was gathered and the two highly active and moderately thermostable XYN I recombinants were developed based on the HDX-MS data which further confirmed the efficiency of the current strategy for the rational designs of catalytic proteins. In summary, the integration of the structure dynamics knowledge to current biochemical and biophysical data of catalysts may provide novel insights to further enzyme improvement applications.

27. Comparative transcriptome analyses of cowpea bruchid to Griffonia simplicifolia lectin II and Wheat germ agglutinin

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Abstract: Griffonia simplicifolia lectin II (GS II) and Wheat germ agglutinin (WGA) both are lectins that can bind to N-acetylglucosamine. Previous research showed that they have anti-insect activities and potential application for pest control. To evaluate the adaptation of cowpea bruchid (Callosobruchus maculatus) to these lectins, cDNA microarray analysis was performed and showed that the expressions of genes involved sugar metabolism, lipid metabolism, transport, development, defense and stress were altered by 24 hours treatment. Compared to cellulose, the control as an inhibitor of digestion and absorption, there were more genes responded to GS II and WGA. These genes are involved in cellular process, neural function etc. Moreover, it was interested that genes related to midgut epidermis repair and senescence were also altered after dietary treatment of GS II and WGA. It is consistent with the observations from electron microscope that the microvilli of midgut were shortening after feeding on a WGA-containing diet. These results suggested lectins have more systemic effects on cowpea bruchid besides nutritional deficiencies, and go along with the hypothesis that lectins can bind to targets, induce senescence, damage the midgut epidermis, and then delay insect development.

28. Genetic dissection of plant effector-triggered immunity

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Abstract: Plants relay on a two-tiered immunity system to fight off pathogen infection. The first tier of plant immunity is triggered by conserved pathogen- or microbe-associated molecular patterns (PAMPs/MAMPs) through pattern recognition receptors (PRRs). To be pathogenic, certain bacterial pathogens have evolved the ability to deliver effectors into plant cells through type III secretion system to interfere with host immunity. However, the effectors can be directly or indirectly recognized by plant nucleotide-binding leucine-rich repeat (NLR) proteins and elicit effector-triggered immunity (ETI), a second tier of plant immunity which is often associated with strong restriction of pathogen growth, hypersensitive response (HR) and transcriptional reprogramming. Although many plant NLR proteins that confer resistance to specific strains of bacteria, fungi, virus and nematodes have been identified, the signaling components downstream of NLR proteins still remained fragmented. To dissect the ETI signaling networks, we have developed a sensitive genetic screen with Arabidopsis transgenic plants carrying a NLR signaling marker gene WRKY46 promoter fused with a luciferase (LUC) reporter. A pilot screen of EMS-mutagenized population identified 15 mutants whose pWRKY46::LUC activities are dramatically changed upon Pseudomonas syringae avrRpt2 infection compared to wild-type plants. We named them as altered WRKY46 gene expression (awe). Different awe mutants display distinct characteristics of pWRKY46::LUC activity, HR and disease resistance upon P. syringae avrRpt2 and avrRpm1 infection, indicating that these mutants likely carry mutations at distinct genes/pathways. For example, awe1 shows increased pWRKY46::LUC activity upon P. syringae avrRpt2 and avrRpm1 infection, whereas it displays delayed HR and enhanced susceptibility to P. syringae avrRpt2 infection compared to wild-type plants. In contrast, awe2 shows much increased pWRKY46::LUC activity upon P. syringae avrRpt2 and avrRpm1 infection. Interestingly, it displays delayed HR only to P. syringae avrRpm1 infection, but not to P. syringae avrRpt2 infection. The causal mutations of these awe mutants will be identified with a combination of map-based cloning and next generation sequencing technology. The identification and characterization of these
29. PIFs and COP1-SPA complexes synergistically repress photomorphogenesis
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Abstract: Light signals perceived by the phytochrome (phy) family of sensory photoreceptors promote photomorphogenic development throughout the life cycle of plants. While phys promote photomorphogenesis in response to light, Phytochrome Interacting Factors (PIFs), CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1) and SUPPRESSOR OF PHYA (SPA) repress photomorphogenesis in the dark. In response to light, phys either inactivate (in case of COP1-SPA complex) or induce rapid degradation (in case of PIFs) of these negative regulators to promote photomorphogenesis. COP1 and SPAs form COP1-SPA complexes that induce degradation of the positively acting components (e.g., HY5, HFR1, LAF1) to repress photomorphogenesis in the dark, PIFs also repress photomorphogenesis in the dark independently. However, the genetic relationship between PIFs and COP1-SPA complexes are still unknown. Previously, it was shown that PIF3 is unstable in cop1 and spa mutants, suggesting that PIFs might act downstream of COP1-SPA complexes to repress photomorphogenesis. Here, we show genetic interactions among cop1, spa and various pif mutants. Our data suggest that PIFs and COP1-SPA complexes act additively and/or synergistically to repress photomorphogenesis in the dark. Keywords: Phytochrome Interacting Factors/COP1-SPA complexes/red/far-red light/proteolysis/photomorphogenesis

