Abstract book

North East American Society Plant Biologist

86th Annual meeting 2023

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Arranged by

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ASPB Ambassador, Vice Chair of the Alliances

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Fluoride Transport in Whole Plants is Impaired in FLUORIDE EXPORTER Mutants.

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OsSULTR3;6 Encodes a Functional Membrane Transporter that Contributes to Mineral Nutrient Homeostasis in *Oryza sativa*

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The Inhibitory Impacts of Curcumin, Ursolic acid, and Crocetin on Breast Cancer Cells

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12:45pm **Leila Feiz**
Genetic and molecular analysis of six maize COI receptors reveals their roles in growth and development

1:00pm **Janeen Braynen**
Regulatory Networks Governing Nitrogen Use Efficiency in Maize and Sorghum

Participant List

Name	Organization
Sunil Kenchanmane Raju	New York University
Rachel Spicer	Connecticut College
Nathanya Thelusma	
Sam Pelletier	Connecticut College
Aditi Bhat	Brookhaven National Laboratory, Upton, NY
Reena Sharma	Brookhaven National Laboratory
Alysha Auslender	Yale
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Stephie Thomas	Adelphi University
Ira Herniter	Rutgers University
Alexander Heyl	Adelphi University
Kyana Gordon	Adelphi University
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Alexandre Miaule	Cornell University
Michael Budziszek	Johnson & Wales University
Makayla Richard	Merrimack College
Leonardo Venturotti	Merrimack College
Azam Noori	Merrimack College
Michael Zulch	Boston University
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Patrick Fardella	Rutgers University
Maja Klosinska	Millersville University
Avilash Singh Yadav	Weill Institute of Cell and Molecular Biology, Cornell University
Leeann Thornton	The College of New Jersey
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MaryAngela Senter	The College of New Jersey
Ellie Kreider	The College of New Jersey
Josie Maguire	The College of New Jersey
Luke Rogers	The College of New Jersey
Shreya Ranadive	The College of New Jersey
Lori Tausta	Yale
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Greg Gregory	

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Anjali Mohan	
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Scarlet Au	Cold Spring Harbor Laboratory
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Nathan Scinto-Madonich	Cornell University
Kerry Lutz	Farmingdale State College
Rahim Khan	
Christos Dimos	Johnson & Wales University
Benoit Lacroix	Stony Brook University

Keynote speakers:

Title: Germline RNA interference and the drive for maize domestication

Benjamin Berube[1,2], Jon Cahn[1], Cristiane Alves[1], Jason Lynn[1], Jeff Ross-Ibarra[3], Jerry Kermicle[4] and **Rob Martienssen**[1]

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[4] Department of Genetics, University of Wisconsin, Madison WI

Meiotic drivers subvert Mendelian expectations by manipulating reproductive development to bias their own transmission. Chromosomal drivers typically function in asymmetric female meiosis, while genic drivers are normally postmeiotic and typically function in males. Cryptic drive is thought to be pervasive and can be unleashed following hybridization with a naïve genome, resulting in sterility and hybrid incompatibility. Here, we describe an instance of gene drive in hybrids between maize (*Zea mays ssp. mays*) and teosinte *mexicana* (*Zea mays ssp. mexicana*). *Teosinte Pollen Drive* depends on RNA interference (RNAi) triggered by non-coding hairpin RNA in the male germline, that targets an essential gene in pollen. Introgression of *mexicana* into early cultivated maize is thought to have been important in maize hybridization and its geographical dispersal throughout the Americas. A survey of maize landraces and sympatric populations of teosinte *mexicana* reveals allelic bias around genes required for RNAi on at least 4 chromosomes that are also subject to gene drive in pollen from synthetic hybrids. *Teosinte Pollen Drive* likely played a role in maize domestication, and may offer an explanation for the widespread abundance of hairpin-encoded and other endogenous small RNA in the germlines of plants and animals.

Title: The domestication and evolution of rice

Michael D. Purugganan

Center for Genomics and Systems Biology and New York University

Domesticated species are an interesting group of organisms that have co-evolved with *Homo sapiens*, and have begun important in human survival and fitness. Using both genomic and archaeological data, we trace the origin and evolution of Asian rice, *Oryza sativa*, the most important food crop on the planet. Using whole-genome sequences of traditional landraces, coupled with geographic, environmental, archaeobotanical, and paleoclimate data, we reconstruct extrinsic factors that impact genome diversity, and reconstruct the dispersal of the crop over the last 9,000 years.

Short Talks:

Title: Efficacy of the *Epichloë festucae* Antifungal Protein Efe-AfpA Against *Clarireddia jacksonii*

Patrick Fardella,[1], Faith C. Belanger[1], and Bruce B Clarke[1]

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Epichloë festucae is a Clavicipitaceous endophyte found in some turfgrasses. In strong creeping red fescue (*Festuca rubra* subsp. *rubra*), the fungal endophyte provides resistance to dollar spot disease caused by the ascomycete pathogen *Clarireedia jacksonii*. Some turfgrasses such as creeping bentgrass (*Agrostosis stolonifera*) do not have naturally occurring endophytes that provide such resistance. Understanding this endophyte-mediated resistance could lead to utilizing the underlying mechanism to protect other grasses from disease. Previously, we identified an *E. festucae* antifungal protein, Efe-AfpA, that was highly expressed in symbio and localized to the plant apoplast. Efe-AfpA was expressed in *Penicillium chrysogenum* and purified from the culture filtrate for downstream investigations of efficacy against plant pathogenic fungi. Efe-AfpA was highly inhibitory against *C. jacksonii* mycelium in plate assays, disrupting the plasma membrane. Applications of pure protein to pots of creeping bentgrass inoculated with dollar spot mycelium showed reduced disease severity. It is possible that purified antifungal proteins can be developed as fungicide alternatives or amendments to current fungicide programs. The resulting decreased use of chemical fungicides will decrease the selective pressure that can result in development of resistant strains of pathogens, the environmental, and financial cost these fungicide programs have.

Title: Involvement of aquaporin in resistance to aluminum and heavy-metal toxicity in plants

Yuqi Wang[1,2], Yan Kang[2,3], Wancong Yu[2,4], Sangbom M. Lyi[2], Hyong Woo Choi[5,6], Enzong Xiao[1], Li Li[2,7], Daniel F. Klessig[5,8], and **Jiping Liu[2,7]**

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Aquaporins (AQPs) constitute a large gene family in plants, encoding relatively small transmembrane proteins with six transmembrane domains. AQPs are involved in diverse biological functions, responsible for transporting water, glycerol, urea, CO2, O2, NH3, H2O2 (signal molecules for resistance to pathogens and abiotic stresses), and metalloids, including B, Si, As(III), and Se. As a result, AQPs are believed to transport polar but non-charged small molecules. However, some AQP members have been suggested to transport monovalent metal ions such as Na+ and K+, and whether AQPs can transport divalent and trivalent metal ions remains unknown. Our recent studies indicate that aquaporins could be involved in transporting trivalent Al3+ and divalent Zn2+ ions and play critical roles in resistance to the toxicity of these metal ions in plants. For example, under low-pH and aluminum (Al) stresses, NIP1;2 functions coordinately with ALMT1, an Al-activated malate transporter, to remove Al in the root cell wall, a significant target of Al toxicity in Arabidopsis. The coordination of the ALMT1-facilitated exclusion mechanism and the NIP1;2-mediated internal tolerance mechanism enhances overall Al resistance in a carbon-use-efficient means. Moreover, we found that, under excess Zn condition, TIP2;2 facilitates Zn immobilization in the root and limits its translocation to the leaf tissues that are more susceptible to Zn toxicity than the root. In addition, TIP2;2 facilitates Zn accumulation in the trichomes of the leaves and shoots. Our findings expand our understanding of plants' biological functions and substrate specificity of aquaporins.

Title: Overexpression of Camelina sativa gamma-glutamyl cyclotransferases 2;1 (CsGGCT2;1) in Camelina Improved its Tolerance to Arsenite.

Gurpal Singh[1], Helen Le[1] , Kenny Ablordeppey [1] , Stephanie Long [2], Rakesh Minocha[2], and Om Parkash Dhankher[1]

[1]Stockbridge School of Agriculture, University of Massachusetts, Amherst, MA, USA
[2]US Forest Service, Northern Research Station, Durham, NH, USA

Plants use various mechanisms to overcome abiotic stresses, and one such pathway revolves around glutathione (GSH) homeostasis. GSH, a tripeptide made of cysteine, glycine, and glutamate, is a crucial antioxidant in all living organisms. It protects the cell from the damaging effects of reactive oxygen species (ROS) produced due to exposure to abiotic stresses and xenobiotics like heavy metals and pesticides. A steady level of GSH is maintained in the cell via GSH homeostasis through the γ-glutamyl cycle. Previous studies using overexpression (OE) lines of gamma-glutamyl cyclotransferases (GGCTs) in *Arabidopsis thaliana* proved the role of AtGGCT2;1 gene in degrading GSH in plants and increasing tolerance to arsenic. AtGGCT2;1 OE lines showed increased biomass under arsenite (AsIII) exposure and had an efficient glutamate recycling, suggesting a role of GGCTs in nitrogen use efficiency (NUE). Currently, we are translating this research into *Camelina sativa* var. *Suneson*. We overexpressed *C. sativa* GGCT2;1 (CsGGCT2;1) in wild-type Camelina plants under a constitutive 35S promoter. Our results showed that the OE lines had strong tolerance to AsIII and had significantly higher fresh biomass (2.5-fold) on AsIII-containing media relative to wild-type plants. Under AsIII treatment, OE lines also had lesser lipid degradation measured as MDA level, higher chlorophyll content, and lower arsenic accumulation (~40-60% less) than wild-type plants. There was no significant difference in Glu, Cys, Gly, and GSH levels between Camelina wild-type and OE lines under control or AsIII conditions. Therefore, GGCT2;1 has the potential to be utilized for enhancing tolerance and reducing the accumulation of arsenic in crops for food safety and security.

