Molecular and metabolic understanding of isoprene emission from trees

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More isoprene enters the atmosphere each year than any other hydrocarbon except methane. This large flux comes mostly from leaves of trees and is related to high temperature, especially high temperature experienced by leaves at the tops of trees on sunny days. The genes involved in isoprene emission (especially isoprene synthase) and the metabolic control of the rate of emission will be described. Putting an isoprene synthase into Arabidopsis, which normally does not make isoprene, causes it to emit isoprene. The substrate for isoprene synthase is dimethylallyl diphosphate (DMADP) and is made by the plastidic methyl erythritol 4-phosphate (MEP) pathway. Isoprene is the primary product of the MEP pathway in plants that make isoprene. Gas exchange measurement techniques have been developed that allow non-invasive measurements of DMADP and a critical MEP pathway intermediate methyl erythritol cyclodiphosphate (MEcDP). These measurements and a metabolomics study of the MEP pathway metabolites have provided explanations for several unusual features of isoprene emission. Two of these will be highlighted. 1. Isoprene emission is subject to overshoots and other transient phenomena in response to temperature and short interruptions of light and these can be explained by variations in the pool of MEcDP. 2. Isoprene emission is extremely sensitive to temperature ($Q_{10} > 4$) and this is explained by simultaneous effects of temperature on isoprene synthase activity and the MEP pathway so that the enzyme is going faster and has more substrate.

Dr. Hideki Takahashi

Dr. Hideki Takahashi has been the PI of research groups at Michigan State University (2010-present) and RIKEN (2000-2010). His research projects are focused on studying molecular mechanisms of sensing, transport and metabolism of two essential nutrients, nitrogen and sulfur, in plants. In the past 15 years of his career, Dr. Takahashi has made significant contribution to the understanding of sulfate transport mechanisms and regulation of sulfur metabolism in plants (reviewed in Takahashi et al. Annu Rev Plant Biol 2011). Dr. Takahashi has recently expanded his research area towards studying nutrient-responsive pathways involved in control of the root system architecture. His recent work, which highlights the function of nitrogen-responsive CLE peptides (Araya et al. PNAS 2014), is a breakthrough in this topical area. His work with a particular emphasis on relationships between nutrient signaling and development of the root system provides new insights into morphological and physiological adaptation mechanisms. Research in this area has strong impacts on biotechnological development of root traits for improvement of nutrient use efficiencies.

CLE-CLAVATA1 signaling pathways in control of plant root system architecture

Hideki Takahashi
Michigan State University

Morphological plasticity of plant roots is critical for nutrient acquisition. Nitrogen (N) is an essential element strongly affecting plant root development in the soil environment. Developing lateral roots in local N-rich soil patches has competitive advantage for N acquisition. The lateral root growth, however, can be significantly restricted when the entire root system is exposed to excess N or deprived of N for a prolonged period of time. This preventive mechanism economizes the cost for root development and reduces the risks of extending roots into N-poor environments. Our recent study indicated critical roles of N-responsive CLE (CLAVATA3/ESR-related) peptides (CLE1, 3, 4 and 7) and CLAVATA1 (CLV1) leucine-rich repeat receptor-like kinase in regulating the expansion of root system in Arabidopsis thaliana [1]. The N-responsive CLE-CLV1 signaling module was demonstrated as an essential mechanism for regulating the emergence of lateral root primordia from the primary root in N-deficient environments. CLEs induced by N deficiency were predominantly expressed in root pericycle cells, while the receptor CLV1 was present in phloem companion cells,
sugestting a systemic mechanism for regulation. Mathematical modeling of root system architecture further supported these phenotypic observations, suggesting the effect of CLE overexpression on arresting lateral root elongation.

Reference

Dr. Lisa Donovan

Dr. Lisa Donovan is a Distinguished Research Professor at the University of Georgia in the Department of Plant Biology. She earned her BS from Salisbury State University and her MS from the University of Delaware college of Marine Studies. She earned her PhD from University of Utah with Jim Ehleringer as her advisor, and then did her postdoctoral training at University of California at Davis with Jim Richards as her mentor. Her current research focuses on the adaptive evolution of plant ecophysiological traits, primarily in Helianthus.