30. BRANCHED1 Partially Mediates Abscisic Acid Regulation of Branching
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Abstract: BRANCHED1 (BRC1) encodes a TCP domain protein which is closely related to the transcription factor TEOSINTE BRANCHED1 (TB1) that represses axillary bud outgrowth in grasses. BRC1 is a negative regulator of axillary bud outgrowth. Bud outgrowth and elongation are regulated by plant hormones including auxin, a strigolactone and abscisic acid (ABA). It has been shown that BRC1 acts downstream of auxin and the strigolactone-mediated pathway to suppress bud outgrowth. We hypothesized that BRC1 may also act downstream of ABA to inhibit branching. ABA (100 pmoles) was applied to the rosette buds of the Arabidopsis Columbia ecotype (WT) and BRC1 deficient plants (brc1). Exogenous ABA strongly suppressed bud outgrowth of WT and partially suppressed bud outgrowth of brc1. ABA also had a strong effect on the correlative inhibition index of WT, which is a measure of systematic suppression of branching. However, the brc1 mutant showed no significant alteration in correlative inhibition index after treatment with ABA suggesting that BRC1 is necessary for this response.

31. Development of SYBR Green I-based one-step real time RT-PCR assay for quantifying SRBSDV in rice
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Abstract: Southern rice black-streaked dwarf virus (SRBSDV) causes southern rice black-streaked dwarf disease and maize rough dwarf disease, which lead to severe yield losses of crops in Southeast Asia. Here, a sensitive and reliable SYBR Green I-based one-step real time RT-PCR assay was developed for rapidly and accurately quantifying SRBSDV in rice. Primers for specific detection of SRBSDV were designed within the conserved region matching the S9 region of SRBSDV genome. The RNA standards targeting the S9 region were obtained by transcription in vitro for generation of standard curve. The assay developed in this study was found to be 100 times more sensitive than the conventional RT-PCR for SRBSDV detection. The specificity of the reported assay system was also established through melting curve analysis. The reported assay shows the potential clinical application as a sensitive diagnostic test for real-time detection and quantitation of SRBSDV in rice samples, and hence could be a useful tool for the studies of pathogenic mechanism of SRBSDV.

32. The HECATE proteins promote photomorphogenesis by negatively regulating the function of PHYTOCHROME INTERACTING FACTOR 1 in Arabidopsis
Ling Zhu, Hui Shen, Jonathan Dang and Enamul Huq

Abstract: The Phytochrome Interacting Factors (PIFs), a small group of bHLH transcription factors repress photomorphogenesis both in the dark and light. Light signals perceived by the phytochrome family of
photoreceptors induce rapid degradation of PIFs to promote photomorphogenesis. Here we show that HECATE proteins, another small group of bHLH proteins antagonistically regulate PIF1 function to promote photomorphogenesis. Both HEC1 and HEC2 heterodimerizes with PIF1 in yeast-two-hybrid assays as well as in vitro and in vivo co-immunoprecipitation assays. Promoter:GUS and GFP fusion proteins showed that PIF1 and HEC genes are co-expressed in the same tissues and the proteins are co-localized in the nucleus. RNA interference-mediated downregulation of HEC1 or HEC2 or hec1 mutant induces hyposensitivity to light-induced seed germination and chlorophyll accumulation, two hallmark processes oppositely regulated by PIF1. By contrast, constitutive overexpression of HEC2 induces seed germination after FR light exposure and increased chlorophyll accumulation compared to wild type. The seed germination phenotypes of HEC2 overexpression lines are GA-dependent as the overexpression lines in ga1 mutant background failed to germinate. In addition, the seed germination phenotype of hec1 or hec2 RNAi lines or hec1 mutant is eliminated in the pif1 background, suggesting that pif1 is epistatic to hec functions. Taken together, these data suggest the HECATE proteins promote photomorphogenesis by negatively regulating the function of PIF1 and possibly other PIFs in Arabidopsis.