Title: Stay FLAT or BUCKLE? The causes and consequences of mechanical buckling in Arabidopsis sepals.

Avilash Singh Yadav[1], Lilan Hong[2], Patrick Klees [1] , Xi He[2], Iselle Barrios[3], Michelle Heeney[1], Richard Smith[4], Arezki Boudaoud[5], Adrienne Roeder[1]

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[2]Zhejiang University, Hangzhou, China
[3]Massachusetts Institute of Technology, MA, USA,
[4]John Innes Centre, Norwich, UK
[5]Ecole Polytechnique, Paris, France

Organ size and shape robustness is a remarkable property that is not well understood. To address how reproducibility in organ shape is achieved, we screened for variable organ size and shape (vos) mutants using Arabidopsis sepals as the model organ of interest. The vos3 mutant exhibits random ectopic lumps and outgrowths on the sepal outer epidermis (abaxial). Mutation underlying vos3 maps to a point mutation in the upstream region of ASYMMETRIC LEAVES 2 (AS2), a key adaxial specific transcription factor involved in adaxial identity specification, resulting in the ectopic expression of AS2 on the sepal abaxial surface. We show that in the initial stages, ectopic expression of AS2 on abaxial surface of vos3 disrupts cell growth patterning, causing mechanical stress mediated buckling. Our mathematical model predicts that overgrowth and reduced stiffness of the abaxial vs adaxial surface can in theory, cause buckling. Outer surface cells of vos3 grow sideways compared to wild type, leading to the initial buckle along the proximal-distal direction. Using osmotic treatment assays, we found that outer epidermis of vos3 is also significantly softer compared to the inner surface, whereas both epidermal layers exhibit similar stiffness in wild type. Thus, both conditions for buckling are met in vos3 and not in wild type sepals. Also, overexpression of KRP1, a cyclin dependent kinase inhibitor, in vos3 both limits the sideways growth and severely reduces buckling, supporting the model. We also find PIN forms ectopic convergence sites in vos3 located to the buckles, where outgrowths start to form. To test whether auxin is necessary, we live imaged vos3 in the presence of NPA, a polar auxin transport inhibitor. While NPA treated vos3 samples show no difference in buckling, the outgrowth formation is largely reduced. Thus, buckling is sufficient to create new PIN convergence sites which initiated outgrowths, suggesting that buckling can be a mechanism driving plant development.

Title: Fluoride Transport in Whole Plants is Impaired in FLUORIDE EXPORTER Mutants

Tausta, S. Lori[1], Kathryn Fontaine[2], Ansel Hillmer[2], Alysha Auslender[3], and Scott Strobel[3],

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Fluoride is an environmental toxin prevalent in water, soil, and air. Organisms must have mechanisms to tolerate the harmful effects of this abundant ion. A fluoride transporter called Fluoride EXporter (FEX) has been discovered across all domains of life, including bacteria, single cell eukaryotes and all plants, that is required for fluoride tolerance. How FEX functions to protect multicellular plants is unknown. In order to distinguish between different models, the dynamic movement of fluoride in WT and FEX mutant plants was monitored using [18F]fluoride with positron emission tomography. Significant differences were observed in the washout behavior following initial fluoride uptake between plants with and without a functioning FEX. [18F]Fluoride traveled quickly up the floral stem and into terminal tissues in WT plants. In contrast, the fluoride did not move out of the lower regions of the stem in mutant plants resulting in clearance rates near zero. This pattern occurred whether fluoride was applied to the roots or the cut stem. The kinetics of fluoride movement in the WT and FEX mutant plants suggests that FEX mediates a fluoride transport mechanism throughout the plant where each individual cell benefits from FEX expression.

Title: OsSULTR3;6 Encodes a Functional Membrane Transporter that Contributes to Mineral Nutrient Homeostasis in Oryza sativa

Nathan J. Scinto-Madonich[1], Miguel A. Piñeros[2], and Adam J. Bogdanove[3]

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[3]Plant Pathology and Plant-Microbe Interactions Section, School of Integrative Plant Science, Cornell University, Ithaca, New York 14853

The Sulfate Transporter (SULTR) gene family encodes for integral membrane proteins involved in the coupled transport of anions across plant cell and organellar membranes and can be split into 4 main sub-groups. The published literature has focused on the role of SULTRs in sulfate uptake and translocation within plants; however, select members of the SULTR3 subgroup have since been functionally characterized as phosphate transporters. This talk will focus on a structure-function investigation of the Oryza sativa Sulfate Transporter 3;6 (OsSULTR3;6), which was initially identified to underlie rice susceptibility to bacterial leaf streak. Expression of OsSULTR3;6 in the heterologous Xenopus laevis oocyte system shows robust transport activity that is driven by external cation concentrations, consistent with other functionally characterized SULTR homologs. Structurally, OsSULTR3;6 homo-oligomerizes and is dependent on the maintenance of the previously uncharacterized cytosolic N-terminus to facilitate transport. CRISPR-edited rice lacking OsSULTR3;6 shows altered phosphorus nutrition and viability of the rice grain, implicating another SULTR3 homolog in phosphate homeostasis. The growing list of distinct substrates transported by the SULTR gene family necessitates the investigation of the molecular features underpinning these functional characteristics, which will be discussed as future steps.

Title: Local Adaptation to Mercury and Cadmium in Nitrogen Fixing Rhizobia is Driven by Copy Number and Enhanced Gene Expression

Aditi Bhat[1], Reena Sharma[1], Michael Clear[1], Mercedes M. Lucas[2], Brendan Epstein, Tanja Woyke[4], Tanja Tiffin[5], and Timothy Paape[1,6],

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[5]University of Minnesota, St. Paul, MN, USA Pueyo, José J., Department of Soil, Plant and Environmental Quality, Institute of Agricultural Sciences, ICA-CSIC, Madrid, Spain
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Legumes have evolved a tight symbiosis with nitrogen fixing bacteria that function to improve resilience through increases in nitrogen for the host plant. Moreover, symbiotic rhizobia may also provide a source of detoxification of heavy metals in the nodules, thereby protecting the plant while still fixing nitrogen. We assembled the genomes of a genetically diverse collection of Ensifer medicae with high variation in mercury (Hg) and cadmium (Cd) tolerance to identify structural and transcriptomic differences between low and high tolerant strains. Furthermore, we experimentally demonstrated the first described a-proteobacteria strains that have a functioning mercury reductase (Mer) operon, which gives them extremely high tolerance to Hg. We found that the most tolerant E. medicae strains showed the fewest differentially expressed genes in response to Hg, which were almost the mer operon genes. In Medicago truncatula host plants, dual-transcriptomics of nodule tissues also showed the Hg-tolerant rhizobia had significantly lower number of DEGs than the non-tolerant rhizobia. Transfer of the mer operon to non-tolerant strains resulted in high Hg tolerance, indicating that the operon is solely necessary to confer Hg tolerance. For Cd responses, even tolerant strains showed high levels of DEGs including the well-known candidates such as cadA, glutathione-S-transferases, and ATP-binding cassette (ABC) transporters. Our findings indicate that the mer operon is far more efficient at evacuating Hg from the cell than the suite of transporters available for detoxifying Cd nitrogen-fixing rhizobia, and that the Hg operon can be horizontally transferred in natural and experimental conditions.

Title: Transcriptomic Responses to Toxic Metal Stress in Medicago Reveals More Resilient Genotypes Use Adaptive Detoxification Mechanism

Reena Sharma[1], Aditi Bhat[1] , Michael Clear, Mercedes M. Lucas[2] , K. Barry[3], A. Lipzen[3], M Blow[3], J. Schmutz[3], S.Curtin[3], J. Pueyo José[4], and Timothy Paape[5]

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Legumes have well-known symbiotic interactions with nitrogen fixing rhizobia, which requires host plants to maintain ion homeostasis in leaves and roots. Plants can sequester toxic ions such as cadmium (Cd) and mercury (Hg) to limit transport using ATPases and ATP-binding cassette proteins (ABCs), which isolate unchelated or chelated ions in vacuoles. Chelated ions that are transported by ABC transporters are also tightly linked with glutathione related genes (GSH) that respond to metal stress. To identify tissue-specific metal detoxification mechanisms, we evaluated variation to Cd and Hg tolerance in the Medicago HapMap germplasm collection and conducted transcriptomics on leaves, roots, and nodules (which contain both plant and bacteria cells). Enrichment of GO-terms such as GSH, xenobiotic transporters, vacuolar transport, and ABC transporters in the differentially expressed genes (DEGs) suggests that these processes are involved in the detoxification of Cd and Hg in Medicago. More tolerant genotypes had fewer DEGs, suggesting more efficient detoxification and reduced stress responses. The role of ABCs in metal ion transport has been shown in many species, but their significance in nodule formation in legumes is uncertain. We identified known orthologs of the metal-related Arabidopsis ABCC transporters exhibiting significant DEGs in response to Cd and Hg. A mutation in MtABCC1 resulted in reduced nodule number, and X-ray fluorescence microscopy revealed altered micronutrient distributions, suggesting the significance of ABCCs for homeostasis during nodulation. Overall, this study aims to provide a comprehensive understanding of the molecular and cellular mechanisms of plant-microbe mediated ion transport by combining genomics, functional genetics, and imaging. The findings may have important implications for developing strategies to mitigate the environmental impact of heavy metal pollution and to improve crop productivity in contaminated soils.