Presentation Abstract

Plant traits, stress resistance, and adaptive differentiation in Helianthus
Lisa Donovan
Department of Plant Biology; Evolution & Ecology Interdisciplinary Group

Helianthus is being developed as a model system for plant ecological and evolutionary genomic investigations of plant stress. I will present an overview of the Helianthus study system and explore two areas of recent research emphasis on the evolution of plant traits as adaptations to low resource habitats. The first area of research emphasis makes use of a hybrid species (H. anomalus) and its ancestral parents to investigate traits as putative adaptations to resource limited sand dune habitats. We have identified genetic variation for resource related traits, potential selectives forces in desert sand dune habitats, phenotypic selection on resource related traits in those habitats, and candidate traits and genes for low nutrient adaptation. The second area of research emphasis makes use of the broader context of the Worldwide Leaf Economic Spectrum (LES) to investigate the ecological and evolutionary lability of leaf traits that are putative adaptations to low resource habitats. Evolutionary lability and adaptive differentiation of LES traits in response to resource availability is supported by phylogenetically explicit species comparisons within the Helianthus genus, population Qst-Fst comparisons within the hybrid species H. anomalus, and genotype comparisons of domesticated H. annuus. However, there is substantial ecological lability in traits that argues for caution when combining evolutionary relationships and species trait data bases to understand adaptive strategies. Our understanding of the role of plant traits as adaptations to stressful habitats will benefit from the continued integration of ecological, evolutionary and genomic approaches.

Dr. Andrew Leakey

The Leakey research group at the University of Illinois studies the mechanisms of plant responses to global change, including rising CO₂ concentrations, temperatures and drought stress. This enhances understanding of how the environment impacts ecosystem goods and services including biodiversity, productivity, water cycling and food supply. It also will inform efforts to adapt to environmental change. To do this we integrate genetic, molecular, biochemical, physiological and ecological tools to assess plant metabolism and productivity in food and biofuel crops, as well as natural ecosystems.

Dr Leakey received his B.Sc. in Plant Sciences in 1998 and his Ph.D. in Tropical Tree Physiology and Ecology in 2003, both from the University of Sheffield, UK. He moved to the University of Illinois at Urbana-Champaign, USA as a Fulbright Scholar in 2002. Staying at Illinois he was a post-doctoral scientist in the Department of Plant Biology and then Research Fellow at the Institute for Genomic Biology, before joining the faculty as an Assistant Professor in 2007. He was promoted to Associate Professor in 2013.

Lab webpage: http://www.life.illinois.edu/leakey/

Presentation Abstract

Should the paradigm of reduced plant drought stress at elevated CO₂ be hung out to dry?
Andrew D.B. Leakey
University of Illinois at Urbana-Champaign

Rising atmospheric CO₂ concentrations and increasing frequency and severity of droughts will alter the environment for plant growth in the coming century and will challenge agricultural production. Models of future food supply currently extrapolate reduced stomatal conductance at elevated CO₂ into reduced plant water use, conservation of soil moisture, and amelioration of physiological stress under drought. Additional carbohydrate availability under elevated CO₂ is also expected to increase root biomass and access to water under low rainfall conditions. By these two mechanisms, elevated CO₂ is expected to compensate for the deleterious effects of drought on yield. Here, we present a 3-year dataset demonstrating that this prediction does not hold true for field-grown soybean as stress becomes more severe. We grew soybean (Glycine max) under ambient (~390 ppm) or elevated (~585 ppm) atmospheric CO₂ in
combination with control or reduced precipitation in the field using Free Air CO\textsubscript{2} Enrichment (FACE) technology at the soyFACE facility in Champaign, IL in 2009-2011. Contrary to expectations, we found that growth at elevated CO\textsubscript{2}: (1) did not reduce plant water use and conserve soil moisture during stronger droughts; (2) resulted in greater stomatal closure associated with ABA signals in response to soil drying; and (3) altered nodulation patterns, potentially impairing nitrogen fixation. In combination, these responses led to equal or greater yield loss to drought under elevated CO\textsubscript{2} compared to ambient CO\textsubscript{2}. These findings suggest current projections of future crop yield in the Midwest U.S. may be overoptimistic and identify potential targets for crop adaptation to environmental change this century.

**Dr. Georg Jander**

Georg Jander received his PhD in Microbiology from Harvard University in 1995. As an NIH-funded postdoctoral fellow with Fred Ausubel at Massachusetts General Hospital, he started studying plant-insect interactions, which has been the focus of his research ever since. After completing his postdoc, Jander worked for four years as a scientist at the Monsanto Company before assuming his current position at the Boyce Thompson Institute in 2002. He has an appointment as an adjunct associate professor in the Department of Plant Biology at Cornell University, where he contributes to both undergraduate and graduate student teaching. Current research in Jander’s lab is focused on studying plant-aphid interactions. In recent years, this has involved two model systems: *Myzus persicae* (green peach aphid) feeding from Arabidopsis and *Rhopalosiphum maidis* (corn leaf aphid) feeding from maize. Additionally, Jander organizes an NSF-funded Research Experience for Undergraduates site that provides hands-on plant molecular biology training to eighteen undergraduates and three high school students each summer at the Boyce Thompson Institute and Cornell University.