Title: The Inhibitory Impacts of Curcumin, Ursolic acid, and Crocetin on Breast Cancer Cells

Richard, Makayla, Foley[1], Emmalee[1], Venturotti, Leonardo[1], Modica-Napolitano, Josephine[1], Noori*, Azam[1],

[1]Merrimack College, North Andover, MA

Breast cancer is one of the most common types of cancer and among the four most prevalent causes of cancer death worldwide. Breast cancer treatments are expensive and impact healthy cells as well as cancerous cells. These treatments are not free of side effects, are costly, and families tend to suffer financially and emotionally during this process. Natural medicine is inexpensive and impacts cancer cells with its antioxidant properties to protect cells against free radicals. Free radicals are molecules produced when the body breaks down food or when exposed to tobacco smoke or radiation. This study focused on the antioxidant and anticancer responses of two breast cancer cell lines exposed to key secondary metabolites extracted from turmeric, rosemary, and saffron. The 231 and MCF-7 cells were exposed to 0.05 mM of curcumin (CU), ursolic acid (UA), and crocetin (CR) for 24 hours. The viability and cytotoxicity were studied using trypan blue and clonogenic assays in both cell lines. The impact of drugs on cellular proliferation was determined using MTT assay. This study showed that the cell viability of both cell lines significantly decreased over time from 1 to 24 hrs exposure. The cell death of 231 cells exposed to CU, UA, & CR for 24 hrs was 87±8%, 59±12%, and 36±8%, respectively. The cell death of MCF-7 exposed to curcumin, ursolic acid, and crocetin for 24 hrs was 96%, 82%, and 38%, respectively. The immunofluorescent imaging data showed that the size of the 231 cells was on average 21%, 27%, and 55% of the control cells upon exposure to UA, CU, & CR, respectively. This indicates the impacts of the metabolites in this study on reducing the size of cancerous cells. This study provides invaluable insight into the effectiveness of NM as a preventive method in cancer development. Keywords: Breast cancer, anticancer, secondary metabolites, cytotoxicity, cell viability

Title: Real-Time, Whole Plant Imaging of CELLULOSE SYNTHASE A8 Expression Throughout the Life of Brachypodium distachyon

Greg A. Gregory[1,2], Joshua H. Coomey[1,2], Kira A. Gardner[1], David Follette[3], Ian W. McCahill1[2], Samuel P. Hazen[1,2]

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Cellulose is the most abundant polymer in plant cells and is the most abundant biopolymer on earth. Cellulose is synthesized at the plasma membrane by multi-protein complexes that include CELLULOSE SYNTHASE A (CESA) proteins. In the cereal model Brachypodium distachyon as well as Arabidopsis thaliana, three similarly named enzymes function in the complex: CESA4/7/8. Expression of these CESA genes is highly correlated with other secondary-wall-associated genes and is extremely high in developing stems. Our understanding of CESA expression dynamics is limited to quantification of mRNA from destructively harvested tissues, which captures a single developmental stage and often lacks fine temporal and spatial resolution. To overcome this limitation, we developed a real-time Luciferase reporter imaging system to quantify a CESA8 promoter LUCIFERASE fusion throughout the lifecycle of a B. distachyon plant using a highly sensitive camera. Additionally, we developed a 3D printed rhizotron that enables imaging of roots. We observed CESA8 expression to be greatest in elongating internodes just above the node, and that it decreased as elongation slowed. This platform enables real-time measurement of gene expression with high spatio-temporal resolution in both above and below ground tissues throughout development.

Title: How Many Microbes Can a Plant Support?

Michael Zulch[1] and Joseph Larkin[1]

[1]Boston University, 610 Commonwealth Avenue

Photosynthetic plants are the primary conduits by which energy enters the biosphere and supports most non-photosynthesizing organisms from bacteria to mammals. The plant-bacteria relationship is defined by a producer-consumer model wherein the plant is the primary producer of energy through photosynthesis and bacteria are primary consumers of that energy. Energy flows unidirectionally starting with the conversion of light to chemical energy in the chloroplasts, the energy is then transferred to the rest of the plant where a majority of it is consumed in the synthesis of biomass. Eventually it's released via the roots into the surrounding environment, finally becoming available to bacteria. Understanding the quantitative relationship between a plant and its rhizobial bacteria is difficult as the size difference between the two organisms span several orders of magnitude. To overcome this, we take a unique approach to measure the quantitative relationship between the energy producer, including the plant and its chloroplasts, and the bacterial consumer by using small individual biospheres which support the growth of a single *Arabidopsis thaliana* plant that in turn provides carbon necessary for the growth of *Bacillus subtilis.*We then use qPCR to enumerate the plant cells, chloroplast, and bacteria in each biosphere to resolve a ratio between the three which can then be used to answer the question "How many microbes can a plant support?"

Title: Shoring Up the Base: the Development and Regulation of Cortex Sclerenchyma in the Basal Region of Nodal Roots

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Grasses develop a shoot-borne root system, which originates from the nodes at the base of the shoot after the primary root system is established. The physical properties of shoot-borne roots, particularly in the basal region where they emerge from the plant, are predicted to have an important role in promoting plant anchorage to the soil. This region features distinct anatomy, characterized by a ring of rigid sclerenchyma tissue formed via lignification and secondary thickening of the first several cortex cell files. We analyzed the development of this tissue layer in soil-grown Brachypodium distachyon plants, revealing that this sclerenchyma layer progressively thickens as the root matures, and therefore is unlikely to facilitate initial penetration into the soil. We further discovered that cortex sclerenchyma is absent in plants grown in agar or hydroponic media, suggesting that its development nonetheless depends on environmental stimuli experienced by roots growing into a solid substrate. To understand the genetic basis for this process, we assayed cortex thickening in plants overexpressing SWAM4, an activator of secondary cell wall thickening in stems, uncovering that wall thickening is driven by common genes in both stem fiber cells and root sclerenchyma cells. Finally, we developed an automated system to reproducibly simulate wind and other mechanical challenges to plant anchorage. Using this system, we found that cortex thickening is strongly induced by tension and compression forces transduced from the shoot to the root crown. Thus we have developed a foundation to characterize a system that governs cortex thickening in roots by employing known above-ground cell wall regulatory genes, but that, unlike in the stem, further depends on environmental factors to control the magnitude and localization of cell wall thickening.

Title: Characterization the DNA binding sites of KNOTTED OF BRACHYPODIUM 7, a negative regulator of secondary cell wall synthesis

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The synthesis and deposition of secondary cell walls is a critical aspect of plant growth. In specific tissues and cell types including xylem and fiber, a thick secondary wall composed of cellulose, hemicellulose, and lignin is deposited. KNOTTED-LIKE HOMEOBOX OF ARABIDOPSIS THALIANA 7 (KNAT7) is a well-characterized transcriptional repressor of several genes that encode secondary wall biosynthetic enzymes and fiber wall thickening. Mutants have interfascicular fiber walls that are thicker than wild-type, but display thin-walled and collapsed xylem vessels. Mutants of the closest ortholog of KNAT7 in rice, OsKNAT7/KNOTTED OF RICE 1, also have thicker interfascicular fiber walls but no reported xylem phenotype. Here we show that mutants of the B. distachyon KNAT7 ortholog, KNOTTED OF BRACHYPODIUM 7 (KNOB7), have increased interfascicular fiber wall thickness while KNOB7 overexpressing plants showed the opposite phenotype. Detailed characterization of these genetic reagents revealed unique control of lignin composition, hydroxycinnamic acid content, and cell wall polysaccharide content. These observations may reflect control of grass specific cell wall characteristics not present in eudicots, such as high levels of wall bound hydroxycinnamic acids and the prevalence of heteroxylan polysaccharides. To further understand KNOB7 function, we characterized protein-DNA interactions using DNA affinity purification sequencing (DAP-seq). We identified 3,228 in vitro KNOB7 binding sites, and these positions were significantly enriched for a W-box motif (TTGAC). Combining protein-DNA interactions and a gene regulatory network for KNOB7 based on co-expression revealed high connectivity with the phenylpropanoid pathways. Our findings suggest that KNOB7 interacts with the TTGAC element to modulate lignin biosynthesis and wall thickening.

Title: Characterization of Arsenate Reductase 2 (ACR2) and HIGH ARSENIC CONTENT1 (HAC1) genes for Their Roles in Arsenic Phytoremediation and Food Safety

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The prevalence of arsenic (arsenate and arsenite) in the environment has led to the evolution of multifunctional enzymes involved in As uptake, translocation, and detoxification in plants. Among these enzymes, ACR2) and HAC1 have been implicated in endogenous As detoxification via the reduction of AsV to AsIII in plants. Previous studies showed that RNAi suppression of Arabidopsis ACR2 (AtACR2) lines caused translocation of 10- to 16-fold more As in shoots and retained less As in roots. However, overexpression of native AtACR2 in Arabidopsis provided strong tolerance to AsV. Further, ACR2 has a conserved arsenate reductase domain 'HCX5R' and a highly Cys-rich C-terminal domain whose function is unknown. We hypothesized that this domain is involved in providing tolerance to arsenic. Therefore, we mutated the Cys residues to understand the mechanism of AtACR2 and the role of conserved domains. Preliminary results showed that transgenic lines overexpressing the mutated AtACR2 lost AsV tolerance. Additionally, we proposed Crambe abyssinica as an ideal high-biomass crop for As phytoremediation. However, Crambe CaACR2 and CaHAC1 are not yet characterized. We hypothesized that 1) the knockdown of the CaACR2 and CAHAC1 by RNAi will lead to an enhanced translocation of AsV to the aboveground tissues by preventing the reduction of AsV to AsIII in the roots and 2) the overexpression of CaACR2 and CaHAC1 will lead to an enhanced reduction of AsV to AsIII only in shoot and thus increased As accumulation in the shoot. To test our hypothesis, we designed and cloned RNAi and overexpression constructs for the genes for use in the Agrobacterium-mediated transformation of Crambe. Further studies will be done to decipher the mechanism of action of the genes. This will aid in the development of a strategy to hyper-accumulate As in shoot tissues for the As phytoremediation for food safety. Keywords: Arsenic, Phytoremediation, Arsenate reductase, Crambe abyssinica, Arabidopsis thaliana.

Title: Soil Amendment with Nanosulfur Alleviates Silver Nanoparticle Toxicity and Improves Seed and Oil Yield in Soybean (Glycine max [L.] Merr.)

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Silver nanoparticles (AgNPs) are ubiquitously used in many commercial products due to their antimicrobial properties, and hence, a significant exposure of AgNPs in agricultural systems is anticipated. AgNPs accumulation in soil and subsequent uptake by plants can be harmful to plant growth and exposure to animals and humans through the food chain is also a concern. Our study evaluated the potential protective role of nanosulfur (NS) and bulk sulfur (BS) at 200 and 400 mg/kg soil in alleviating silver nanoparticle (AgNPs; 32 and 64 mg/kg) phytotoxicity to soybean (Glycine max [L.] Merr.). The treatments were added in the soil before soybean transplantation; growth, yield, nutrient, and silver accumulation were measured in the shoot, root, and seeds at vegetative and maturity stages. Exposure to AgNPs significantly affected plant growth and yield, reducing nodule weight by 40%, fresh shoot weight by 66%, and seed yield by 68% when compared to controls. However, nanosulfur application in soil alleviated AgNPs toxicity, and importantly, this impact was nanoscale specific as the benefits of corresponding bulk sulfur (BS) were marginal. Specifically, nanosulfur at 400 mg/kg significantly increased seed yield (~3-fold more than AgNP64) and shoot biomass (2.6-fold more than AgNP64) upon co-exposure with AgNP, essentially alleviating AgNP toxicity. Moreover, NS increased nodule mass by 3.5 times compared to AgNP-treated plants, which was 170% greater than the Ag- and NS-free controls. Plants treated with NS with AgNP co-exposure accumulated significantly less Ag in the shoots (~ 80% reduction) and roots (~ 95% reduction); no Ag contents were detected in seeds. These findings demonstrate the potential of NS as a sustainable soil amendment to reduce the accumulation and toxicity of AgNPs and as a valuable nano-enabled strategy to promote food safety and security.

Title: Seed Priming with g-C3N4 Nanosheets Doped with Essential Elements Enhanced Salinity Stress Tolerance in Rice

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The current study explored the effect of graphitic carbon nitride (g-C3N4) and zinc-doped graphitic carbon nitride (Zn-g-C3N4) in reducing NaCl-induced salinity stress in Oryza sativa L (rice). High-temperature pyrolysis was used to synthesize the nanomaterials, which was characterized by transmission electron microscopy, scanning electron microscopy, and XRD. Rice seeds were primed with g-C3N4 and Zn-g-C3N4 at a concentration of 250 µg/L for a period of 12 hours. Primed seeds were germinated and grown for 21 days in half-strength Hoagland's solution amended with or without 100 mM NaCl. Un-primed rice seeds resulted in a 25% decrease in germination percentage, and a 74% and 48% decrease in fresh weight and dry weight, respectively, with 100 mM NaCl treatment. Seed priming with g-C3N4 and Zn-g-C3N4 (250 µg/L; 12hr) under normal conditions increased the fresh biomass by 13% and 16%, whereas seed priming with g-C3N4 and Zn-g-C3N4 under 100 mM salt stress conditions showed 177% and 240%, respectively. Furthermore, the addition of g-C3N4 and Zn-g-C3N4 reduced ROS accumulation in the roots and shoots by 50-62% and 59-61%, respectively, whereas the non-enzymatic antioxidant content in the shoots increased to 45% and 65%. Both g-C3N4 and Zn-g-C3N4 significantly affected rice macronutrient (K, P, Ca, and Mg) and micronutrient (Cu, Fe, Zn, and Mn) contents, as well as the Na+/K+ ratio in salinity-stressed rice plants. Further, differential regulation of gene(s) and gene networks at molecular levels in rice plants treated with g-C3N4 and Zn-g-C3N4 under salt stress is currently undergoing. These results show the significant potential of the use of these nanomaterials for increasing crop productivity under extreme environmental conditions for increasing global food security. Keywords: Antioxidants, Climate Change, Gene Expression, Nanotechnology, ROS, Salinity Tolerance.

Title: Convergence and divergence in abscission zone morphology in domesticated rice and de-domesticated weedy rice

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Throughout the evolutionary history of rice (Oryza sativa), the modification of seed shattering has been a recurring theme. Reduced seed shattering was selected during rice domestication to facilitate harvesting and minimize yield loss. Conversely, increased seed shattering was preferred during the evolution of weedy rice (Oryza spp.) to optimize seed dispersal and spread. The abscission zone (AZ) is a specialized structure in the flower-pedicel junction that is necessary for seed separation, but our understanding of its morphological characteristics has been limited to a few rice lines. This has led to a simplified paradigm, particularly in cultivars, that their low shattering level is due to loss or disruption of the AZ. To obtain a comprehensive view of AZ morphological diversity, we quantitatively characterized individual AZ morphologies by sectioning the flower-pedicel junction longitudinally in populations of both cultivated and weedy rice. We employed three AZ characteristics - AZ relative length, AZ discontinuity, and AZ intensity - to compare morphologies between high and low shattering rice. Among these, AZ relative length was the most distinguishing AZ character. Comparisons of AZ morphologies among different groups revealed that the japonica cultivated rice group diverges from other cultivars, having a more disrupted AZ, with more breaks among AZ cells and less distinctiveness from its surrounding cells. In contrast, all weeds typically exhibited a complete AZ regardless of their origins, consistent with their convergence in high shattering levels. A low phylogenetic signal for AZ traits suggests that these AZ features are easily modifiable throughout rice's evolutionary history without phylogenetic constraints. Our study provides valuable insights into the evolutionary history of rice seed shattering by examining the morphological characteristics of the abscission zone at the population level.

Cardiac Glycoside Biosynthesis in Erysimum cheiranthoides

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The biosynthesis of cardiac glycosides, steroid-derived plant metabolites that are potent inhibitors of essential Na+/K+-ATPases in animal cells, evolved independently in more than a dozen plant families. We established Erysimum cheiranthoides (wormseed wallflower; Brassicaceae) as a new model system for investigating previously unknown genes of the cardiac glycoside biosynthesis pathway. After sequencing the genome of an inbred E. cheiranthoides lineage, we used genetic mapping, phylogenetic comparisons, and co-expression analysis to discover candidate genes involved in cardiac glycoside biosynthesis. We verified the in vivo functions of these candidate genes by making stable transgenic knockouts using CRISPR/Cas9 mutagenesis and quantifying the resulting changes in the E. cheiranthoides cardiac glycoside profile by HPLC-MS. Additionally, the enzymatic functions of some E. cheiranthoides genes were confirmed by transient expression in Nicotiana benthamiana. Ongoing insect bioassays with E. cheiranthoides mutants will determine the defensive properties of different cardiac glycoside profiles.

Title: Unraveling Root Architecture Complexity at Single-cell Resolution in Maize

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Root architecture and anatomy determine spatiotemporal control of resource exploration and acquisition, leading to crucial implications in plant productivity. Monocot roots, especially in the grasses, differ substantially from the well-studied model Arabidopsis thaliana by producing embryonic seminal and non-embryonic crown roots that are essential for anchorage and nutrient acquisition. Little is known about cell type differences among these roots at the molecular level and how these affect the cellular organization, development, and root function. Here, we present a comparative study of single-cell transcriptomes from the embryonic primary and seminal roots with their respective laterals, and post-embryonic crown roots in maize. Integration of scRNAseq datasets combining about 24 000 cells revealed conserved and unique transcriptional programs in cell type specification and allowed dissection of gene expression patterns changes within cell types. Interestingly, while primary and secondary roots morphologically differ in their number of cortical layers, we didn't see significant differences in the number of cells with cortical identity. However, cells with endodermis identity were significantly higher in lateral roots, particularly in the seminal laterals, revealing a potential root-type specific transcriptional specialization. Post-embryonic crown roots showed an overrepresentation of genes involved in water transport, epidermis differentiation, and response to stress compared to primary and seminal roots, while genes involved in stress response, immune system process, catalytic activity, and antioxidant activity were significantly overrepresented in lateral roots compared to primary and seminal roots. These results together show the potential of scRNAseq in unraveling the molecular complexity of root architecture in maize.

Title: Genetic and molecular analysis of six maize COI receptors reveals their roles in growth and development

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The F-box protein Coronatine Insensitive (COI) is a key receptor for the jasmonic acid signaling pathway in plants. To investigate the function of the six maize COI proteins (COI1A1, COI1A2, COI1B1, COI1B2, COI2A, and COI2B), we made single, double, and quadruple loss-of-function mutants. Double-mutant coi2A coi2B pollen was inviable, and no homozygous mutant plants were obtained. The coi1 quadruple mutant (coi1-4x) exhibited shortened internode lengths, decreased photosynthesis, leaf discoloration, microelement deficiencies, and accumulation of DELLA, a repressor of the gibberellic acid signaling pathway. Co-expression of maize COI and DWARF9 (DELLA) genes in Nicotiana benthamiana showed that the COI proteins lead to proteasome-dependent DELLA degradation. Downregulated genes in the coi1-4x mutant were primarily ones that are normally induced by gibberellic acid, most of which were bundle sheath or mesophyll-specific genes, including those encoding C4-specific photosynthetic enzymes. Ectopic expression of maize COI genes in N. benthamiana showed that COI2A is fully localized in the nucleus and interacts with maize JAZ proteins, the canonical COI repressor partners. However, maize COI1A and COI1B proteins showed only partial nuclear localization and lacked binding to most of the JAZ proteins. Together, these results show divergent functions of the six COI proteins in regulating maize growth and defense.