**Presentation Abstract**

A genetic and biochemical basis for natural variation in maize aphid resistance  
Georg Jander  
Boyce Thompson Institute for Plant Research  
Cornell University

Corn leaf aphids (*Rhopalosiphum maidis*) show considerable variation in their ability to feed on different maize (*Zea mays*) cultivars. Benzoxazinoids, a class of defensive metabolites found in many grasses, are frequently associated with insect resistance. Genetic mapping with diverse maize inbred lines showed that improved aphid growth is associated with benzoxazinoid methylation. Analysis of an inactivating transposon insertion in a maize benzoxazinoid methyltransferase gene confirmed that this specific enzyme causes aphid sensitivity. This is in marked contrast to previous experiments showing increased caterpillar resistance through methylation of benzoxazinoids by the same enzyme. Thus, there are ecological tradeoffs in benzoxazinoid production, with the methylated form of the benzoxazinoids providing caterpillar resistance and the non-methylated form providing aphid resistance. The identification of specific genes that influence this defensive tradeoff will facilitate future breeding efforts to produce maize with enhanced or more targeted resistance to insect herbivores.

**Dr. Thomas Juenger**

Tom Juenger is a Professor in the Department of Integrative Biology at the University of Texas at Austin. He received his undergraduate degree in Ecology, Evolution, and Ethology at the University of Illinois and his PhD from the University of Chicago. He completed postdoctoral research as a Miller Fellow at the University of California Berkeley. Tom’s research explores the interface of ecology and evolution in natural populations. He is generally interested in phenotypic evolution and the role of natural selection and genetic architecture in shaping evolutionary responses and adaptation. A current focus of his lab is the identification and characterization of genes underlying variation in stress responses in plants.

**Presentation Abstract**

Studying ecophysiology and adaptation in plants using genetic and genomic tools  
Thomas Juenger  
Section of Integrative Biology  
University of Texas at Austin

Water availability imposes strong and recurring selective pressure as it is fundamental to almost all aspects of plant physiology. Nearly all terrestrial plants are exposed to drought stress, and it is clear that the natural distribution and abundance of many plants is largely driven by precipitation regimes. Over several years, Juenger has studied *Arabidopsis thaliana* using whole-plant physiology, quantitative genetics, and genome-wide gene expression experiments to explore drought adaptation. An underlying theme has been the use of natural variation to explore plant function, to understand the mechanistic basis of genotype-by-environment interaction, and to discover the genes important in stress responses. The lab has been especially interested in genes involved in drought avoidance and tolerance characteristics and linking the functional effects of these allelic variants to their evolutionary history. Here, I report on our progress cloning physiological QTL in *Arabidopsis thaliana* and toward a better understanding of drought adaptation in plants.
CADMIUM DETERMINANT1 (CDM1) is a cadmium transporter controlled by a RNA metabolism regulator, CARBOXYL-TERMINAL DOMAIN PHOSPHATASE-LIKE 1 (CPL1) in plants

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Abstract: Toxic for most organisms, cadmium (Cd) is a widespread heavy metal contaminant in arable lands as a result of recent anthropogenic activities. It is readily absorbed by the plants and accumulates in edible parts, whereby it is introduced into the food chain; consequently, it causes severe health risks to humans. Thus, understanding mechanisms of Cd absorption, translocation, and tolerance is essential for sustaining production of crops that are safe for human consumption. In our previous study, a knock-out mutant of Arabidopsis thaliana CARBOXYL-TERMINAL DOMAIN (CTD) PHOSPHATASE-LIKE1 (CPL1), a plant-specific RNA polymerase II CTD phosphatase, showed higher tolerance to the Cd toxicity by enhancing the root-to-shoot translocation of Cd. Here we present that a Cd accumulation determinant in cpl1-2 is a putative metal transporter. CADMIUM DETERMINANT1 (CDM1). CDM1 was highly induced in cpl1-2 roots upon exposure to Cd. Transgenic Arabidopsis expressing GFP-CDM1 showed specific fluorescence in the plastids, indicating a role of plastids in Cd transport and accumulation. The root growth of cdm1 mutants showed higher tolerance to the Cd toxicity whereas overexpression of CDM1 caused Cd sensitivity. Interestingly, cdm1 mutants accumulated less Cd, Fe and Zn, indicating the involvement of CDM1 in the transport of these metals. Overall, these results suggest that CPL1 regulates the Cd distribution in plants by repressing the expression of CDM1. Further characterization of cpl1 and cdm1 mutants under Cd toxicity will extend our understanding of plant heavy-metal accumulation and lead to the development of new phytoremediation techniques.

Transcriptome-wide characterization of functions of Arabidopsis CPL4 in coding gene and snRNA transcription
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Abstract: Phosphoregulation on C-terminal domain of RNA polymerase II (pol II CTD) is a pivotal mechanism to control eukaryotic transcription, however, understanding of CTD phosphorylation in plants is still in its infancy. We have previously established that Arabidopsis CTD-phosphatase like 4 (CPL4) is an essential CTD phosphatase that interacts with pol II and dephosphorylates pol II CTD.