Title: Regulatory Networks Governing Nitrogen Use Efficiency in Maize and Sorghum

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Nitrogen availability is vital for crop production and overall plant development; however, excessive application of nitrogen can create nitrogen toxicity in plants and can lead to negative impacts on the environment. Nitrogen use efficiency (NUE) is a complex trait; to study this, we constructed a yeast one hybrid gene regulatory network (GRN) for NUE in maize and used time-series gene expression data to profile the early response to nitrogen limitation and recovery in maize and sorghum. The Maize NUE GRN consists of 1625 protein DNA interactions (PDI) between 301 transcriptional factors (TF) and 70 promoters. When comparing the NUE GRN of maize with the previously published Arabidopsis NUE GRN, we observed less than 18% conservation among various nitrogen-related processes, with three TF families displaying a high degree of connectivity in the GRN. Additionally, the conservation is greater in subnetworks associated with carbon metabolism and nitrate assimilation. The conservation observed between dicot and monocot suggests the possibility of projecting these GRN to other species. We were able to project the maize GRN via orthology to a sorghum GRN consisting of 1596 PDIs between 93 promoters and 226 TFs. To characterize subnetworks involved in response to nitrogen limitation and recovery, we were able to overlay time-series expression data from leaf and root tissues sampled from hydroponically grown maize and sorghum. Preliminary results indicated that the nitrogen-responsive genes in the two monocots display common PDI interactions, but exhibit dissimilar patterns of temporal expression for conserved interactions in the nitrate assimilation subnetwork. In our future work we plan to explore high accumulation of nitrogen in A-lines of sorghum to answer a core question on nitrogen toxicity in cattle. Together, the conserved interaction highlights the response of nitrogen-responsive genes to adapt to the availability of nitrogen while maintaining the stability of the regulatory structure in crops. This GRN may provide critical insight into the response to nitrogen regulation for agronomic and NUE improvements in major crop species.

Title: STUDY OF Ma1 REGULATION FOR MODULATING APPLE FRUIT ACIDITY

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Fruit acidity and sweetness in apples (Malus domestica) are largely conferred by the malate content of the vacuole. Previous studies have found that allelic variation in the tonoplastic membrane transport protein Ma1, an orthologue of ALUMINUM-ACTIVATED MALATE TRANSPORTER9 (ALMT9) in Arabidopsis (Arabidopsis thaliana), results in large differences in fruit acidity . The recessive allele, ma1, contains a single nucleotide substitution which results in a premature STOP, truncating the cytosolic C-terminal domain. When expressed in heterologous systems the transporter encoded by ma1 shows a decreased transport activity, as well as a reduced expression at the vacuolar membrane, which is associated with a reduced fruit acidity . Here, we examined the regulatory role of the ALMT C-terminus. Using a fluorometric quantification approach, we were able to estimate protein longevity at the membrane. Preliminary results indicated that truncation of the C-term in the ma1 protein does not result in increased degradation at the membrane, but rather may result from a reduction in trafficking to the tonoplast. A pharmaceutical approach is being implemented to elucidate trafficking differences between the two alleles and provide insight into the role that the C-terminus plays in trafficking of membrane-bound proteins to the tonoplast membrane. Furthermore, additional loci have been associated with variations in fruit acidity, among which is one locus whose causal phenotype has been narrowed to a protein homologous to a mammalian scaffolding protein family. Preliminary data suggests a potential interaction between this putative protein and the membrane transporter Ma1 indicating a possible additional regulatory mechanism for Ma1 transport activity. Our current aim is to elucidate if the putative scaffolding protein plays a stabilizing role on Ma1, and therefore its association with a given fruit acidity phenotype.

Poster Abstracts:

P1

Title: A Genetic Test for Serpentine-Specific Natural Selection in the Lyre-Leaf Rockcress, Arabidopsis lyrata, an Important Element of the State Line Serpentine Barrens of Eastern North America.

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A short-lived perennial in the mustard family, Arabidopsis lyrata, is often used in studies of plant ecology and evolution due to its high level of morphological and genetic polymorphism. A. lyrata can grow in disturbed or disadvantaged habitats, such as on nutrient poor serpentine soils high in heavy metals. We aim to provide insight into adaptation and evolution of this species through examining gene flows between A. lyrata populations growing in the same geographic area (Mid-Atlantic) but in drastically different habitats, on more hospitable non-serpentine soils and nutrient poor and toxic serpentine soils of serpentine barrens. Gene flow between populations was investigated through comparison of alleles of selected microsatellite loci. We aim to determine whether serpentine soil populations are highly adapted and genetically closer to each other, with genetic exchanges occurring mainly within and between serpentine soil populations despite geographic distance, or they are the result of adaptation of local non-serpentine soil populations. Our preliminary data show serpentine soil populations exchange genetic material mainly with other serpentine soil populations, though there is also some input from their non-serpentine neighbors.

P2

Title: CYP72A9 Functions as a Modulator of Plant Growth During Abiotic Stress in Arabidopsis

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Plants regularly experience mild stresses for which they are equipped to adjust gene expression and biochemistry to maintain growth and reproduction. Metabolic tradeoffs between growth and defense allow plants to optimize resource use in response to adverse environmental conditions. Little is known about the subtle changes in secondary metabolites and growth promoting hormones during stress acclimation. Many cytochrome P450 enzymes (CYPs) are induced by environmental stresses to facilitate shifts in metabolism. Plant genome sequencing has revealed the presence of thousands of CYP genes with an average of about 300 genes per plant. The CYP72A subfamily appears to have members in all angiosperms and provides the potential for diverse biochemical functions in each plant species in response to external stresses. CYP72A9 from Arabidopsis is expressed in immature seeds and inactivates gibberellins as part of the dormancy process, and its GA hydroxylase activity appears to be conserved in CYP72As across the plant kingdom. The gene encoding CYP72A9 is induced by various abiotic stresses, such as heat and osmotic stress. Our work tests the hypothesis that CYP72A9 modulates plant growth in response to environmental stress. We measured plant growth at various stages of development when CYP72A9 mutant and wild type plants are exposed to abiotic stresses individually or in combination. Plants deficient in CYP72A9 are larger than wild type plants when five-day old plants are exposed to individual and a combinations of abiotic stresses. These results suggest that under a variety of adverse environmental conditions, young plants are slowing growth through GA inactivation. There is little support for a response later in plant development. Unraveling the stress-induced regulation of gibberellin homeostasis contributes to our understanding of the growth-defense tradeoffs in plant acclimation to adverse conditions.

P3

Title: Recognizing Your Substrate: Functional Analysis of Plant MATE Transporters

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Membrane transporters are vital for maintaining cellular homeostasis. In plants, they dynamically modulate the uptake of essential nutrients and maintain ionic balance. In acidic soils, aluminum (Al) becomes soluble and toxic. It inhibits root growth, induces oxidative stress, and disrupts water and essential nutrient absorption, ultimately reducing crop yield. Membrane transport proteins belonging to the multidrug and toxic compound extrusion (MATE) family mediate the release of carboxylates like citrate, which binds Al, forming non-toxic compounds at the root surface. This subgroup of plant MATEs and the substrates it transports is unique in function compared to other plant MATEs. MATEs are present in all kingdoms; however, only a few have been characterized functionally despite their dominating presence in plants. Citrate and non-citrate transporting MATEs are remarkably similar in structure. However, there are distinct protein regions unique to MATEs capable of transporting citrate that may dictate the differences in substrate recognition. Four major protein domains have been identified based on the correlations between these functional data and amino acid sequence similarities. We hypothesize that a unique structural domain enables citrate-transporting MATEs to recognize their substrates. I am currently characterizing the functionality of chimeras, formed by swapping protein domains among citrate-transporting and non-transporting MATEs, to identify the structural motifs underlying plant MATE transporters' fundamental functional characteristics.

P4

Title: Thickening of Secondary Cell Walls in Leaf Nodal Roots in Response to Mechanical Challenge and Overexpression of the SECONDARY WALL INTERACTING bZIP

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Cereal crops are prone to falling over in the field due to environmental stresses such as wind and heavy rain. This phenomenon, known as lodging, occurs as a result of the stem bending near the ground or the roots detaching from the soil and leads to a significant reduction in yield. Leaf nodal roots, which emerge from leaf nodes at the base of the plant, can provide stability, and an increase in secondary cell wall thickening in those roots may provide lodging resistance. Here, we examine leaf nodal roots in the context of thigmomorphogenisis, a response to repeated mechanical stimuli that can result in dramatic developmental effects throughout the plant. When mechanical stimuli increase membrane tension, the transcription factor protein SECONDARY WALL INTERACTING bZIP (SWIZ) translocates into the nucleus and activates secondary cell wall thickening. To understand the effect of repeated mechanical stimuli on leaf nodal root secondary cell walls, we grew Brachypodium distachyon plants with and without overexpression of SWIZ in an automated brushing system that provides repeated mechanical challenge to the above-ground portion of the plant. We found that mechanical stimulation leads to an increase in leaf nodal root cortex cell wall thickness in wild-type plants, and that an overexpression of SWIZ leads to a decrease in the number of cell files of the cortex of leaf nodal roots. We will further investigate the effect of mechanical stimuli on secondary wall thickening in leaf nodal roots by characterizing the expression of genes involved in secondary cell wall synthesis in plants undergoing mechanical stimulation.