In order to understand the function of CPL4 in global transcription, we employed transcriptome approach. Microarray analysis on CPL4 knockdown (CPL4RNAi) line identified several co-expression gene networks involved in xenobiotic stress response, ABA response and flavonoid biosynthesis pathways are up-regulated by CPL4RNAi. In addition, the highest up-regulated gene is found to be AT1G61280, which has uncharacterized function and is located at downstream of U12 snRNA (AT1G61275).

To obtain comprehensive transcripteme landscape of CPL4RNAi plant, we performed RNA-seq analysis on ribosome RNA-depleted samples prepared from the CPL4RNAi line. Interestingly, this identified not only the up-regulation of AT1G61280, but also a considerable 3' read-through from the upstream U12 snRNA region. Reverse transcription PCR confirmed that some U12 snRNA transcripts extend into AT1G61280, making long read-through transcripts. In total, among 16 pol II-dependent snRNA transcripts detected, fourteen snRNAs showed 3' read-through, and some resulted in up-regulation of downstream coding gene expression.

Taken together, these transcriptome approaches imply pathway-specific and general functions of CPL4 in regulating transcription of both protein-coding genes and snRNAs.

The drought response of physiological and structural traits in loblolly pine (P. taeda L.) clones with a focus on mesophyll conductance to CO2
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Abstract: Climate change will likely affect the productivity of forests through changes in precipitation and moisture availability. An important measure of a plant’s ability to assimilate carbon in photosynthesis with limited water loss, water use efficiency (WUE), is assessed through the use of carbon stable isotopes by the Farquhar model. However, recent work has shown that mesophyll conductance to CO2 (gm) changes in response to environmental conditions, and the simplified model does not take into account this variability. Variation in this parameter could decrease the effectiveness of the stable isotope tool. This study, conducted in a greenhouse, examined the effects of drought on gm and other physical and biochemical traits in three clones of loblolly pine (P. taeda L.).

The three clones exhibited plasticity in stomatal conductivity and hydraulic conductivity in response to drought. There was some evidence for an interaction between clone and drought such that one clone exhibited higher photosynthetic capacity and gm in response to drought but there were not significant differences between all other treatments. We report on the relationship between the
Abstract: The *opaque2* (*o2*) mutation increases lysine 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. In this study, based on zymogram assay, western blotting and quantitative enzyme activity assay, K0326Y (a QPM inbred line) had higher pullulanase and SSIII activity than W64Ao2. The analysis of recombinant inbred lines (RILs) derived from a cross of K0326Y and W64Ao2 revealed that RILs with homozygous QPM-derived Zpu1 allele had lower onset and maximum endotherm temperatures, as well as lower average glucan chain length of starch granule, whereas RILs with homozygous QPM-derived SSIII allele had higher onset and maximum endotherm temperatures, as well as higher average glucan chain length. Also, the enzyme activity assay of RILs and correlation test showed that the kernel vitreousness was positively correlated with pullulanase activity, but negatively correlated with SSIII activity. Therefore, pullulanase and SSIII could be two of the important factors that influence glucan chain length distributions, altering the fine structure of starch granule, which in turn affects the formation of vitreous endosperm in QPM.

### FUNGUS DEFENSE-RELATED KINASE1 represses *Arabidopsis* resistance to green peach aphids

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**Abstract:** FUNGUS DEFENSE-RELATED KINASE1 (FDRK1) plays important roles in induced defense against fungal and bacterial pathogens in *Arabidopsis*. However, it remains unknown whether FDRK1 functions in plant defense against aphids, a group of insects with a specialized phloem-feeding style. In this study, the potential role of FDRK1 was investigated in *Arabidopsis* infested with the green peach aphid, *Myzus persicae*. In contrast to the previously reported positive role of intact FDRK1 in defense response, loss of FDRK1 function adversely impacted aphid settling, feeding and reproduction. Relative to wild-type plants, *fdrk1* displayed higher aphid-induced H₂O₂ accumulation and more severe lesions, resembling a hypersensitive response (HR) against pathogens. These symptoms were limited to the infested leaves. The *fdrk1* mutant showed elevated basal as well as induced salicylic acid and ethylene accumulation. Intriguingly, elevated salicylic acid levels did not contribute to the HR-like symptoms or to the heightened aphid resistance associated with the *fdrk1* mutant. Elevated ethylene levels in *fdrk1* accounted for an initial, short-term antixenotic activity. Introducing a loss of function mutation in the aphid resistance and senescence-promoting gene *PHYTOALEXIN DEFICIENT4 (PAD4)* into the *fdrk1* background blocked both aphid resistance and HR-like symptoms, indicating *fdrk1*-mediated resistance to aphids is PAD4-dependent. Taken together, *Arabidopsis* FDRK1 confers susceptibility to aphid infestation through its suppression of PAD4 function. Furthermore, the results underscore the role of reactive oxygen species and cell death in plant defense against phloem-feeding insects.