P5

Title: A Firefly Luciferase Reporter to Characterize Expression of the Gene p-Coumaroyl-CoA Monolignol Transferase in Brachypodium distachyon

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There is significant interest in using plant biomass to produce sustainable biomaterials and biofuels. In mature plants, most biomass is contained within the secondary cell wall. One major obstacle to the industrial use of plant biomass is the phenolic polymer lignin, a key component of the secondary cell wall. In nature, lignin provides much-needed mechanical support and hydrophobicity to the vasculature; however, the chemical recalcitrance of lignin poses a challenge to the deconstruction of cell wall polysaccharides. Thus, an improved understanding of lignin biosynthesis will be important for generating biomass-derived products. In grasses, lignin polymers consist of three subunits: p-hydroxyphenyl, guaiacyl, and syringyl units. Some monolignols may be acylated with p-coumaric acid prior to incorporation into a growing lignin chain, resulting in partial acylation of the polymer. The function of this acylation is currently unknown. In the model grass Brachypodium distachyon, the enzyme p-coumaryl-CoA-monolignol transferase (PMT) acylates monolignols, but does not act on other acylated polymers in the cell wall, such as heteroxylans. Here, we characterize the expression of PMT in B. distachyon using a luciferase reporter, in which the promoter region of PMT is fused to the luciferase coding region. This imaging allows for spatiotemporal visualization of gene expression in the whole plant across developmental stages and with higher resolution than is practical with methods such as RNA-seq. Using this luciferase system, we observed that PMT expression exhibited a diurnal rhythm with peak expression during the day in the presence of photo- and thermocycles. With a photocycle and constant temperature PMT expression remained constant. Collectively, these results suggest that the PMT diurnal rhythm is temperature regulated.

P6

Title: BdSND2R is a transcriptional repressor that forms a negative feedback loop with SWAM1 to control secondary wall thickening in Brachypodium distachyon

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Plant secondary cell walls are complex matrices composed of mostly cellulose, lignin, and hemicellulose. Biosynthesis of these polymers in Arabidopsis thaliana is regulated by a transcriptional network containing several MYB and NAC family transcription factors. However, genetic and structural evidence suggests that genes controlling this process might be different between eudicots and monocots. In the model grass Brachypodium distachyon, we characterized the NAC transcription factor BdSND2R. Co-expression analysis revealed that BdSND2R clustered with putative cell-wall-associated genes. Phylogenetic analysis identified BdSND2R as the homolog of the AtSND2 transcription factor, which functions as an activator of secondary wall biosynthesis. Genome synteny suggests SND2 was duplicated in grasses following divergence from eudicots, and one copy was lost during the evolution of the family Pooideae. To characterize transcriptome regulation by BdSND2R, we performed DNA affinity purification sequencing (DAP-seq). Binding sites were significantly enriched with the VNS element. We identified a significant peak near the transcriptional start site of SWAM1, a MYB activator of cell wall thickening. However, transcriptomic analysis showed that SWAM1 expression was reduced by BdSND2R overexpression and was elevated in BdSND2R mutants. While AtSND2 is understood to function as a positive regulator of secondary cell wall deposition, histological analyses further showed BdSND2R is a repressor of cell wall biosynthesis. Plants overexpressing BdSND2R had decreased lignin content and thinner cell walls, whereas snd2r-4 and snd2r-6 mutants showed opposite secondary cell wall phenotypes. Collectively, these results suggest that unlike in eudicots BdSND2R is a repressor of cell wall biosynthesis and forms a negative feedback loop with SWAM1.

P7

Title: Inoculating Brachypodium distachyon Roots with Fusarium oxysporum Fo47 PARP-1 Mutant Increases Above-Ground Plant Area and Seed Yield

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Plant-microbe interactions in the rhizosphere can lead to the mutual benefit of both organisms. Understanding such interactions can provide key insight into plant growth and development. Fusarium oxysporum Fo47 is a non-virulent endophytic fungus commonly found within the rhizosphere. By exploiting accessible openings, it is able to infiltrate roots of plants such as Arabidopsis thaliana and Solanum lycopersicum. While capable of colonizing the root interior, F. oxysporum Fo47 does not penetrate the vascular system, therefore having a neutral – or, in some cases, beneficial – effect on its hosts. Although well-characterized in A. thaliana, S. lycopersicum, and other eudicot species, neither the effects of this fungus nor its capability to colonize grass root systems have been reported. To that end, we investigated the effects of a mutant strain of F. oxysporum Fo47 on wounded and unwounded Brachypodium distachyon roots. The mutant was created by excision of Poly(ADP-ribose) polymerase-1 (PARP-1), which encodes an enzyme that repairs breaks in nuclear DNA. We then developed a semi-automated imaging system to analyze plant area from multiple perspectives. We observed a significant increase in above-ground growth in response to wounding, with a greater increase in both area and seed production when wounding was combined with F. oxysporum Fo47 inoculation. The degree of response differed among B. distachyon accessions, indicating that there is genetic variation within the species. These findings suggest that B. distachyon and F. oxysporum are tractable systems for studying beneficial plant-microbe interaction.

P8

Title: Molecular and Biochemical Studies on the Interaction between Cucumber and Plant Growth Promoting Rhizobacteria (PGPR) under Cadmium (Cd) Stress

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Excess use of chemical fertilizers and pesticides in agriculture cause heavy metal accumulation in soil and in food crops and pose a threat to human health. Cadmium (Cd) is a hazardous metal that disrupt plants physiological, biochemical and molecular pathways. Plant growth promoting rhizobacteria (PGPR) has been applied as ecofriendly and promising technique to remove or detoxify the heavy metals from soil. In this study 16 Cd tolerant PGPR strains were isolated from rhizosphere soils of the contaminated cultivating field. According to plant growth promoting traits, four isolates were selected and their 16srRNA gene sequence showed that they belonged to Serratia rubidaea. Among these, strain B12 showed the highest response to phosphate solubilization and synthesized essential plant growth promoting molecules like IAA. The effect of Serratia on plant growth, chlorophyll concentration, and Cd uptake and accumulation were compared in a hydroponic experiment with different concentration of CdCl2. It was observed that Cd stress significantly reduced the germination rate, seedling length, and vigor index of Cucumis sativus L., while biopriming cucumber seeds with S. rubidaea considerably enhanced the germination, fresh weight, and length of shoot and root exposed to different levels of Cd stress. Furthermore, S. rubidaea inoculation significantly induced the biosynthesis of chlorophyll under Cd stress. This indicating a promoting role of Serratia in plant growth. Therefore, our study suggests that S. rubidaea mitigated the negative impacts of Cd stress by modulating photosynthesis activity, nutrient uptake, seed germination and growth parameters. Further analysis of the effect of S. rubidaea on ameliorating the Cd toxicity at the molecular and biochemical levels are undergoing. This proposed strategy could alleviate the heavy metals uptake in food crops and improve food safety. Key words: Cucumber, Fertilizers, Heavy metal stress, PGPR.

P9

Title: Root Growth Under Heat Stress is Enhanced by RCI2A in Arabidopsis thaliana Seedlings

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RARE COLD-INDUCIBLE 2A is a gene induced by low temperatures, ABA, salt stress, and dehydration. RCI2A encodes a small hydrophobic protein containing two transmembrane domains and may regulate membrane properties by interacting with other proteins. To investigate the impact of insertion alleles on the expression and function of RCI2A in roots, SALK_052241 (rci2a-2), SALK_063028 (rci2a-3), and SALK_059113 (rci2a-5) were genotyped by PCR and RCI2A expression levels were determined by qPCR which confirmed the insertions were present and that they downregulated RCI2A. We investigated root architecture phenotypes in response to heat stress using an in vitro image-based approach. The heat-stressed rci2a-2 seedlings had significantly shorter total root lengths than the control treatment. The experiment was repeated and on day 14, the primary root lengths of all mutant alleles were significantly shorter in the heat-stressed seedlings than in the control treatment. The varying results might have to do with differences in recovery time, however, the overall results suggest that RCI2A contributes to the heat tolerance of root growth in Arabidopsis thaliana seedlings. Therefore, our data indicate a new RCI2A function to protect Arabidopsis from abiotic stressors including heat.

P10

Title: Genotype Analysis of PpCSLD5/8 CRISPR/Cas9 Knockout Mutants

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Genotype Analysis of PpCSLD5/8 CRISPR/Cas9 Knockout Mutants Kerrina Whelan*, Kayleigh Kearney, Michael Budziszek, and Christos Dimos Department of Science, Johnson & Wales University, Providence, RI, USA The moss Physcomitrella patens is an attractive model organism due to its small genome and dominant haploid phase. P. patens has eight genes that form a family known as the Cellulose Synthase-like Ds (CSLDs). A previous study using global knockdown of the entire PpCSLD family by RNA interference (RNAi) showed a decrease in protonemal tip growth. In order to study the roles of specific CSLDs in P. patens development, potential double knockout (KO) mutants for PpCSLD5/8 were generated by CRISPR/Cas9 mutagenesis. The goal of this project was to screen potential ppcsld5/8 KO lines for gene deletions using PCR. A total of 59 potential ppcsld5/8 KO lines were screened and 4/59 were positive for PpCSLD5 deletions, while 1/59 was positive for a PpCSLD8 deletion. None of the potential ppcsld5/8 KO lines screened were positive for both PpCSLD5 and PpCSLD8 gene deletions. The high number of potential ppcsld5/8 KO lines reporting as wild type could be the result of the CRISPR/Cas9 system only cutting at one target site. This would result in minor indel events that cannot be detected by conventional PCR. As a result, Competition-based PCR (cbPCR) assays are being designed to look for indels in these potential ppcsld5/8 KO lines.

P11

Title: Exploring the Role of CYP72A Enzymes in the Zea mays Stress Response

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Global warming impacts crop productivity due to the worsening of stressful environmental conditions. To combat stress, such as high heat, drought, salinity, caterpillar and/or aphid feeding, plants evolved a variety of defense mechanisms that include physical and chemical defenses, and growth modulation. Biosynthesis of many protective secondary metabolites that are induced by environmental stresses require the diverse superfamily of enzymes, cytochrome P450s (CYPs). Specifically, the CYP72A subfamily has been shown to possess functional diversity among flowering plants. Previous experiments in Arabidopsis thaliana demonstrated that CYP72A9 is differentially expressed by abiotic stress. CYP72A genes have also been implicated in gibberellin inactivation in rice, suggesting a role in defense-related growth modulation. Phylogenetic relationships between CYP72As in three Zea mays inbred lines suggests natural variation in the contributions of these enzymes to plant metabolism, so we set out to determine which CYPs are contributing to specific stress responses. We hypothesize that CYP72A enzymes in maize are differentially induced by abiotic and biotic stress as part of the acclimation process. Studying the CYP72A gene expression in maize can give us a better understanding of their role in plant defense pathways and metabolism. Gene expression analysis revealed that CYP72As are differentially expressed under different stress conditions. To study the effects of environmental stresses on CYP72As and their ability to modulate growth, we performed single and combined stress experiments to compare the growth of wildtype and mutants missing CYP72A349. We also combined abiotic stress with caterpillar choice experiments to study the role of CYP72A349 on the interaction of abiotic stress and herbivory responses. Our ongoing mutant analysis will contribute to a better understanding of the metabolic contributions of the CYP72A subfamily in plant stress acclimation.