### Maize GCN2 phosphorylates eukaryotic translation initiation factor 2α under amino acid starvation and regulates the endosperm specific transcription factor Opaque2

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**Abstract:** General control non-derepressible-2 (GCN2) plays an important role in cellular responses to amino acid availability as a regulatory protein kinase. It phosphorylates the α subunit of the trimeric eukaryotic translation initiation factor-2 (eIF2), which in turn decreases the general rate of protein synthesis in response to amino acid starvation. The phosphorylation of eIF2α enhances the translation of the transcription factor GCN4 by overcoming the inhibitory effect of the GCN4 upstream open reading frames (uORFs), resulting in increased expression of over 30 amino acid synthesis genes. Although the GCN2-like kinases are highly conserved among eukaryotes, there are no candidates of plant GCN4 homologues identified. *Mutator* tagged GCN2 null mutants were used to characterize the GCN2 homologue in maize (*Zea mays*). ZmGCN2 shared sequence
identity in the conserved domains with other GCN2 homologues. An increase of eIF2α phosphorylation in response to herbicide treatment that inhibited amino acid biosynthesis was only detected in wild type maize endosperms, not mutant, indicating that it was GCN2-dependent. Opaque2 (O2) was reported to have sequence and function similarity with GCN4, and its protein accumulation increased during induced endosperm amino acid starvation, but O2 transcript level was unchanged. This suggested that O2 was post-transcriptionally regulated through the GCN2 kinase pathway and that O2 could be a maize GCN4 homologue. Bioinformatics approaches are being taken to identify other possible GCN2 responsive transcripts with highly conserved uORF spacing patterns similar to O2.

Understanding Hydrocarbon Production in the Green Microalga *Botryococcus braunii* Using Genomics Tools
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**Abstract:** Due to concerns over global climate change, increasing petroleum prices, and diminishing supplies of petroleum reserves, there has been interest in recent years in identifying a photosynthetic organism capable of producing large amounts of oils which can be used as a feedstock for production of combustion engine fuels, thereby creating a source of clean renewable energy. The green colonial microalga *Botryococcus braunii* is well known as a prodigious producer of liquid hydrocarbons that can be found as major constituents of currently used petroleum reserves. Thus, *B. braunii* hydrocarbons can be easily converted into petroleum-equivalent fuels such as gasoline, kerosine, and diesel and is an exciting potential source of renewable hydrocarbons. *B. braunii* is subclassified into 3 chemical races based on the nature of the hydrocarbon produced; the A race produce fatty acid derived alkanienes/alkatrienes; the B race produces the triterpene botryococcenes; and the L race produces the tetraterpene lycopadiene. Our laboratory is interested in deciphering the biochemical pathways for hydrocarbon biosynthesis in each race to support the future use of *B. braunii* as a hydrocarbon source. To aid in these efforts we have developed genomics-based tools for each race such as high quality transcriptomes for each race and a genome sequence for the B race. These tools have been used to successfully identify and elucidate the genes encoding enzymes for hydrocarbon biosynthesis in each race. These results will be presented and discussed to show how genomics approaches are valuable for deciphering biochemical pathways.

Raman Spectroscopy Studies of Race B Botryococcenes
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**Abstract:** *Botryococcus braunii* is a green colonial microalga which produces up to 86% hydrocarbons that can easily be converted to combustion engine fuels. There are three races of *B. braunii* that are classified based on the hydrocarbons they produce. Race A produces alkanienes and alkatrienes from fatty acids; race B produces triterpenes known as botryococcenes, and race L produces a tetraterpene known as lycopadiene. Among these three races, race B has been considered as the best potential source of renewable energy due to the high accumulation of botryococcenes which can easily be transformed to high quality engine fuels via basic physical processes such as hydrocracking and distillation. Thus, the B race botryococcene hydrocarbons are the focus of this study. It has been shown that there are several identified isomers of botryococcenes based on C31, C32, and C34 structures. Laser Raman spectroscopy is a powerful technique that helps to analyze the differences in the closely related isomers not only in vitro but also in vivo. This valuable feature makes Raman spectroscopy an important tool for the analysis of botryococcenes in a live *B. braunii* cells in order to understand their biosynthesis and physiology. In vitro Raman analysis and DFT calculations have shown that C30, C31, C32 isomers have several significant spectral differences in the region from 200-1700 cm⁻¹ and these will be discussed. These specific signatures will then be used to map individual botryococcene molecules in vivo using confocal Raman microspectroscopy.

Posters (Alphabetical Order)

A novel Arabidopsis mutant *sup1* plays role in iron homeostasis
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**Abstract:** Plants respond to low Fe environments by regulating genes responsible for Fe utilization. We previously established that CPL1 (CTD Phosphatase like protein-1) is a key regulator of Fe homeostasis and *cpl1* mutation upregulates *FIT1* promoter. To identify Fe utilization related genes we used a firefly reporter gene system. The reporter gene contained bioluminescent luciferase (*LUC*) gene fused to *FIT1* promoter. In Arabidopsis this *FIT-LUC* system functions in Fe response. To conduct genetic screen we used ethyl methanesulfonate (EMS) mutagenised *cpl1* seeds. We have identified a suppressor mutant *sup1* by *FIT-LUC* luminescence screening. *sup1* shows decreased *FIT-LUC* luminescence with severe chlorosis and dies within 3 weeks on Fe less media. When grown on soil chlorotic phenotype was also observed. With the help of simple sequence length polymorphism (SSLP) marker based mapping we mapped this suppressor gene on chromosome 2. Cross of *sup1* mutant with previously known iron responsive mutant *fit1* recovered the phenotype of *sup1*. Cross results eliminate the possibility that *sup1* is a *fit1* mutant which is also found on chromosome 2. Confirmation of the causal mutation gene will be achieved by testing T-DNA mutants or by complementation tests. Identification of these genes will help us to understand how plants assimilate Fe from soil which ultimately will help in agriculture and human health.
The MORC1/CRT1 family play a role in modulating transposable elements via altering genome accessibility

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ABSTRACT: A genetic screen for components involved in resistance (R) protein-mediated immunity in Arabidopsis led to isolation of ctrl (compromised recognition of TCV). CRT1/MORC1 was shown to be a MORC ATPase/endonuclease that physically interacts with multiple immune components. While CRT1 is mainly located in endosome-like vesicles in the cytoplasm, a subpopulation resides in the nucleus, which increases after infection. The combined findings that MORC1 i) is an endonuclease, ii) physically interacts with several components of DNA repair and recombination (R/R) pathway, iii) is localized to heterochromatin, and iv) is implicated in epigenetic regulation, including suppression of heterochromatic transposable elements (TEs), suggest that MORC1 has an important nuclear function(s). Thus, we are investigating MORC1’s role in the nucleus, particularly its involvement in stress-triggered genome stability, to assess the importance of this function in plant immunity and evolution.

To assess the function of MORC1 on genome under biotic stress, DNase I-seq was performed on pathogen-inoculated wild type and mutant plants (morc1/2) lacking MORC1 and its closest homolog MORC2. This genomic approach reveals that TE-associated areas are better accessible in morc1/2, as compared to wild type. Interestingly, this differential access to TEs were also enhanced in WT by infection with Pseudomonas syringae. Moreover, MORC1 genetically interacted with epigenetic components in RNA dependent DNA methylation. Taken together, these results suggest that biotic stress leads to altered chromatin accessibility to TEs and that the MORC1 family likely plays an important modulator in this process. Evolutionary implication of these findings will also be discussed.

Physiological controls of endophyte-mediated drought tolerance in Panicum virgatum

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Abstract: Plant responses to drought are key to ecosystem productivity. Most climate models predict an increase in the frequency and severity of drought in upcoming decades, particularly in agriculturally significant areas like the central United States. Fungal endophytes living within plant leaf tissues are thought to directly affect plant drought responses by modulating plant physiology, growth, and water loss. However, the physiological mechanisms by which endophytes affect plant drought responses are not well-characterized. Additionally, the frequency of endophytes that confer drought tolerance remains unknown. To address these questions, we isolated, identified, and tested the function of endophytic fungi in Panicum grasses across central Texas. Thirty-six endophyte species were paired with sterile Panicum virgatum seedlings under dry and well-watered conditions to determine their affect on plant drought responses. A majority of fungal endophyte significantly decreased plant water loss (~70% of taxa) and improved plant survival under drought (~65% of taxa), but there was substantial variability between individual taxa. Responses in plant transpiration efficiency under drought ranged from approximately -70% to 500% relative to a sterile control. To understand how these isolates induced such varied plant responses, we began characterizing fungal physiological traits. Screens of growth rate, osmotic stress tolerance, and resource use were all initially characterized in culture and will later be screened within the plant. In culture, taxa varied widely in growth rate (~275% difference) and stress tolerance (~125% difference). Further fungal trait screens will be performed and the gathered data will be used to predict fungal function within the plant.

Plants as biofactories: mechanical wounding applied on leaves as an innovative way to increase the levels of nutraceuticals in fruits

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Abstract: Stresses like wounding and herbivores induce changes in plant metabolism. Wounded tissues affect the production of phenylpropanoid secondary metabolites as a local response, and also as a systemic response in the same organ type (e.g. leaves). The importance of phytochemicals for human health has led the study of pre and post-harvest factors that influence the production of bioactive phenylpropanoids. In this study, a preharvest leaf wounding was applied to measure the effects on production of bioactive compounds on fruits. The experiment was conducted on strawberry experimental plot subjected to two levels of mechanical wounding applied on leaves, 7-12 days before harvest time. The fruits were evaluated after harvest for quality parameters (color, soluble solids, firmness, and fresh weight), total phenolics (TP), vitamin C, and specific phenylpropanoids. No differences with the control were detected (p>0.05) for color, soluble solids, fresh weight, firmness and vitamin C. However, the level of TP in fruits of treated plants increased significantly >20% over the control. Moreover, significant increase (p≤0.05) in the level of specific phenylpropanoids was observed: epicatechin (+186%), quercetin (+194%) and rutin (+190%) and the ellagitannins derivatives, ellagic acid and gallic acid (+128% and +17%, respectively). These results support the idea that higher levels of phytochemicals reported in organic fruits and vegetables could be due to the wounding component of the biotic stress attributed to insects to which the plant are exposed. In addition, the controlled mechanical wounding applied during preharvest in leaves could be used to increase phytochemicals in fruits.