P12

Title: Development of a Routine Protocol for Chloroplast Transformation from Leaf Tissue in the Model Plant Arabidopsis thaliana

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Chloroplast transformation is a powerful tool for plant genetic engineering because plastid genes have high expression levels, maternal inheritance and can be expressed from operons. An efficient chloroplast transformation protocol requires source tissue that can regenerate efficiently in tissue culture and is sensitive to the antibiotic spectinomycin, which is needed for transformant selection. The model plant Arabidopsis thaliana is naturally tolerant to spectinomycin and has poor regeneration ability in tissue culture. To enable chloroplast transformation in Arabidopsis, spectinomycin-sensitive plant lines were created by inactivating the nuclear-encoded acc2 gene. In this study, we over-expressed a steroid-inducible Baby Boom (BBM) gene in the spectinomycin-sensitive Arabidopsis plant lines to improve their regeneration capacity. Leaves from the new Arabidopsis acc2-BBM plant lines were used as source tissue for chloroplast transformation experiments. Eighteen spectinomycin-resistant calli were obtained from 18 bombardments and plants regenerated from eight of the spectinomycin-resistant calli. Molecular characterization of DNA from the regenerated plants determined that spectinomycin resistance was due to spontaneous mutations in the chloroplast encoded 16S rRNA gene. Two of the regenerated plants formed viable seed and four are currently forming siliques. Coupling of the spectinomycin-sensitivity with the improved plant regeneration system reported here resulted in reproducible regeneration of fertile spectinomycin resistant plants from regenerated leaf tissue in the model plant Arabidopsis thaliana. It is expected that utilization of this protocol will enable chloroplast transformation in Arabidopsis, which will have a major impact on basic science and applications in biotechnology due to the extensive genomic resources available.

P13

Title: Tissue Specific Expression of Fluoride Exporter (FEX) Reveals That More is Better

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Fluoride is toxic to plants, yet nearly ubiquitous in the environment with both natural and anthropogenic sources. Plants are generally tolerant to fluoride, but excessive amounts can accumulate in tissues causing localized chlorosis and cell death. We have seen in yeast that the Fluoride EXporter, FEX, is essential for survival in fluoride concentrations as low as 0.05 mM via ion efflux out of the cell. FEX homologs have been identified in all plants for which genomic sequences are available, but the means by which FEX confers fluoride tolerance in a multicellular organism are unclear. To determine how FEX functions globally in plants and in which tissues it is necessary, we have made several tissue-specific promotor constructs controlling the expression of a translational fusion of eYFP and AtFEX, in our Arabidopsis thaliana knockout mutant. These FEX mutant plants exhibit distinctive fluoride-sensitive phenotypes, at all developmental stages, including non-viable pollen and reproductive sterility when exposed to fluoride concentrations as low as 5.0 μM. Tissues expressing FEX are able to rescue the mutant phenotype to varying degrees giving us insight into how FEX functions to provide fluoride tolerance.

P14

Title: The Chromatin Associated DEK3 Protein is Likely Necessary for Root Growth and Cold Tolerance in *Arabidopsis thaliana*

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The DEK protein is an evolutionary conserved chromatin-associated protein whose function in plants is still unknown. Previous studies have shown that DEK3 can bind to DNA and histones and altered levels of DEK3 are associated with abiotic stress tolerance. With the changing climate affecting plant life around the globe, our goal was to determine if DEK3 contributes to the tolerance of temperature extremes in Arabidopsis thaliana. In this study, phenotypic assays were developed using a transient heat stress and a transient cold stress. Arabidopsis seedlings were either treated to a 48 hour heat stress of 35°C or a 48 hour cold stress of 12°C and then allowed to recover for several days. Analysis with qPCR was performed to quantify the level of expression in the DEK3 mutants relative to WT. The mutant dek-3-3 had levels of expression around 29% that of WT while the mutant dek3-4 had levels of expression 8% that of WT. Results show that dek3-4 seedlings had shorter root lengths compared to WT in control conditions suggesting that a possible function of DEK3 is to promote longer roots. In the heat stress assay, all treated seedlings had shorter total root lengths compared to their controls. In the cold stress assay, dek3-3 was more tolerant to cold stress than WT and dek3-4 when compared to the control treatment group. These results suggest a possible function of DEK3 in regulating root growth and cold tolerance.

P15

Title: RCI2A Contributes to Heat Stress Tolerance in Adult *Arabidopsis thaliana* Plants

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RCI2A, a gene in Arabidopsis thaliana, is highly expressed under low temperatures, dehydration, salt stress, and ABA. RCI2A encodes a small, hydrophobic protein with two possible transmembrane domains. Overexpression of RCI2A has been shown to increase cold tolerance in tomato. However, the effects of heat on RCI2A expression have not yet been investigated. We hypothesized that if RCI2A aids in heat resistance then rci2a mutant lines that have undergone a heat stress event will have a smaller area and exhibit more tissue necrosis. This study investigated the phenotypic effects of a heat stress event on rosettes of adult rci2a mutants. Plants were grown under controlled conditions and image-based phenotyping was used to collect data over time. We found that heat stress negatively affected morphological traits and increased the fraction of leaf necrosis. RCI2A expression may correlate to plant resistance to heat stress. Among the mutant lines, rci2a-2 may be less heat tolerant than wild type, and rci2a-4 and rci2a-1 may be more heat tolerant than wild type. In future experiments, other time points from this experiment will be analyzed to look at recovery over time. Deep learning will also be explored for more accurate and efficient image segmentation.

P16

Title: Possible Antagonistic Roles of Arabidopsis PUM4 in Root Architecture Phenotypes Across Temperature Extremes

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The role of the gene PUMILIO4 (PUM4) in Arabidopsis thaliana has yet to be determined. Similar genes, such as PUM24 and PUM9, are expressed in roots and are influenced by increased temperatures and salt concentration. We hypothesized that PUM4 plays a role in temperature and salt tolerance in the root architecture of the plant, more specifically primary and lateral root length, as well as lateral root density. T-DNA genotyping and Sanger sequencing confirmed the location of homozygous alleles–SALK_004686 (pum4-1) and SALK_056506 (pum4-2) of PUM4. These genotypes were exposed to heat at 35oC for 48 hours. The root length of the seedlings was statistically analyzed and produced no significant differences across genotypes. The root length of seedlings was again analyzed statistically after exposure to increased salt concentration (75 mL NaCl). Root length significantly decreased in pum4-1 seedlings when compared to wild-type. The lateral root density of seedlings was observed after exposure to an increase in temperature for 48 hours. There was a significant reduction in lateral root density in pum4 mutant alleles while wildtype seedlings showed no significant change. The final experiment exposed seedlings to a decreased temperature over various time intervals. The results showed that pum4-1 had a significant increase in its root length, volume, and perimeter after exposure to cold. The wild type and pum4-2, however, showed no significant change in any aspect of their phenotypic root architecture. The results show that PUM4 is utilized by the seedlings to change root architecture when exposed to various stresses. This allows the seedlings to maintain and improve their root functionality to continue to grow after exposure to harsh conditions.

P17

Title: Effect of *Agrobacterium tumefaciens* infection on its host-plant DNA damage response activity.

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The plant pathogenic bacterium Agrobacterium tumefaciens possesses the unique ability to transfer a segment of its own DNA (T-DNA, transferred DNA) into the genome of its host plant cells. The generation and transport of the T-DNA to the plant cell nucleus, as well as of several effector proteins, is mediated by Agrobacterium virulence machinery. The final step, T-DNA integration in the host genomic DNA, is not completely understood yet and relies mostly on host factors. Host proteins involved in DNA damage repair pathways are likely to mediate T-DNA integration. So far, studies using mutant plants yielded conflicting results, maybe because of the high level of redundancy between DNA repair pathways in plants. In this study, we investigate the regulation of different plant DNA repair pathways' activity upon infection with A. tumefaciens. We have identified marker genes of the main DNA repair pathways in Nicotiana tabacum. The expression of several of these genes was induced upon A. tumefaciens infection at different intensities and temporal patterns; this induction was lower with an avirulent bacterial strain compared to wild type. Interestingly, inoculation with a mutant impaired in T-DNA and effector proteins delivery to host cells also resulted in lesser activation of DNA repair gene expression than wild-type Agrobacterium. Other markers of DNA repair activity, such as histone H2A phosphorylation levels, are under study. Overall, our data suggest that Agrobacterium infection induces DNA breaks and/or regulates DNA damage response, which could be at least partially mediated by bacterial effector proteins. It is tempting to speculate that modulation of DNA damage response by Agrobacterium ultimately facilitates integration of the bacterial T-DNA into the host genome.