Characterization of Arabidopsis oligosaccharyl transferase subunits: in vivo interactions and topological features

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Abstract: The oligosaccharyl transferase (OT) catalyzes N-glycosylation of nascent polypeptides in the lumen of endoplasmic reticulum (ER). Despite its importance, the OT is poorly described in plants due to the difficulties of membrane protein characterization. Here, we purified the proteins associated with the STT3A, a catalytic subunit of the Arabidopsis OT complex, by tandem-affinity-purification and identified them as three essential OT subunits (OST1, HAP6 and DGL1) and STT3B, a homolog of STT3A, through mass spectrometry analysis. In order to address the in vivo interactions and the topological features of the OT components, we conducted the luciferase complementation image (LuCI) analysis. The pairwise analysis of in vivo interactions of individual OT subunits with STT3A demonstrated that all the OT subunits which we tested here (OST1, OST2, OST4, HAP6, DGL1 and STT3B) display specific interactions with STT3A. Especially, the positive interaction between STT3A and STT3B suggests a dimerization of OT complexes in Arabidopsis. The LuCI analysis also revealed important topological features of OT subunits; the N terminus of STT3 locates in the cytosol, C termini of OST1, OST4, HAP6 and DGL1 locate in the cytosol and both N and C termini of OST2 locate in the cytosol. These results may supply critical clues to elucidate molecular and cellular functions of OT in plants.

Characterization of the cell wall related transcriptome in high biomass energy sorghum

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Abstract: The need to generate a large and sustainable supply of biomass to make biofuels generation from lignocellulose profitable will require the development of crops grown specifically for bioenergy production. The low-input requirements, drought tolerance, and high biomass yield potential of sorghum (Sorghum bicolor L. Moench) make this species suited as a bioenergy crop. Since plant cell walls comprise a significant proportion of the lignocellulosic biomass, RNA-Seq analysis was used to characterize cell wall biosynthesis gene regulatory networks in the high biomass energy sorghum, R07020, and the grain sorghum genotype, BTx623. Three internodes along the stem were collected in triplicate at 60 days after planting during the linear stem elongation phase. RNA was extracted from each internode pool and sequenced on Illumina HiSeq2000 using a 70bp paired-end strategy on two biological replicates. Differential gene and transcript expression analysis between non-elongating (basal), elongating (middle) and upper stem internodes was conducted using the CLC Workbench and edgeR. A total of 430.6 million reads from R07020 and 445.6 million from BTx623 were generated across all three stem samples from two biological replicates. From the total reads, 410.3 and 429.7 million from R07020 and BTx623, respectively, were mapped to the sorghum reference genome. Transcriptional activity for more than 27,600 sorghum genes was detected. Comparative analysis between the different internode sections and the high biomass and grain sorghums revealed differential expression of several cell wall related genes. This improved understanding of grass cell wall biogenesis will facilitate the manipulation of traits favorable for sustainable biofuel production.

DiOC6 may be a marker for an endoplasmic reticulum-mediated internalization pathway in some cell types in Arabidopsis.

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Abstract: A study of the labeling of the endoplasmic reticulum (ER) with fluorescent analogues reveals interesting dynamics that may illuminate a previously-suspected, but unproven ER-labeling pathway. The suspected pathway involves the close association of the ER with the plasma membrane, placing it in a position to deliver or receive lipid through the process of unconventional secretion or internalization, independent of endosomes or Golgi. Three aspects of the dynamics of labeling of the ER with the vital dye, DiOC6, are consistent with the unconventional internalization pathway. 1) Uptake is energy-dependent, but does not follow the same pathway as FM 4-64, a marker for endocytosis. 2) The labeling of the ER with DiOC6 is primarily cortical, not endoplasmic in nature, and the label in that compartment exhibits different diffusional dynamics than other labels (e.g. rhodamine hexyl ester). 3) The labeling of the cortical ER is diminished in mutant lines lacking the protein RHD3 (ROOT HAIR DEFORMED 3). In the latter case, other work has indicated that RHD3 may be involved in part of a complex that associates the ER with the plasma membrane. It shows, as does DiOC6, limited diffusion in the cortical compartment of the ER, which may be interpreted as due to steric interactions caused by the association between the two membranes. The energy-dependence of the uptake is consistent with, but does not prove, an involvement of a plasma membrane flippase that delivers the label across the membrane into the ER. Our model is that the DiOC6, if taken up via a flippase, then travels via the ER to other organelles with which the ER associates, such as the mitochondria and outer envelope of the chloroplast.

Development of the wild tomato Solanum pennellii as a novel and sustainable biofuel feedstock
**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 C4 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 have fused promoters for the first two genes in the pathway to GFP and transformed these reporter constructs into tobacco (another member of the Solanaceae) to determine whether expression of the genes is restricted to trichomes. We are using comparative transcriptomics between high glucolipid-producing accessions and low glucolipid-producing accessions to identify the remaining two or three genes in this 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.

**Identification of quantitative trait loci associated with anthracnose resistance in sorghum [Sorghum bicolor (L.) Moench]**

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*Arabidopsis thaliana* CRT1 (*Compromised for Recognition of TCV*) is a MORC (*Microrchidia*) ATPase protein that is required for a multiple level of plant immunity including effector-triggered immunity (ETI), PAMP (pathogen-associated molecular pattern)-triggered immunity (PTI), basal resistance, non-host resistance, and systemic acquired resistance. Consistent with its role in ETI and PTI, MORC1/CRT1 interacted with 11 R proteins and the PAMP-recognition receptor FLS2. Furthermore, MORC1 and its homolog MORC6 are recently found to be involved in epigenetic regulation of heterochromatin.

In this study, we employed yeast two-hybrid to assess a protein interaction profile of MORC1. MORC1 interacts with some of the MORC1 family members through its C-terminal domain. In particular, MORC1 and MORC6 display the strongest interaction, suggesting that the MORC1-MORC6 heterodimer is one of predominant proteins functioning in Arabidopsis. The MORC1 homologs tested, despite their close sequence homology to MORC1, show poor interaction with fourteen MORC1-interacting proteins identified through yeast two-hybrid screening. Together, these results show for the first time that the MORC1 family is likely subject to more complicated mode of action through dimerization/multimerization and that the C-terminal domain of MORC1 is responsible for these interactions.

**Identification of quantitative trait loci associated with anthracnose resistance in sorghum [Sorghum bicolor (L.) Moench]**

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**Abstract:** Anthracnose in sorghum, caused by the fungal pathogen *Colletotrichum sublineolum* Hemm. is a major biotic constraint to forage and grain production in warm and humid environments. Of the many control strategies for anthracnose, employment of host plant resistance has been regarded to be the most effective. Multiple sources of genetic resistance to different races of the pathogen exist among various sorghum genotypes. To identify the genetic determinants of anthracnose resistance, two mapping populations, each consisting of 100 recombinant inbred lines (RIL) were derived by crossing BTx623, an anthracnose susceptible line with two different anthracnose resistant lines, SC155 and SC414. RILs and parents were evaluated in replicated field trials in three environments. Linkage maps for the two populations were constructed using Digital Genotyping, a genotyping-by-sequencing method. For the BTx623 × SC155 RIL population, a major quantitative trait locus (QTL) was identified on chromosome 10 in two of the three environments examined, which explained 20% of the phenotypic variation. In the BTx623 × SC414 RIL population, a major QTL explaining 40% of the phenotypic variation was identified on chromosome 5 in two of the three environments. The information provided by these QTLs will be of significance in marker-assisted pyramiding of multiple sources of anthracnose resistance into elite sorghum germplasm.

**PHYTOCHROME INTERACTING FACTOR1 enhances the E3 ligase activity of CONSTITUTIVE PHOTOMORPHENIC1 to synergistically repress photomorphogenesis in the dark**

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ABSTRACT: CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1) is a RING/WD40 repeat containing ubiquitin E3 ligase that is conserved from plants to humans. COP1 forms complexes with SUPPRESSOR OF PHYA (SPA) proteins, and these complexes degrade positively acting transcription factors (e.g., HY5, HFR1 and LAF1) in the dark to repress photomorphogenesis. Phytochrome interacting bHLH transcription factors (PIFs) also repress photomorphogenesis in the dark. In response to light, the phytochrome (phy) family of sensory photoreceptors simultaneously inactivate COP1-SPA complexes and induce rapid degradation of PIFs to promote photomorphogenesis. However, the functional relationship between PIFs and COP1-SPA complexes is still unknown. Here, we show genetic evidence that the pif and cop1/spa mutants synergistically promote photomorphogenesis in the dark. HY5 is stabilized in the cop1pif1 and spa123pif1 mutants. PIF1 interacts with COP1, HY5 and SPA1, and enhances the substrate recruitment, auto- and trans-ubiquitylation activity of COP1. These data uncover a novel function of PIFs as potential cofactors of COP1 that synergistically repress photomorphogenesis in the dark. The proposed regulatory mechanism not only expands the diversity of COP1 substrates but also the strength of their regulation by COP1 to fine tune photomorphogenesis.