P18

Title: A Dual Role of Benxozainoids in Direct Defense and Defense Regulation

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Grass species, including maize, produce a wide variety of constitutive and inducible defenses to protect themselves against herbivores and pathogens. DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) and its derivatives constitute a class of compounds called benzoxazinoids that have long been known to function as weapons for direct defense in maize. While some benzoxazinoids are toxic to caterpillars, others are known to induce callose formation, hindering aphids in their phloem sap uptake. Based on this callose induction, the question arises whether benzoxazinoids also have a regulatory function and are involved in triggering additional defense reactions. Comparison of metabolomic data generated from a benzoxazinoid mutant line, bx1::Ds, and wildtype maize inbred line W22 identified two compounds whose biosynthesis is dependent on the occurrence of benzoxazinoids. Both compounds are derived from catechol and are identified as catechol monoglucoside and likely as 6-O-acetylcatechol-glucoside, respectively. A genome-wide association study (GWAS) using a 282-line maize panel identified an acetyltransferase that acetylates catechol monoglucoside, producing the other compound. Further, a glucosyltransferase detoxifying catechol into pyrocatechol monoglucoside was verified using in vitro enzyme assays. Bioassays with caterpillars showed an increase of catechol monoglucoside after herbivory, suggesting this compound's role in defense against insects and/or pathogens. To test this hypothesis, feeding catechol monoglucoside to insects in an artificial diet assay will be necessary.

P19

Title: Expanding Capabilities of DAP-seq with Improved Protein Expression

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Gene regulatory networks (GRNs) can show the interactions between transcription factors (TF) and the genes they regulate. They are used in a wide variety of applications such as studying plant response to stimuli. To build a GRN, characterizing the transcription factor binding sites within promoter regions of a corresponding gene can suggest a high probability candidate for TF targets. There are a number of methods people use to determine these binding motifs including DNA affinity purification sequencing, also known as DAP-seq. This employs column-bound bacterially expressed affinity-tagged TFs that are mixed with genomic DNA fragments. The TF-bound DNA fragments are eluted and sequenced. Sequences with higher TF occupancy and other characteristics are determined, and likely binding motifs are identified. For high-throughput screening, this method is highly effective, yet is ineffective for TFs that do not express well or degrade in standardized conditions. For this reason, there is little known about the binding motifs of certain TF families. Here, we demonstrate an improved version of this method that results in stable expression of tagged transcription factors with little-to-no degradation. This was achieved by the addition of chemical additives. The tagged TFs were then effectively used in the remaining steps of the DAP-seq pull down to elute genomic DNA fragments that are suitable for short read sequencing. The DNA fragment libraries had clear profiles, demonstrating good representation of a distribution of fragments. Subsequent analysis of the sequencing data further demonstrated the effectiveness of the improved protein expression conditions. Known and novel peaks were identified, highlighting capabilities of using the improved method to characterize previously understudied TFs and their recognition motifs. This data, combined with transcriptomic and other data, can be incorporated to update GRNs to more accurately reflect the dynamic pathways that regulate cell response.

P20

Title: Biochemical Responses of Hybrid Poplar Clone NM6 (*Populus nigra L.* x *P. maximowiczii A. Henry*) to Lead Treatment and its Amelioration Using Foliar Putrescine Spray

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We examined the effects of one-time soil application of two different concentrations (50 and 150 μM) of lead (PbCl2) accompanied by 1 mM putrescine (Put) foliar spray on 2-year-old hybrid poplar NM6 (Populus nigra L. x P. maximowiczii A. Henry) plants. Our objectives were to analyze: (1) metabolic changes in leaf and root tissues in response to lead; and (2) the effects of foliar Put spray to ameliorate the effects of lead in the leaves. These plants were produced by tissue culture of leaves from a 3-year-old tree grown at the UNH Kingman Farm. The tissue-culture plants were rooted and grown in pots at the UNH Greenhouse facility for two years. Leaf samples were analyzed: (1) on days 3, 7, 14, and 21 after lead application for free polyamines, amino acids, phytochelatins, gas exchange, total soluble proteins, and chlorophyll contents; (2) the roots were analyzed on the day of harvest (22 days) for free polyamines and phytochelatins. The results show that: (a) although lead caused changes in the metabolism (amino acids, phytochelatins, chlorophyll, and carotenoids) of foliage, the effects were not always dose-dependent. In roots, a significant change was observed in proline, and (b) amelioration of lead effects by Put was minor and visible only on certain days of analyses for some metabolites. The effects of lead in leaves on glutamic acid, glutamine, alanine, and phytochelatin (gamma-glutamyl-cysteine) were significant on some days. In roots, only proline was significantly affected at 22 days post-treatment. Though poplars can be used for phytoremediation, the lack of significant responses in several metabolites (gas exchange, sugars, polyamines) could be because the level and frequency of lead treatment may not have been sufficient for the size of the plants used in this study.

P21

Title: Investigating the Evolution of Cytokinin Signaling Using Streptophyte Algae

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Cytokinins are crucial in many developmental processes and a key regulator in the responses of plants to changes in their abiotic and biotic environment. The main metabolic and signaling pathways of this class of phytohormones have been well characterized in modern land plants, such as rice and Arabidopsis. However, we are only beginning to understand the origin and evolution of cytokinin signaling. While the steadily increasing number of sequenced algal genomes allows us to investigate the presence of putative members of the signaling pathway, it is unclear if those genes function the same way as they do in land plants. Thus, the first step to characterize the cytokinin signaling in algae is to test if those species respond to the presence of the hormone. Toward this goal, two Streptophyte algal species, Coleochaete scutata and Spirogyra pratensis, were treated with different cytokinins, and the effect on plant growth was measured. Adenine, the backbone of cytokinins, and KOH were used as controls. Cytokinin treatment led to an increase in growth as compared to the solvent control. Surprisingly, the treatment with adenine, often used as a negative control in cytokinin treatment experiments in land plants, led to increased growth. The possible implications of these results will be discussed.

P22

Title: YUCCA Gene Expression in Expanding Leaves of Hybrid Poplar

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Members of the YUCCA gene family encode a flavin monooxygenase-like enzyme that catalyzes the final step in auxin biosynthesis, the conversion of indole-3-pyruvic acid (IPyA) to indole-3-acetic acid (IAA). As this is generally considered the rate limiting step in IAA biosynthesis, the YUCCA genes are thought to be central to this process. In order to better understand the sites of IAA biosynthesis in shoot systems of hybrid poplar, we measured the expression levels of multiple YUCCA genes in the leaves of young, greenhouse-grown Populus tremula x alba clone INRA 717-1BA using qRT-PCR, normalizing expression against three reference genes. Previous work had shown that shoot apices (defined as tight clusters of young leaves at the apex with indistinguishable internodes) contained the highest concentrations of IAA, and that IAA dropped down to low and stable levels by L16, the 16th leaf beneath the apex. Both YUC1 and YUC12 followed a pattern similar to IAA concentrations, with peak expression in apices, but YUC12 showed more than 10x the expression of YUC1 and was consistently the dominant YUCCA expressed in whole leaves that were still undergoing expansion. In order to determine relative expression levels within leaves, L8 – which is about one-half fully expanded in poplars of this size and age – was further dissected into margin, blade, midvein and petiole. YUC2 and YUC12 were both highly expressed in the blade and leaf margin, but YUC2 dropped down to near-negligible levels in the midvein and petiole while YUC12 expression remained high. A neighbor-joining phylogenetic tree supports multiple pairs of YUCCA homologs in Populus, and suggests differential expression in leaf development within at least some of these pairs. These results provide important insight into the source(s) of leaf-derived IAA during both leaf expansion and cambial development in the stem.

P23

Title: Small Molecules in Aphid Saliva

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Aphid saliva is important for mitigating plant perception of and defense against aphid attack. While the saliva is composed of a diverse array of proteins and small molecules, much of what is known about aphid saliva has been focused on identifying and characterizing the protein components. However, a few studies have demonstrated that the small molecules in the saliva are also influential in the plant-aphid interaction. Therefore, this study aimed to characterize the composition of small molecules in aphid saliva and identify candidates with potential bioactivity. Saliva from the green peach aphid, Myzus persicae, and the pea aphid, Acyrthosiphon pisum, was collected after feeding different host plants or artificial diet. Saliva samples, analyzed by HPLC-MS in both the positive and negative ionization modes, were matched with a reference library. Among the detected small molecules, the majority were identified as sugars, amino acids, dipeptides, and, surprisingly, intermediates in the tricarboxylic acid cycle. In fact, the most abundant metabolites in aphid saliva samples were citrate and malate. These results were consistent throughout the samples, including saliva from aphids reared entirely on artificial diet, indicating that the observed citrate and malate are at least partly aphid-derived. As citrate and malate act as calcium chelators in the soil, it is possible that aphids are injecting them to dampen calcium signaling in the phloem. However, more work is needed to determine the in planta effects of these metabolites. These findings are important because they show the complexity of aphid saliva and suggest new mechanisms by which aphids manipulate their host plants.

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Title: Environmental Genome-Wide Association Study in Sorghum

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Climate change and growing population have put an increased pressure on land and food resources. Major staple crops such as sorghum are often suboptimally adapted when grown in local environments and have an untapped genetic yield potential. In addition to its shared ancestry with maize, sorghum is known as the "Camel of Crops" due to its ability to adapt to drought and high temperature conditions, making it a staple crop that is of particular interest. The state-of-the-art sequencing technologies and high-throughput phenotyping aided by robust statistical models and computational tools have proved critical in aiding and informing the plant breeding process. This ongoing study conducts an environmental genome-wide association analysis (GWAS) in major staple crop sorghum. Germplasm was curated for geospatial and genetic variation data. Bioinformatics and statistical tests were conducted using the TASSEL pipeline to identify the sorghum genetic variants for an environmental variable of interest and, conversely, environmental variables associated with a given genetic variant. We identify genotypes in the sorghum germplasm collection that are associated with heightened fitness levels across 304 geo-spatial climate variables through our initial results produced by fitting Generalized Linear Models (GLM) and Mixed Linear Models (MLM). The data will be compared to published QTL data to identify overlaps with other agricultural traits and identify candidate genes of interest. Continued germplasm curation to include additional populations and the fitting of more robust models will also be executed. This dataset will be used to develop CLIMtools for sorghum and will be made available to breeders and researchers using Gramene's web interface.

Notes: