American Society of Plant Biologists

Western Regional Meeting

2018

February 3-4, 2018

California State University, Fullerton

ASPB Western Regional Meeting 2018 Organization Committee

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Western Section

USA: *Arkansas, Arizona, California, Colorado, Hawaii, Idaho, Montana, New Mexico, Nevada, Oregon, Utah, Washington, Wyoming*
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Saturday, February 3, 2018

Schedule at a Glance

8:30 AM	Registration	Holiday Inn Foyer
8:45 – 9:00 AM	**Welcome, Opening Remarks**	Crown Ballroom
9:00 – 9:45 AM	**Plenary 1**: Katayoon Dehesh, UC Riverside *Choreography of plastidial retrograde signaling*	Crown Ballroom, Chair: J. Brusslan
9:45 – 10:30 AM	**Short Oral Presentations 1**	Crown Ballroom,
9:45-10:00 AM	Melanie Sacco	Chair: J. Brusslan
10:00 – 10:15 AM	Yingnan Hou	
10:15 – 10:30 AM	Susanne Matschi	
10:30 – 10:45 AM	Break, Poster session 1 set-up	Royal Ballroom
10:45 AM – Noon	**Poster Session 1**	Royal Ballroom
Noon – 1:00 PM	Lunch, Poster removal	Royal Ballroom
1:00 – 1:45 PM	**Plenary 2**: Sabeeha Merchant, UCLA *Synchronized cell 'omics*	Crown Ballroom, Chair: M. Sacco
1:45 – 2:30 PM	**Short Oral Presentations 2**	Crown Ballroom,
1:45 – 2:00 PM	David Rhoads	Chair: M. Sacco
2:00 – 2:15 PM	Honghong Wu	
2:15 – 2:30 PM	Will Hinckley	
2:30 – 2:45 PM	Break, Poster session 2 set-up	Royal Ballroom
2:45 – 4:00 PM	**Poster Session 2**	Royal Ballroom
4:00 – 4:45 PM	**Short Oral Presentation 3**	Crown Ballroom,
4:00 – 4:15 PM	Phillip Weckwerth	Chair: ME Zavala
4:15 – 4:30 PM	Marci Parra	
4:30 – 4:45 PM	Greg Goralogia	
6:00 – 9:00 PM	Dinner, **Keynote Address**: Ann Hirsch, UCLA *The Nodule Microbiome: What We Learn from Nature, not from Dogma.*	Royal Ballroom, Chair: ME Zavala

Sunday, February 4, 2018

Schedule at a Glance

9:00 – 9:45 AM	**Plenary 3**: Zhi-Yong Wang, Carnegie Institute *Molecular Integration of Nutritional, Hormonal, and Environmental Signals in Plant Growth Regulation*	Crown Ballroom, Chair: ME Zavala
9:45 – 10:15 AM 9:45-10:00 AM 10 – 10:15 AM	**Short Oral Presentations 4** Diwaker Tripathi Pablo Martinez	Crown Ballroom, Chair: ME Zavala
10:15 – 10:30 AM	Break	Crown Ballroom
10:30 – 11:00 AM 1:45 – 2:00 PM 2:15 – 2:30 PM	**Short Oral Presentations 5** Danielle Garceau Jochen Schenk	Crown Ballroom, Chair: M. Sacco
11:00 – 11:45 AM	**Plenary 4**: Meng Chen, UC Riverside *Remodel of the nuclear architecture by light signaling during photomorphogenesis*	Crown Ballroom, Chair: J. Brusslan
11:45 AM - Noon	**Awards/ wrap up**	Crown Ballroom, Chair: J. Brusslan

Oral Presentation Abstracts

Plenary Session 1:

Title: Choreography of Plastidial Retrograde Signaling

Author(s) and affiliations: ***Katayoon Dehesh**
Institute of Integrative Genome Biology and Department of Botany and Plant Sciences, UC Riverside
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Abstract:
Being sessile, plants have evolved complex and intricate response networks to biotic and abiotic stresses. Identification of the signaling networks regulating general components of these stress responses has been a challenge.

We have identified a novel retrograde stress-sensor methylerythritol cyclodiphosphate (MEcPP), ***previously known solely as an intermediate in the isoprenoid biosynthetic pathway, as a stress sensor that communicates environmental perturbations sensed by plastids back to the nucleus***. MEcPP specifically coordinates expression of key stress-responsive nuclear genes encoding plastid-localized proteins. To identify the underlying molecular mechanism of the MEcPP-mediated stress responses, we have performed a multi-omics approach. These studies have led to identification of a transcriptional hub activated by MEcPP, and have established a previously unrecognized link between this plastidial retrograde signal and transcriptional reprogramming of endoplasmic reticulum genes critical for readjustment of protein-folding capacity in stressed cells, and further provided an understanding of the molecular mechanism by which MEcPP regulates plant growth and development in response to stress.

In conclusion, I will outline our current understanding of a functional module concept of biological organization and regulation by MEcPP, the plastidial retrograde signaling metabolite.

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Short Oral Presentations 1:

Title: Understanding Roles for the Polerovirus P0 Protein in Virulence and Plant Defense Evasion and Elicitation

Author(s) and affiliations: ***Melanie A. Sacco**, Erick Ortiz, Shyamal Oza, Kevin Valdez, Mansour Dughbaj, Timothy Choi, Tan Tri V. Nguyen, Ken-Der Wang, & Roman Empleo
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Abstract:

The Polerovirus protein P0 ($P0^{Tu}$) is a viral suppressor of RNA silencing (VSR) that targets Argonaute (AGO) proteins for degradation. We have previously shown that the P0 protein from *Turnip yellows virus* ($P0^{Tu}$) is recognized in *Nicotiana glutinosa* and elicits hypersensitive response (HR). To understand how P0 interacts with the host plant cell as a VSR versus elicitor of HR, $P0^{Tu}$ was subjected to serial deletion analysis and site-directed mutagenesis at either several conserved sites or by systematic substitutions with the 6 amino acid sequence NAAIRS along the entire coding sequence. Mutant clones were co-infiltrated with GFP into the leaves of *N. glutinosa* accession TW59 and *N. benthamiana* to observe for suppression of RNA silencing and induction of cell death. The majority of deletions and NAAIRS substitutions resulted in loss of $P0^{Tu}$ VSR function and elicitation of HR. However, NAAIRS substitution at the amino terminus inactivated VSR activity while maintaining HR elicitation, while modifications within or deletion of the last 22 carboxy-terminal amino acid residues (227-249) maintained VSR activity, but impaired HR elicitation. To further examine P0 functions in virulence, we expressed HA-tagged ubiquitin from tomato (2xHA:SlUb) in *N. benthamiana* in the presence or absence of P0 from *Potato leafroll virus* ($P0^{PL}$) and GFP. We observed a 2xHA:SlUb-induced augmentation of GFP accumulation that was eliminated in the presence of $P0^{PL}$. Moreover, we immunoprecipitated 2xHA:SlUb-tagged host proteins that were eliminated in the presence of $P0^{PL}$ and identified possible new targets of P0-induced protein degradation by tandem mass spectrometry that include metabolic enzymes that have not been implicated in defense functions. Identification of accelerated degradation of these proteins together with our observation of GFP turnover implicate P0 proteins in a general mechanism of increased ubiquitin-tagged protein turnover beyond their proposed role in targeting AGO proteins for degradation to overcome antiviral defenses.

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Title: Secondary Small RNAs Contribute to Host-induced Gene Silencing in Oomycete Pathogens

Author(s) and affiliations: ***Yingnan Hou**[1,2], Yi Zhai[1], Liping Zeng[1], Li Feng[3], Du Seok Choi[1,2],Wenwu Ye[6], Bailong Zhang[4], Weifeng Gu[5], Xuemei Chen[2,4], Jixian Zhai[3], & Wenbo Ma[1,2]
[1]Department of Microbiology and Plant Pathology, University of California, Riverside, USA,
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[4]Department of Botany and Plant Science, University of California, Riverside, USA
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[6]Department of Plant Pathology, Nanjing Agricultural University, Nanjing, China
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Abstract:

Constantly facing challenges from potential pathogens in the environment, plants have evolved a myriad of defense mechanisms. Increasing evidence has emerged to suggest that small RNAs (sRNAs) are integral components of plant immunity. As a counter-defense strategy, pathogens have evolved the ability to interfere with host sRNA pathways. We previously identified secreted effector proteins from the destructive Phytophthora pathogens that can suppress RNA silencing in plant hosts. Here, we report the investigation of mechanisms underlying the virulence activity of the effector Phytophthora suppressor of RNA silencing 2 (PSR2), which allowed us to identify a specific sRNA pathway that is particularly important for defense response in Arabidopsis. In particular, secondary small interfering RNAs (siRNAs) generated from transcripts of pentatricopeptide-repeat proteins (PPRs)-encoding genes and triggered by the parent microRNA miR161 contribute to disease resistance by silencing specific genes in Phytophthora, thereby reducing their virulence. miR161 is induced during Phytophthora infection, leading to increased production of PPR-derived secondary siRNAs as the executors of a host-induced gene silencing. However, this defense mechanism is compromised by Phytophthora through the virulence activity of PSR2, which targets Double-stranded RNA-binding protein 4 (DRB4) and specifically impairs the biogenesis of secondary siRNAs derived from protein-coding transcripts. This study discovers a role of secondary siRNAs in plant immunity and establishes a new paradigm of host-pathogen arms race.

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Title: Development and Structure-Function Relationships of the Adult Maize Leaf Cuticle

Author(s) and affiliations: ***Matschi, Susanne**[1], Vasquez, Miguel F.[1], Bourgault, Richard[2]; Qiao, Pengfei[3], Scanlon, Michael J.[3], Molina, Isabel[2] & Smith, Laurie G.[1]
[1]Section of Cell and Developmental Biology, University of California San Diego, La Jolla, CA 92093, USA
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Abstract:

The cuticle is the outer physical barrier of plants, establishing an important interaction interface with the environment. This hydrophobic layer consists of the lipid polymer cutin embedded with and covered by waxes, providing protection against environmental stresses like desiccation, UV radiation, and pathogen attack. Thickness, structure, and chemical composition of the cuticle vary widely among plant species, and even within a species, depend on organ identity, developmental stage, and growth conditions. The functional contribution of the maize cuticle to abiotic and biotic stress responses have been rarely studied so far. Moreover, the cuticle's impact on the adult plant, agronomically the most important growth phase, is largely unknown. We are characterizing the biogenesis of the adult leaf cuticle and its genetic basis, and aim to elucidate its impact on important agricultural traits. A first part of the project is the characterization of cuticle maturation along the adult leaf developmental gradient as measured by cuticle permeability and resistance to water loss. Changes in cuticle composition, analyzed by GC-MS, are mapped to the developmental gradient of the adult maize leaf. In a collaborative effort we are conducting an epidermal-specific transcriptomic analysis, which will be related to the compositional changes along the leaf. A second part of the project tries to identify the relationship of cuticle structure, composition and function of different epidermal cell types of the adult maize leaf. Ultrastructural data show distinct alterations in cuticular organization dependent on the cell type, which we want to relate to differences in cuticle composition to identify crucial components of cuticular function in these epidermal cell types.

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Plenary Session 2:

Title: Synchronized cell 'omics

Author(s) and affiliations: Daniela Strenkert, Sean D. Gallaher, Stefan Schmollinger & ***Sabeeha Merchant**

Institute for Genomics and Proteomics, UCLA, Los Angeles, USA

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Abstract:

We generated a multi-layered dataset, consisting of transcriptomic data and physiological measurements, over the course of a day during synchronized growth of the green alga *Chlamydomonas reinhardtii*. Cultures were grown in a flat panel bioreactor system subject to an alternating 12h-light / 12h-dark regime with corresponding change in temperature (28 °C / 18 °C) for highly reproducible and tight synchronization, as assessed by markers of DNA synthesis, resulting in 1 round of division (two-fold increase in biomass) each 24h. The use of ribo-depleted RNAs allowed us to capture organellar transcripts as well as nucleus-encoded polyA-minus RNAs (such as those encoding histones). We noted that nearly the entire genome (81% of all transcribed genes) is differentially expressed at one or more time points, and these could be clustered into major expression patterns describing the sequence of events during a day in the alga's life. A few exemplary patterns will be demonstrated. 1) Expression of genes for the proton-pumping complexes in the thylakoid membranes peaks before the expression of genes for the photosystems, 2) respiration and fermentation metabolism show clear diurnal separation, and 3) the PSBS and LHCSR components of photoprotection show distinct patterns of expression, suggestive of at least two inputs into the signaling pathways leading to their up-regulation.

Short Oral Presentations 2:

Title: Mitochondrial retrograde regulation initiated by sHSPs in the response of plants to heat stress

Author(s) and affiliations: ***Rhoads, David**
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Abstract:

A common responses of plants to abiotic stresses is altered nuclear gene expression. Specifically, plants attempt to alleviate the effects of heat stress by increasing expression of genes encoding heat shock proteins (HSPs), including HSP70s and small HSPs (sHSPs). Arabidopsis transgenic lines with constitutive expression of a transgene encoding a maize mitochondrial sHSP (mt sHSP), ZmHSP22, or the endogenous homolog, AtHSP23.6, were produced. The constitutively expressed mt sHSPs are directed to the mitochondria of the transgenic plants. These plants demonstrate increased thermotolerance compared to wild-type plants. Further, during heat stress, these plants exhibit super-induction of nuclear genes encoding sHSPs and HSP70s. Because the mitochondrial protein content is changed in the transgenic plants, this demonstrates that expression of HSPs can be affected by heat-stress-induced mitochondrial retrograde regulation initiated by sHSPs. This suggests that this form of regulation could be a source of signaling that contributes to the alteration of nuclear gene expression during heat stress in plants generally.

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Title: Anionic cerium oxide nanoparticles protect plants from abiotic stresses by engineering ROS-mediated physiological responses

Author(s) and affiliations: ***Honghong Wu**[1], Lana Shabala[2], Sergey Shabala[2], & Juan Pablo Giraldo[1,3]
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Abstract:

Plant abiotic stress leads to accumulation of reactive oxygen species (ROS) and a consequent decrease in photosynthetic performance. We demonstrate that a plant nanobionics approach of localizing negatively charged, sub-11 nm, spherical cerium oxide nanoparticles (nanoceria) inside chloroplasts *in vivo* augments ROS scavenging and photosynthesis of *Arabidopsis thaliana* plants under excess light, heat, dark chilling, and salinity stress. Poly (acrylic acid) nanoceria (PNC) with a hydrodynamic diameter (10.3 nm) - lower than the maximum plant cell wall porosity - and negative zeta potential (-16.9 mV) exhibit significantly higher colocalization (46 %) with chloroplasts in leaf mesophyll cells than aminated nanoceria (ANC) (27 %) of similar size (12.6 nm) but positive charge (9.7 mV). Nanoceria are transported into chloroplasts via non-endocytic pathways, influenced by the electrochemical gradient of the plasma membrane potential. PNC with a low Ce3+/Ce4+ ratio (PNC1, 35.0 %) reduce leaf ROS levels by 52 %, including superoxide anion and hydroxyl radicals, for the latter ROS there is no known plant enzyme scavenger. Plants embedded with these PNC that were exposed to abiotic stress exhibit an increase up to 19 % in quantum yield of photosystem II, 67 % in carbon assimilation rates, and 61 % in Rubisco carboxylation rates relative to plants without nanoparticles. In contrast, PNC with high Ce^{3+}/Ce^{4+} ratio (PNC2, 60.8 %) increase overall leaf ROS levels and do not protect photosynthesis from oxidative damage during abiotic stress. Under salinity stress, PNC1-Leaves infiltrated plant leaves showed one fold higher ($P < 0.05$) cytosolic K^+ intensity signals in leaf mesophyll cells relative to controls. Non-invasive microelectrode ion flux electrophysiological measurements indicate that PNC1-Leaves have about three folds lower NaCl-induced K^+ efflux from leaf mesophyll compared to controls. The ROS-activated nonselective cation channels in the plasma membrane of leaf mesophyll cells were identified as the main •OH–induced K^+ efflux channels. Overall, this study demonstrates that anionic, spherical, sub-11 nm PNC with low Ce^{3+}/Ce^{4+} ratio can act as a tool to study the impact of ROS mediated physiological processes including photosynthesis and mesophyll K^+ retention, and to protect plants from abiotic stresses.

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Title: Deciphering the Molecular Connection between Flowering and Age-Dependent Leaf Senescence in Arabidopsis thaliana

Author(s) and affiliations: ***Will Hinckley**, Keykhosrow Keymanesh & Judy Brusslan
California State University, Long Beach, 1250 Bellflower Blvd, Long Beach CA, 90840
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Abstract:

ATX (*ARABIDOPSIS TRITHORAX*) enzymes catalyze the trimethylation of H3K4, a histone tail mark associated with active gene expression. Higher order *atx* knockout mutants display additive early flowering and early senescence phenotypes. It is hypothesized that gene expression changes that occur at the time of flowering may signal the onset of senescence, temporally coupling the two developmental milestones. Two sibling homozygous *atx* triple mutants (*atx1/atx3/atx4*) were studied to define the molecular connection between flowering and senescence. A BrAD-seq (RNA-seq) time-course experiment compared expression of flowering *atx* triple mutants to vegetative wildtype plants of the same age. NOI-seq and MaSig-pro were used to interrogate data for genes showing significant temporal expression changes in triple mutants but not in wildtype. Results show *NRT1.7* (a nitrate transporter that is important in leaf senescence) and *SMR5* (cell cycle inhibitor) are induced in flowering triple mutants but not in vegetative wildtype. *GATA15*, which is responsible for leaf greening, was decreasing earlier in triple mutants than in wildtype. This indicates that flowering triple mutants were arresting vegetative growth and preparing for nitrogen recycling, an important component of leaf senescence. Genes with regulatory domains (*WRKY*, *MYB*, *AGL*, *ERF*) were selected and T-DNA insertion alleles for 21 genes were obtained and sown. Single mutants will be isolated and screened for flowering and senescence phenotypes. An altered phenotype that uncouples the timing of flowering and age-dependent senescence may indicate that a particular gene is contributing to the regulation of this temporal connection.

Keynote Address:

Title: The Nodule Microbiome: What We Learn from Nature, not from Dogma.

Author(s) and affiliations: P. Martínez-Hidalgo[1,a], M. Maymon[1], N. Khan[1], L. Briscoe[1], E. Humm[1], & **A.M. Hirsch**[1,2]*

[1]Molecular, Cell and Developmental Biology Dept.

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Abstract:

Legume root nodules are plant organs in which atmospheric nitrogen is converted to ammonia by rhizobia. Both alpha-rhizobia (*Rhizobiaceae*) and beta-rhizobia (*Burkholderiaceae*) fix N_2 in nodules that they initiate on legume roots. Discovery of the beta-rhizobia in legume nodules in 2001 is a well-known case of bacteria other than *Rhizobiaceae* inhabiting nodules. However, the "other" bacteria are even more diverse, and indeed a large percentage of them do not fix nitrogen. In the past, most of these nodule-housed microbes were considered contaminants and thrown away, but studies of legume nodule isolates worldwide have repeatedly shown that non-fixing microbes in addition to rhizobia make up the nodule microbiome. Many have plant growth-promoting (PGP) traits or biocontrol activity. To test the possibility that these non-rhizobia might be useful either singly or together with rhizobia as potential inoculants for enhancing crop production or for disease control, we isolated bacteria from nodules and performed co-inoculation experiments with rhizobia on roots grown in N-depleted medium. We also investigated the mechanisms whereby these non-rhizobial bacteria stimulate plant growth by sequencing their genomes and performing biochemical analyses of PGP activity. Together with nitrogen-fixing bacteria, nodule-isolated Plant-Growth Promoting Bacteria (PGPB) may be useful for furthering sustainable agriculture and for reducing soil contamination brought about by the overuse of chemical fertilizers and pesticides.

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Short Oral Presentations 3:

Title: Maize Terpene Synthases 6 and 11 are Required for Zealexin Production and Protection Against Multiple Pathogens

Author(s) and affiliations: ***Philipp Weckwerth**[1], Bing Yang[2], Eric Schmelz[1], & Alisa Huffaker[1]
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Abstract:

Acidic terpenoid phytoalexins, zealexins and kauralexins, are dominant inducible antimicrobial metabolites in maize (*Zea mays*) following the combined pressures of herbivory and microbial infection. Zealexins were first isolated and identified through efforts to characterize stalk rot (*Fusarium graminearum*)-induced defenses. Zealexins are predicted to be synthesized de novo by terpene synthases from farnesyl diphosphate precursors into the rarely encountered β-macrocarpene olefin and modified by a series of oxidative reactions mediated by cytochrome P450 enzymes. Zealexin accumulation is associated with increased expression of the terpene synthases *Tps6* and *Tps11*, which have been demonstrated *in vitro* to catalyze production of β-macrocarpene. Despite proposed relationships, it remains to be empirically demonstrated that the endogenous biosynthesis of zealexins requires the exclusive activity of Tps6/11. Moreover, while select zealexins demonstrate significant in vitro antimicrobial activity, a combination of genetic and biochemical evidence causally linking zealexins to pathogen resistance *in vivo* is required. Using CRISPR we created *tps6* and *tps11* double mutants with frame shift mutations yielding non-functional copies of both tandemly-arrayed genes. Recent analyses utilizing liquid chromatography mass spectrometry (LC/MS) established that maize *tps6/tps11* double mutants plants lack pathogen-induced zealexins. In contrast, synthesis of diterpenoid defenses, such as the *ent*-15-kaurene-derived kauralexins, remained unchanged. Plant-pathogen bioassays revealed impaired disease resistance of *tps6/11* double mutants compared to wild type Tps6/11 plants, as measured through increased symptoms and colonization following necrotrophic fungi (*Fusarium graminearum*) and also xylem-dwelling bacteria (*Pantoea stewartii*) challenge. Twelve years ago, Tps6/11 were demonstrated to be among the most strongly elicited transcripts in maize following pathogen attack and remain dominant inducible markers in a majority of studies. We now provide conclusive genetic, biochemical and physiological evidence that maize zealexin production requires Tps6/11 and mediates broad-spectrum defense against both fungal and bacterial pathogens.

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Title: Identifying Regulatory Steps in Vitamin B6 Biosynthesis

Author(s) and affiliations: ***Marcelina Parra**, Sutton Mooney, Dipika Jadav, & Hanjo Hellmann
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Abstract:

Vitamin B6 is a pivotal compound involved in over 140 biochemical reactions that affect processes such as metabolism, development, and stress sensitivity. Two enzymes, PDX1 (Pyridoxine Biosynthesis Protein 1) and PDX2, assemble together to form a vitamin B6 synthase. Through a series of reactions, these enzymes form the active vitamer of vitamin B6. Vitamin B6 biosynthesis is well understood, however, hardly anything is known about the regulation of this process. *PDX1.3* is one of three homologs of *PDX1* in Arabidopsis. Its loss results in *pdx1.3* mutant plants with shorter roots, delayed flowering, reduced chlorophyll content, and reduced vitamin B6 levels. To better understand regulatory steps in vitamin B6 biosynthesis, *pdx1.3* mutants were mutagenized a second time and screened for plants that developed again longer roots and have normal vitamin B6 levels. We hypothesized that such suppressor mutations of the pdx1.3 mutant phenotype likely represent regulatory steps in vitamin B6 biosynthesis. We found eight suppressor (*sup*) mutant lines. One of these mutants, *sup49*, has been mapped and cloned, and is affected in a protein of unknown function. Initial interaction studies showed that this protein can interact with PDX1.1 and PDX2. Although the exact impact of the mutation in *SUP49* is currently unclear, it appears to be that the mutated form has less affinity to interact with PDX2. Based on these observations we hypothesize that SUP49 is a novel, negative regulator of vitamin B6 synthase complex assembly and activity.

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Title: CYCLING DOF FACTOR 1 Uses TOPLESS to Concert Morning Repression of Photoperiodic Flowering Genes in Arabidopsis

Author(s) and affiliations: * Goralogia, Greg S., Liu, Tongkun., Zhao, Lin., Panipinto, Paul M., Groover, Evan D., Bains, Yashkarn S. & Imaizumi, Takato.
Department of Biology, University of Washington, Seattle, USA
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Abstract:

CYCLING DOF FACTOR 1 (CDF1) and its homologs play an important role in the floral transition by repressing the expression of floral activator genes such as *CONSTANS (CO)* and *FLOWERING LOCUS T (FT)* in Arabidopsis. The day-length specific removal of CDF1-dependent repression is a critical mechanism in photoperiodic flowering. However, the mechanism by which CDF1 represses *CO* and *FT* transcription was unknown. We present that Arabidopsis CDF proteins contain non-EAR motif-like conserved domains required for interaction with the TOPLESS (TPL) co-repressor protein. This TPL interaction confers a repressive function on CDF1, as mutations of the N-terminal TPL binding domain largely impair the ability of CDF1 protein to repress its targets. TPL proteins are present on specific regions of the *CO* and *FT* promoters where CDF1 binds during the morning. In addition, TPL binding increases when *CDF1* expression is elevated, suggesting that TPL is recruited to these promoters in a time-dependent fashion by CDFs. We show a reduction of TPL activity induced by expressing a dominant negative version of *TPL (tpl-1)* in phloem companion cells results in early flowering and a decreased sensitivity to photoperiod in a manner similar to a *cdf* loss-of-function mutant. Our results indicate that the mechanism of CDF1 repression is through the formation of a CDF-TPL transcriptional complex, which reduces the expression levels of *CO* and *FT* during the morning for seasonal flowering.

Plenary Session 3:

Title: Molecular Integration of Nutritional, Hormonal, and Environmental Signals in Plant Growth Regulation.

Author(s) and affiliations: *Zhi-Yong Wang
Department of Plant Biology, Carnegie Institution for Science, Stanford, CA 94305, USA
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Abstract:

Plant growth is controlled by a wide range of environmental signals as well as endogenous cues such as hormones and nutrients. How these environmental and internal signals are integrated into coherent growth decisions is a central question in plant biology. Using the Arabidopsis hypocotyl elongation as model system, we have dissected the molecular circuitry that controls cell elongation in response to major environmental and endogenous signals. At the center of this regulatory circuit is a module of interacting transcription factors, including the BR-activated BZR1 family transcription factors, the auxin response factor 6 (ARF6), the phytochrome-interacting factors (PIFs), and the GA-sensitive DELLA proteins. BZR, ARF, and PIF factors act cooperatively to promote cell elongation, while their DNA-binding activities are inhibited by the DELLA proteins. We named this interaction complex BAP/D module, for the synergy among BZR1, ARF6, and PIFs and their antagonism by DELLA. The BAP/D module elegantly explains how cell elongation is controlled by a wide range of signals: BR, auxin, and shade/darkness promote cell elongation by activating BZR1, ARF6, and PIFs, respectively, while GA induces degradation of DELLAs; sugar signalling through Target Of Rapamycin (TOR) stabilizes BZR1; warm temperature increase cell elongation by activating PIF4; the circadian clock controls PIF activity through both transcriptional regulation and direct protein-protein interaction with clock component TOC1. Downstream of the BAP/D module, a tripartite HLH-HLH-bHLH (HHbH) transcription factor module regulates the activities of PIFs and additional bHLH factors, including HBI1 which promotes growth but inhibits immunity. Many components of the BAP/D module are modified by O-GlcNAc, which is a conserved mechanism of cellular response to sugar and nutrient. As such, the BAP/D module coupled with the HHbH module forms a central molecular circuit that integrates nutritional, hormonal, and environmental signals in plant growth regulation.

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Short Oral Presentations 4:

Title: Jazzing up Plant Defense via Extracellular ATP Signaling

Author(s) and affiliations: ***Diwaker Tripathi**[1,2] & Kiwamu Tanaka[2]
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[2]Department of Plant Pathology, Washington State University, Pullman, WA, U.S. - 99164
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Abstract:

Plants produce damage signals upon being targeted by various environmental stressors such as pathogens. One of the damage signals, extracellular ATP, acts as a Damage Associated Molecular Pattern (DAMP) and activates downstream signaling via its receptor, P2K1 (a.k.a. DORN1). There is accumulating evidence suggesting the involvement of ATP in damage and plant defense responses. However, a direct link between extracellular ATP and plant defense signaling is missing. In the present study, we observed a synergistic effect of extracellular ATP with a plant defense hormone, jasmonic acid (JA) on plant resistance against a necrotrophic fungus, *Botrytis cinerea*. This resistance was enhanced in the *P2K1* overexpression line. In order to explore the role of jasmonic acid in this defense response, stability of a negative regulator of JA signaling, JAZ1, was analyzed upon ATP treatment. Our result showed a direct effect of extracellular ATP on the JA signaling during the plant defense. Interestingly, extracellular ATP induced COI1-JAZ1 interaction that leads to JAZ1 degradation without activation of JA biosynthesis. In addition, the ATP-induced JA signaling required formation of secondary messengers (calcium, reactive oxygen species, and nitric oxide) to facilitate JAZ1 degradation. This study provides a new direction to our understanding of defense signaling activated by DAMPs. Further research will be focused on more detailed investigations of ATP-JA signaling and on roles of other plant defense hormones such as salicylic acid and ethylene.

ASPB-Western Region Meeting 2018

Title: Analysis of Three-Dimensional Cell Shape to Study Division Plane Orientation in Plant Cells

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Abstract:

Proper division plane orientation is an important aspect of plant development. A mathematical modeling approach has been developed to understand the contribution of cell shape in predicting symmetric cell divisions. The mathematical model is based on two rules of symmetric cell division: the resulting daughter cells have equal volume and the resulting division follows the shortest local path. Cell shapes are reconstructed in three-dimensions and the surface is imported into Surface Evolver, a program which uses gradient descent to predict local minimal surface areas that divide the cell into two equal volume daughter cells. By comparing the location of the preprophase band to the mathematically calculated division planes, this approach has revealed that most symmetric cell divisions can be accurately predicted based on the geometry of the cell. This three-dimensional approach also predicts rare longitudinal or periclinal divisions at higher proportions than previously developed two-dimensional models. When applied at a population level, this model can be used to reveal the contribution of cell shape to division plane orientation at different developmental stages. This application has shown that cell geometry can account for most of the symmetric cell divisions seen in the maize leaf. This model is also being applied to mutants which display altered cell shape to determine if there are division plane establishment defects.

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Short Oral Presentations 5:

Title: Defense Hormone Transcriptional Profiling Reveals Novel Complexities in Cassava

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Abstract:

Outbreaks of superabundant whitefly populations throughout Eastern and Central Africa in recent years have dramatically increased the pressures of whitefly feeding and virus transmission on their host, cassava. Indeed, whitefly-transmitted viral diseases such as Cassava Mosaic Virus (CMV) and Cassava Brown Streak Virus (CBSV) continue to decimate African cassava yields, threatening the food security of millions. Considerable cassava yield losses due to such pests and pathogens evidences the need for both virus- and whitefly-resistant cassava lines for distribution to African small shareholder farmers. However, basic knowledge of the defense programs of non-model crops like cassava is lacking, limiting the characterization of cassava resistance mechanisms to whiteflies, viruses, and other pests/pathogens of cassava. Here, a collaborative effort between the African Cassava Whitefly Project (ACWP) groups at UCR and CIAT has generated the first defense-hormone-responsive transcriptomes in cassava. We have characterized the responses of the whitefly-susceptible cassava genotype Col2246 to the two major defense hormones, salicylic acid (SA) and jasmonic acid (JA), over a 24 h time course. Interestingly, comparison of the well-known SA and JA responses in Arabidopsis to those in the highly heterozygous tetraploid cassava has revealed marked complexities of these responses specific to cassava. These results provide evidence that defense programs in Arabidopsis may not always mirror those in crop species, and provide a baseline for characterizing the defense responses of cassava to yield-limiting pathogens and pests.

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Title: Lipidomics and Surface Tension of Lipids from Xylem Sap of Five Woody Angiosperm Species

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Abstract:

Angiosperms transport water under negative pressure without constantly creating gas bubbles that would disable their hydraulic system, the xylem. Xylem sap is saturated or sometimes supersaturated with atmospheric gas and contains surface-active molecules that can lower surface tension. Because surface tension plays a dominant role both for the formation and stability of gas bubbles in water, we studied the lipidomics of xylem sap from five woody angiosperm species chosen to represent major clades of angiosperm phylogeny, including magnoliids, fabids, malvids, lamiids, and campanulids. Dynamic surface tension characteristics of xylem lipids were studied for all five species using constrained drop surfactometry. Xylem sap from all species contained very similar compositions of lipids, including phospholipids (mainly phosphatidylcholine, phosphatidic acid, phosphatidylethanolamine, and phosphatidylinositol) and galactolipids, including both monogalactosyldiacylglycerol and digalactosyldiacylglycerol. Xylem sap concentrations of these lipids were also similar, except for *Liriodendron tulipifera*, which had much lower concentration than the other four angiosperms. Lipids extracted from xylem sap of all species showed similar strong dynamic surface activity, ranging from about 20 mN / m to approaching the surface tension of pure water, depending on the lipid concentration at the gas-water surface. We hypothesize that xylem lipids in angiosperms act as surfactants that reduce the sizes of any nanobubbles that do form by lowering surface tension, thereby keeping the bubbles below the critical size at which they would expand under negative pressure to form embolisms. Xylem sap lipids therefore may be crucial for transporting water by the cohesion-tension mechanism.

Plenary Session 4:

Title: Remodeling of the nuclear architecture by light signaling during photomorphogenesis.

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Abstract:

Plant genomes are extremely sensitive to and can be developmentally reprogrammed by environmental light cues. Increasing evidence in yeast and mammalian models suggests that environmental cues can regulate gene activity by altering the spatial positioning of individual genes within the nucleus. However, if and how the spatial positioning of individual genes contributes to gene regulation in plants is still poorly understood. We have developed a method to label the positioning of individual genes by utilizing rolling-circle amplification of gene-specific circularizable oligonucleotides coupled with fluorescence in situ hybridization. Using this approach, we demonstrate that light triggers a rapid repositioning of the light-inducible *CAB1* locus in Arabidopsis from the nucleoplasm to the nuclear periphery during its transcriptional activation. *CAB1* repositioning is mediated by the red/far-red photoreceptors, the phytochromes, and is inhibited by the repressors of phytochrome signaling, including COP1, DET1, and PIFs. *CAB1* repositioning appears to be a separate regulatory step occurring prior to its full transcriptional activation. Moreover, the light inducible loci *RBCS*, *PC*, and *GUN5* undergo similar repositioning behavior upon their transcriptional activation. Our results support a novel gene regulatory mechanism in which phytochromes activate light-inducible loci by relocating them to the nuclear periphery. This study provides the initial evidence for the biological importance of gene positioning in plants and shows that plant genes can be spatially reorganized in response to environmental cues.

Poster Presentations

Poster Session 1 (Odd) – Poster Session 2 (Even)

P-1: ANALYSIS OF *tan1* MUTANT DIVISION PLANES WITH MATHEMATICAL MODELING

***Jocelyne Aranda** [1], Pablo Martinez[1,2], Lindy Allsman[1,3], & Carolyn Rasmussen[1,3]

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During plant cell division the division site is established by the preprophase band (PPB), which serves as a marker for future cell wall placement. TANGLED1 protein is a microtubule binding protein that is present throughout all of division and has been shown to colocalize to the PPB, a microtubule structure that marks the predicted division plane. The tangled1 (tan1) mutant in Zea mays exhibits a division plane defect due to the fact that the cell wall does not always return to the position initially marked by the PPB. As a result, tan1 mutant cells have altered cell shapes. Symmetric cell division is described as two daughter cells containing equal volumes. Given the altered cell shape of tan1 it is difficult to assess whether the PPB is placed according to the observed properties of symmetric plant cell division. I used mathematical modeling to determine if symmetric division planes are established according to cell geometry. In addition, using the math model I want to determine whether tan1 mutant cells are able to predict all typical division types (transverse, longitudinal, periclinal, and alternate), which refers to the placement of the new cell wall. Data collected from tan1 was compared to wild-type, in the same developmental stage, in order to observe the differences in PPB accuracy, possible predictions, and eigenvalue ratios. The results exhibited that tan1 had distinct shapes, such as tan1 cells being more cubic than wild-type cells and placement of the symmetric division plane was less accurate in tan1 than wild-type. Longitudinal divisions, while predicted by the model, are rarely observed in maize leaf samples; however, these divisions are expected given the developmental stage. The data displayed that there is no geometric inhibition pertaining to specific division planes even though the cell shape is able to predict longitudinal divisions, and thus leads us to believe that TAN1 (directly or indirectly) affects these different divisions.

P-2: IDENTIFYING CRITICAL UBIQUITIN LINKAGES IN POLYUBIQUITIN MODIFICATION OF PROTEINS TARGETED BY P0, A VIRAL SUPPRESSOR OF RNA SILENCING

***Nathan Bui**, ***Elaine Nguyen**, & Melanie A. Sacco

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Turnip yellows virus (TuYV) and *Potato leaf roll virus* (PLRV), members of the *Polerovirus* genus, use the protein P0 as a viral suppressor of RNA silencing (VSR) to degrade ARGONAUTE1 (AGO1), a key component of the RNA-induced silencing complex (RISC). P0 expression in plants has been shown to be associated with increased K48-specific polyubiquitination of host protein levels, implicating the proteasome-mediated degradation pathway. However, K63-specific polyubiquitination was detected for AGO1 and its degradation was blocked by 3-methyladenine, an inhibitor of the autophagic pathway. To understand these conflicting observations, we are investigating further the roles of K48- or K63-polyubiquitination in P0 protein-mediated degradation of AGO1 or other host proteins through site-directed mutagenesis of the *Solanum lycopersicum* ubiquitin protein (SlUb). A series of polymerase chain reactions generated K48A, K63A, K48A/K63A SlUb constructs, as well as a K_{Null} construct in which all seven ubiquitin lysine residues were mutated to alanine. Mutant SlUb constructs were ligated into the binary expression vector pBTEX with amino-terminal Flag- or HA-epitope tags. The mutants will be co-expressed with P0 and green fluorescent protein (GFP) in *Nicotiana benthamiana* to visualize RNA silencing. Detection of HA:ubiquitin or Flag:ubiquitin-tagged proteins will be conducted via immunoprecipitation and immunoblot analysis, in the presence and absence of autophagy and proteasome inhibitors. We hypothesize that these experiments will shed light on which polyubiquitin linkages are necessary for P0-mediated elimination of host proteins and provide insight into the virulence functions of P0 in Polerovirus-host interactions.

P-3: THE KARRIKIN RESPONSIVE GENE *KUF1* REGULATES SEEDLING GROWTH AND KARRIKIN RESPONSES IN *ARABIDOPSIS THALIANA*

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Karrikins (KARs) are abiotic signals found in smoke that promote germination in a wide variety of land plants and can also enhance photomorphogenic responses. *KARRIKIN UPREGULATED F-BOX 1* (*KUF1*) is a commonly used transcriptional marker of KAR responses in seeds and seedlings, but its function is unknown. To investigate *KUF1* function, we created loss-of-function *kuf1* alleles in *Arabidopsis thaliana* through CRISPR-Cas9 mutagenesis. Under red light conditions, *kuf1* mutants have a short hypocotyl phenotype. Moreover, *kuf1* seedlings are hypersensitive to KAR treatments but have normal responses to *rac*-GR24, a strigolactone analog mixture that contains both KAR-like and strigolactone-like components. These observations contradicted our hypothesis that *KUF1* may be a mediator of KAR responses, and instead suggest that *KUF1* may be part of a negative feedback mechanism that attenuates KAR responses. We are performing an in-depth characterization of *KUF1* to examine its interaction with genes in karrikin and light signaling pathways, and searching for the protein(s) that SCF targets for polyubiquitination and degradation.

P-4: FORWARD GENETICS APPROACH USING EMS LEADS TO IDENTIFICATION OF MUTANTS SHOWING DIVISION PLANE DEFECTS IN MAIZE

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Division plane orientation of plant cells is important for proper growth and development. The orientation of the division plane determines how cells are aligned with respect to one another. Since plant cells cannot migrate and do not move relative to each other, the orientation of the division plane within a cell, apropos to neighboring cells, and also within the context of the whole plant, are necessary factors in establishing proper plant cell patterning. Our lab used ethyl methanesulfonate to induce mutations and then screened mutants for defects in division plane orientation. Glue impressions of leaves were taken at young and adult stages of development to check if the misorientation of cell wall placement was rectified by cell expansion. A total of 159 mutants were identified and were separated into phenotypic categories. Mutants with defects in cytokinesis had cell wall stubs in addition to division plane defects and accounted for 0.4%. Mutants with asymmetric and/or symmetric division plane defects accounted for 9.2%. Finally, mutants with apparent expansion defects, called warty mutants, were found in 0.13% of the families screened. Mutants were backcrossed to B73 and fluorescent protein marker lines to investigate cell division defects. These mutants will be analyzed for division plane orientation defects, and the most interesting will be sequenced to determine which genes are altered. Our goal is to identify new proteins required for division plane orientation.

P-5: CHARACTERIZING THE FUNCTION OF KINECTIN IN PLANTS VIA *ZEA MAYS* AND *ARABIDOPSIS THALIANA*

Marschal Bellinger, Sukhmani Sidhu, Sareen Leon, Jocelyne Aranda, Lindy Allsman, Bing Yang, Carolyn Rasmussen

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Establishment and maintenance of the division plane is essential for proper growth and development in multicellular organisms. Tangled1 (Tan1) is an essential component of this process, in plants, which localizes to the future division site. Mutants of tan1 have disorganized cell architecture that leads to rough leaf texture and short stature in Zea mays. These mutants have proven to be a valuable tool for understanding cell division plane orientation mechanics. A yeast-2 hybrid experiment identified Kinectin1 (Knn1), an integral endoplasmic reticulum (ER) protein and motor protein regulator in animals, as a candidate Tan1 interacting protein. We chose to investigate Knn1 for its potential role in the division plane orientation interactome and contributions to plant growth. Our hypothesis is that Knn1 is an ER localized protein that contributes to proper growth and development through Tan1 interaction. Various new alleles of Knn1 were generated in Zea mays using CRISPR/Cas9. Homozygous loss-of-function knn1 single mutants have apparently normal ER morphology with no obvious cell division plane orientation defects in Zea mays. Knn1 appears to localize to the ER in Arabidopsis thaliana. RNAseq data together with confocal microscopy of transformed Arabidopsis (Col-0) carrying a native promoter, C-terminal YFP, fusion reporter gene provides evidence that Knn1 accumulates to a higher degree in the earliest differentiating cell types of the root, such as the endodermis and maturing xylem. Our findings suggest that Knn1 has a role in plant growth. Preliminary data provides clues for the spatial and temporal positioning of potential Tan1 interaction, in Arabidopsis.

P-6: GERMACRENE SYNTHASE GENE EXPRESSION PATTERNS IN HEAT STRESSED SUNFLOWER (*HELIANTHUS ANNUUS*)

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The burning of fossil fuels is one of the major causes of rising levels of atmospheric carbon dioxide. The consequence of elevated levels of carbon dioxide contributes to global warming and has potentially a damaging effect on crop productivity. The sunflower (***Helianthus annuus***) is an economically important crop due to the fact that of its many uses such as vegetable oil for food and holds the potential of its oil that can also be transesterified to produce biodiesel for fuel. During heat stress, certain genes are expressed and regulated. This project involves understanding expression patterns of germacrene synthase gene in sunflower tissues while being compared to a housekeeping gene, the elongation factor (ELF) in mature sunflower plants. Germacrene synthase is an enzyme involved in production of a sesquiterpene, namely, germacrene and is produced by plants under various abiotic stressful conditions. According to preliminary data analysis of qPCR using the Livak Method, we can conclude that the germacrene synthase gene is upregulated under heat stress. The information from these studies can help researchers better understand molecular plant physiology of sunflower plants under heat stress. The future direction of this project will involve RNAseq to compare gene expression in heat stress versus control plants.

P-7: EXPRESSION ANALYSIS OF HEAT STRESS INDUCIBLE GENES IN *PAULOWNIA ELONGATA*

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Global Warming causes the accumulation of heat stress in plants, leading to decreased global crop productivity and food security. Selection and development of heat tolerant crops require a deeper understanding of genetic and molecular mechanism underlying the heat stress defense pathways. *Paulownia elongata* are fast growing hardwood trees present throughout the world. Thus they are exposed to a wide variety of extreme temperatures. The primary objective of our study was to analyze and understand the ability of these trees to handle the heat stress, particularly as global warming continues to rise. A global transcriptomic analysis was done using the RNA-seq approach on the leaves of control and heat stressed *P.elongata*. A total of 2438 genes were identified to have a differential gene expression under heat stress. Heat stress kills the plants mainly by decreasing its photosynthetic ability. Most of the photosynthetic compounds are heat-susceptible and so are easily affected Most of the genes that were upregulated in *P.elongata* during heat stress were directly or indirectly involved in supporting photosynthesis during stress. This includes (i) the heat shock protein (HSP) family such as HSP 101,21, 22,23.6 etc. that protect photosystems and act on all phases of heat tolerance response such as induction, regulation and recovery, (ii) Magnesium chelatase a chloroplast enzyme that catalyzes the photosynthetic pathway and (iii) PPDK a photosynthetic enzyme regulating the C4 cycle in plants. These results can be useful to screen and breed the heat tolerant plant genotypes thereby restoring the lost crop productivity. To the best of our knowledge, this research on *Paulownia elongata* is brand new and has not been studied before.

P-8: RECEPTOR LIKE PROTEINS (RLPS) REGULATE LEAF SENESCENCE IN *ARABIDOPSIS THALIANA*

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Leaf senescence is observed when plants lose their green pigmentation and nutrients are recycled from older to newer leaves and developing tissue. *RLP7* (receptor-like protein 7) is a K4-SURG, a gene that has an increase in mRNA levels accompanied by an increase in the H3K4me3 activating histone mark in older leaves. These marked genes may have an important function during senescence. In previous research, *RLP7* was observed to be highly expressed at the start of senescence (Wang et al., 2008); it is hypothesized that it signals the plant to undergo senescence, and this project has shown that RLP7 is a positive regulator of leaf senescence. One T-DNA insertion (SALK_123145; *rlp7-1*) was selected to disrupt *RLP7*, and genomic and gene expression analysis demonstrates that *rlp7-1* is a null allele. The null allele was evaluated for senescence by measuring chlorophyll levels and gene expression during senescence, and was compared to wild-type to determine if there were any significant differences. *rlp7-1*had higher chlorophyll levels and lower SURG gene expression than WT. *RLP6* is similar to *RLP7* (with 83% similarity) and is hypothesized to also be a positive regulator of senescence. Two T-DNA insertion alleles, *rlp6-1* (SALK_080898) and *rlp6-2*(SAIL_84_E01) will be evaluated for senescence phenotypes, and an *rlp6/rlp7* double mutant will be produced.

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P-9: TRANSCRIPTOME ANALYSIS OF FLESHY AND DRY FRUIT DEVELOPMENT IN SOLANACEAE

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Many animal species, including humans, depend on fleshy fruits for nutrition. Fleshy fruits have evolved numerous times during the evolution of angiosperms. However, we do not know the molecular mechanisms responsible for these evolutionary events. In Solanaceae (nightshades), there was a shift to fleshy fruit in the sub-family Solanoideae from the ancestral dry capsule. The availability of multiple sequenced genomes, as well as well-developed molecular tools for Solanaceae, provides an excellent opportunity to study the molecular basis of fleshy fruit evolution. As part of a larger project aimed at elucidating the genetic architecture that distinguishes fleshy fruit from dry fruit, we compared the transcriptomes of fleshy tomato (*Solanum lycopersicum*), its closest wild-relative (*S. pimpinellifolium*), and dry-fruited desert tobacco (*Nicotiana obtusifolia*). Relatively few studies have looked at fleshy fruit development prior to ripening but studies have shown that key features such as shape, size, and pericarp thickness are determined at those early stages. Therefore, we performed transcriptome profiling at early and late stages of fruit development. We compared the transcriptomes of both tomato species to desert tobacco to elucidate how gene networks may have changed in the shift to fleshy fruit. The transcriptome of *S. pimpinellifolium* allows us to identify elements of the genetic networks that may have undergone changes during domestication. Our RNA-Seq analyses will illustrate fleshy fruit molecular dynamics on both developmental and evolutionary timescales.

P-10: INVESTIGATING THE EFFECTS OF ABA AND PHOSPHORYLATION ON THE STABILITY OF ABF TRANSCRIPTION FACTORS IN *ARABIDOPSIS THALIANA*

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The ABA Responsive Element Binding Factor (ABF) family of transcription factors plays an important role in abscisic acid (ABA) signaling during vegetative growth. Under conditions of elevated ABA, ABF proteins induce transcription of ABA-responsive genes to enhance a plant's ability to respond to abiotic stresses. We are interested in how ABA leads to an increase in ABF activity. Previous studies in our lab showed that the degradation of ABF1 and ABF3 proteins is slowed after seedlings are treated with ABA (Chen 2013). However, the link between ABF stability and changes in activity has yet to be established. To better understand how ABA activates ABFs and whether protein stability plays a role, we are studying the degradation and post-translational regulation of ABF1 and ABF2 in *Arabidopsis thaliana* using both *in vitro* and *in vivo* methods. It has been shown that ABFs are phosphorylated at four conserved serines or threonines in an ABA-dependent manner, and this phosphorylation is important for ABF activity (Furihata 2006). Our current experiments focus on determining whether these phosphorylations play a role in ABF protein stability. We are using recombinant ABFs with substitutions at these sites to study ABF degradation *in vitro*, and plants expressing the substituted ABFs to study degradation *in vivo*. Results from these experiments will provide insight into the mechanisms by which ABA induces ABF activity, and the role of proteolysis in ABF activation.

P-11: FUNCTIONAL CHARACTERIZATION OF THE NITRATE-INDUCED GLUTAREDOXINS IN *ARABIDOPSIS THALIANA*

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Glutaredoxins are small redox enzymes that use glutathione as a substrate to reduce disulfide bonds in target proteins. Plants have a far greater number of glutaredoxins than other organisms, mostly due to a unique clade of class III glutaredoxins that are exclusively found in higher plants. Previous studies in our lab demonstrated that a small group of class III glutaredoxin genes are strongly upregulated by nitrate in *Arabidopsis thaliana*, and that reducing expression of these nitrate-regulated glutaredoxins leads to increased primary root growth. Thus, glutaredoxins appear to link nutrient sensing with plant growth and root system architecture. To further explore this hypothesis, we are generating two groups of transgenic plants that 1) constitutively overexpress several nitrate-induced glutaredoxins and 2) completely inactivate targeted glutaredoxins via CRISPR-Cas9 technology. The coding sequence of the *AtGRXS5*, *AtGRXS6*, and *AtGRXS8* genes have been sub-cloned into an expression cassette and used to transform A. thaliana. Preliminary results suggest that high-level constitutive overexpression of these genes causes a dwarf phenotype in transgenic plant lines, again implicating the nitrate-regulated glutaredoxins as important regulators of plant growth. Work with CRISPR-Cas9 knockout lines is at an earlier stage, but CRISPR vectors targeting *AtGRXS6* and the *AtGRXS3/4/5/7/8* gene cluster have been constructed and utilized for plant transformation. We are currently screening these transgenic plant lines to identify knockouts in the target gene(s). Because the nitrate-regulated glutaredoxins appear to be important regulators of primary root growth, this work could have significant broader implications related to important agricultural traits such as root depth (drought tolerance) and nitrogen use efficiency.

P-12: DEFINING THE EXPRESSION DOMAINS OF THE ARABIDOPSIS GLUTAREDOXIN GENES *ATGRXS5*, *ATGRXS6*, AND *ATGRXS8*

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Glutaredoxins are oxidoreductase enzymes that can regulate the activities of target proteins through the reversible breakage of disulfide bonds. We recently identified a group of seven glutaredoxin genes that are upregulated by nitrate and control primary root length in the model plant *Arabidopsis thaliana*. The objective of this study was to characterize the gene expression patterns and subcellular localization of three nitrate-regulated glutaredoxins: *AtGRXS5*, *AtGRXS6*, and *AtGRXS8*. Reporter lines consisting of the glutaredoxin promoter sequences fused to the β- glucuronidase (GUS) gene were used to study glutaredoxin gene expression. Colorimetric GUS assays and histological sectioning showed that *AtGRXS5*, *AtGRXS6*, and *AtGRXS8* are expressed exclusively in the phloem of A. thaliana roots and leaves. GUS activity assays demonstrated that *AtGRXS6* and *AtGRXS8* are strongly and specifically upregulated by nitrate. In contrast, *AtGRXS5* gene expression was activated by both ammonium and nitrate. Nitrate concentrations as low as 50 µM activated glutaredoxin gene expression, with no further increase in expression at higher concentrations of nitrate. To characterize the subcellular localization of *AtGRXS5*, *AtGRXS6*, and *AtGRXS8*, translational fusions between the glutaredoxin gene and yellow fluorescent protein (YFP) were transiently expressed in *Nicotiana benthamiana* leaves. Preliminary results demonstrated that all three glutaredoxins are localized to cell nuclei as well as small, as yet unidentified structures in the cytoplasm. Overall, these studies will further explain how plants control the growth of their root system to maximize the uptake of nitrogen from the soil, a behavioral response that is highly significant in agriculture.

P-13: NOVELTIES OF THE OLDEST LIVING-FRUIT: SACRED LOTUS *NELUMBO NUCIFERA*, VAR. CHINA ANTIQUE

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The Oldest Directly Radiocarbon-Dated Living-Fruit is a 1,300-yr-old Sacred Lotus, *Nelumbo nucifera* var. China Antique, excavated from a dry lakebed in NE China. Its genome is sequenced, annotation of which and its physiology show novelties worthy of mining for aging and survival. In particular, it has: 1) ~14 blue-light-photoreceptor cryptochromes (CRY) & 8 phototropins (PHOT), 27 CAB (Chl a/b binding proteins), 121 bHLH (basic-helix-loop-helix transcription factor), 9 TIC (Time for Coffee), 5 Bru1 (brassinodsteriod regulator), 635 PPR pentapeptide repeat (RNA editing/ processing), and 10 stress-abating Annexins up-regulated at germination and 90C; 2) A deep green embryo-axis developed in the dark, that contains a hefty amount of chlorophyll a/b preserved for hundreds and thousand years before germination; 3) Germinating seedlings shown able to perform dark photosynthesis; 4) Seed maturation that continues for at least 13 yr [preparation for longevity?]; 5) Abundant reduced-proteins at germination; 6) A hardy fruit coat pericarp, with ROS properties impermeable to water and air; and 7) Amount of total protein in the embryo axis that remains unchanged after 550 years, 30% of which exhibits thermal stability to 110C. The genomics, metablomics and proteomics of this plant await exploration.

P-14: BSU1 FAMILY PHOSPHATASES MEDIATE SIGNALING FROM THE IMMUNE RECEPTOR FLS2 TO MAP KINASES

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The first line of defense against pathogen attack relies on detection of pathogen-associated molecular patterns (PAMPs) by pattern-recognition receptors (PRRs) and their rapid signal transduction to activate innate immune responses. The plant PRR, FLS2 receptor recognizes bacterial flagellin (flg22) and forms receptor complex with receptor-like cytoplasmic kinases to trigger rapid and transient immune responses including activation of a MAP kinase cascade. However, the signaling mechanism by which FLS2 receptor activates the MAP kinases remains poorly understood. Here, we show that the growth-promoting brassinosteroid signaling components, BSU1 family phosphatases, mediate the signal transduction from the membrane-localized FLS2 receptor complex to cytosolic MAP kinases. Constitutive and inducible quadruple mutants of BSU1 family display compromised immune responses. Quantitative mass spectrometry analysis using metabolic stable isotope-labeled plants indicates that BSU1 is phosphorylated at serine 251 (S251) residue upon flg22 treatment. Yeast-two-hybrid assays show that BSU1 family proteins interact with multiple receptor-like cytoplasmic kinases of subfamily VIIs. *In vitro* and *in vivo* assays demonstrate that BIK1 kinase interacts with and phosphorylates BSU1. *In vitro* and immunocomplex kinase assays indicate that the BIK1 phosphorylation of BSU1 requires S251. Overexpression of BSU1 rescues the reduced flg22-induced MAP kinase activation of an inducible BSU1 family quadruple mutant. However, overexpression of a mutant BSU1 containing S251A substitution fails to do so. These results uncover a role of BSU1 family in signaling from FLS2 receptor complex to MAP kinases.

P-15: IDENTIFICATION OF *BURKHOLDERIA TUBERUM* MOTILITY AND EXOPOLYSACCHARIDE MUTANTS

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Intro: *Burkholderia tuberum* is a nodulating bacterium, known as a rhizobia, that creates symbiotic relationships with legume plants by causing the formation of root nodules. Bacteria in the root nodules fix atmospheric nitrogen in exchange for carbon compounds that the plant produces. The production of nitrogen through the nodule is thought to be a major contributor to the global nitrogen cycle. Up until 2001, known nodulating rhizobia were limited to only the Alphaproteobacteria class, thus *Burkholderia,* a Betaproteobacterium, has greatly expanded the knowledge of rhizobia. *B. tuberum,* STM678T, originated from South Africa from *Aspalathus carnosa. Burkholderia* contains two main groups, pathogenic and non-pathogenic species. Members of the non-pathogenic clade are able to fix nitrogen, nodulate legumes, and degrade aromatic compounds, and are thus of interest for their potential use as biofertilizers and for biotechnological purposes. **Objective:** The purpose of this research is to use transposon mutagenesis to identify genes in *B. tuberum* that are involved in the bacterium's association with plants. **Methods:** A 96-well plate assay was first optimized to screen bacterial mutants. Then, a pool of mutants was generated through introducing a Tn5-RL27 transposon gene into *B. tuberum.* The transposon mutants were screened for exopolysaccharide production (EPS) and motility, as both these processes have previously been implicated in plant-microbe interactions. **Results:** It was determined that agar worked better than liquid in 96-well plates for stamping cultures to screening media as the agar method was seen to have a higher percentage of colonies transferred. A total of 3,360 transposon mutants were screened. The motility test had inconclusive results therefore needing further testing. In addition, mutants were screened for nodulation using hydroponic boxes. **Conclusions:** Mutants of interest, that tested positive in EPS or motility, will be further characterized and the mutated gene identified. Specifically, molecular methods including DNA isolation, restriction digest, ligation, transformation, and sequencing will be used to identify where the transposon inserted.

P-16: IDENTIFYING GENES THAT ARE DIFFERENTIALLY EXPRESSED IN *NICOTIANA GLUTINOSA* DEFENSE RESPONSES AGAINST POLEROVIRUS INFECTION

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Plants defend themselves against infectious pathogens either through pre-existing defenses, such as physical barriers that prevent infections, or through inducible defenses that operate at the molecular level through immune receptors that are activated by pathogen elicitors. Examples of inducible responses to pathogens include extreme resistance (ER) in which the pathogen is eliminated without cell death, and hypersensitive response (HR), which limits the pathogen to the infected area by local programmed cell death. When infected with different members of the *Polerovirus* genus, two accessions of *Nicotiana glutinosa* displayed either HR or ER. *N. glutinosa* accession TW59 exhibited HR when infected with *Turnip yellows virus* (TuYV) as well as *Potato leaf roll virus* (PLRV). Accession TW61 exhibited HR only when infected by PLRV, but showed ER when infected by TuYV. To determine the gene expression changes in response to each virus, RNA was isolated at 36 hours before the visible onset of cell death from both uninfected leaves and leaves infected by TuYV or PLRV from the two accessions for analysis by Next Generation RNA-sequencing (RNA-seq). We hypothesize that there will be specific changes in gene expression associated with ER versus resistance accompanied by HR, as well as some shared gene expression changes for these responses. Genes of interest will be further tested for differential expression through real-time reverse transcription-quantitative polymerase chain reaction (RT-qPCR). We aim to identify candidate genes with overlapping roles in ER and HR or with unique roles in one defense pathway in order to obtain a better understanding of these separate but intersecting plant defense responses.

P-17: ANALYSIS OF THE CPH1 CRYPTOCHROME-BINDING PARTNERS IN *CHLAMYDOMONAS REINHARDTII*

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Chlamydomonas reinhardtii contains several RNA binding (RB) proteins that regulate the translation of *psbA* mRNA to the photosystem II-associated D1 protein. *rb38* and *rb60* are transcriptionally regulated by red light and Ca^{2+}/calmodulin (CaM), whereas *rb47* appears to be translationally regulated. The induction of the *rb* genes by red light and Ca^{2+}/CaM suggests that a phytochrome may be involved. Phytochromes are red-light photoreceptors deactivated by far-red light and found in cyanobacteria, green algae, fungi and vascular plants. Despite being identified in four kingdoms, a phytochrome has yet to be recognized in*C. reinhardtii*. In *A. thaliana,* phytochromes physically interact with plant-type cryptochromes in a light-dependent fashion. If *C. reinhardtii*possesses a phytochrome-like photoreceptor, then the phytochrome-cryptochrome interactions may also be conserved. We aim to isolate and identify the putative phytochrome through a pull-down assay using the*C. reinhardtii* plant-type CPH1 cryptochrome as bait. An inducible, tagged pRCPH1 construct was transformed into competent *E. coli* cells. Tagged CPH1 protein will be induced, purified, and used in an *in vitro*interaction assay with *C. reinhardtii* whole cell protein lysate under various light conditions. Binding partners will be visualized via an SDS-PAGE. If the interactions are conserved, we expect to pull down a phytochrome-like photoreceptor under dark conditions or far-red light. Any isolated proteins will be sent out for sequencing and a bioinformatics analysis will be performed. The identification of a novel photoreceptor will further elucidate the mechanism of red-light regulated gene expression in *C. reinhardtii.*

P-18: CLAVATA3-SIGNALING AND NUCLEAR EXPORT MAY CONTRIBUTE TO WUSCHEL GRADED ACCUMULATION IN ARABIDOPSIS SHOOT APICAL MERISTEMS

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How transcription factor levels are regulated and how they affect gene expression is critical in cellular and developmental processes. WUSCHEL (WUS), a homeodomain-containing transcription factor, is necessary for the maintenance of plant stem cells in shoot apical meristem (SAM). In Arabidopsis, WUS is synthesized in the niche (rib meristem-RM) below the stem cell domain (central zone-CZ) and migrates into overlying stem cells to form a gradient. Differential nuclear accumulation of WUS across cell layers is critical for its spatial patterning and regulation of CLAVATA3 (CLV3), a negative regulator of WUS. In clv3 mutants, lack of WUS repression leads to extremely high WUS accumulation in the RM but low levels in the most apical layer of the CZ. Precise regulation of WUS levels is important and recent work has shown that DNA-dependent dimerization, subcellular localization, and stability of WUS regulates WUS levels and gradient in the SAM. Additionally, extrinsic signals such as cytokinin or high levels of nuclear-localized WUS lead to increased stabilization of WUS. However, how extrinsic signals or subcellular partitioning contribute to the maintenance of WUS levels and its spatial patterning in the SAM is not well understood. Through transient manipulation of nuclear export function and CLV3 levels, followed by live-imaging of Arabidopsis SAMs to monitor fluorescently tagged WUS, we show that disrupting the process of nuclear export or reintroduction of CLV3-signaling into clv3 mutants are able to increase WUS levels in the outer layers. CLV3-signaling and loss of nuclear export could function to decrease net movement to the cytoplasm leading to increased nuclear levels of WUS in the outer layers which suggest a concentration-dependent stabilization of the protein.

P-19: WILD TYPE VS. OVEREXPRESSION OF *KINECTIN1* IN *ARABIDOPSIS THALIANA*

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Living organisms undergo cell division to help development and growth. TANGLED1 (TAN1), plays a central role in determining proper cell division plane orientation. The loss of function in tan1 mutants exhibit characteristics typical of cell division plane orientation defect mutants, which include crêped leaf texture, aberrant cell wall placement, and short stature. A central focus of our laboratory is to study TAN1 and potential TAN1 interacting proteins to understand the mechanisms of cell division plane orientation.

KINECTIN1 (KNN1) has been identified as a potential TAN1 interacting protein, in Arabidopsis thaliana (At). Previous research animals has shown that KNN1 orthologs localize to the endoplasmic reticulum (ER) during interphase. To understand the role of KNN1 in plants, my research project focuses on observing how KNN1 gene expression may impact cell division processes in At. Since KNN1 may interact with TAN1, my hypothesis is that KNN1 overexpression will alter plant growth and cell division in At. By comparing overexpression of the KNN1 gene to native expression through genotyping, I am able to see if the gene expression appears to be similar or different between the two. Observation of root growth rate, flowering rate, and shoot rate will show a phenotypic comparison between the KNN1 overexpression and the native expression to prove the effect of KNN1 gene expression in Arabidopsis.

P-20: LOSS OF FUNCTION *kinectin1* MUTANTS DEVELOP FASTER IN *ARABIDOPSIS THALIANA*

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Cell division occurs in different ways, but there are basic steps that need to be followed. Previous research has shown that TANGLED1 (TAN1), a microtubule-binding protein, plays an important role for the establishment of proper cell division plane orientation, in plants. Through the use of plants as model systems, the study of cell division plane orientation can be thoroughly analyzed throughout many rounds of division in a tissue. Loss of function tan1 mutants in maize exhibit a small stature with crepe-like leaf texture and aberrant cell wall placement, indicative of cell division plane orientation errors during mitosis. Our laboratory identified KINECTIN1 (KNN1), as a potential TAN1 interacting protein. Putative loss-of-function mutants for KNN1 exist in *Arabidopsis thaliana* via the TDNA insertion library at the SALK institute in San Diego, California. Although the kinesin interaction was found in animal systems, kinesin interaction with kinectin in plants has not been studied yet. We seek to understand how KNN1 contributes to cell division mechanisms and division plane orientation in *Arabidopsis thaliana*. As TAN1 plants show growth defects and KNN1 may interact with TAN1, our hypothesis is KNN1 plays a role in the root growth of Arabidopsis. Results from root growth analysis of homozygous TDNA insertion, shows KNN1 mutants have longer root length and faster rate of growth compared to wild type. Our data supports KNN1 loss of function knn1 grows at a faster rate. By staining the mutant cell walls with propidium iodide, KNN1 does not play a role in division plane orientation on its own, but interactions with TAN1 may affect the proper placement of division site. Next I will study the double mutant (*knn1, tan1*) to test for genetic interaction.

P-21: THE HAC1 HISTONE ACETYL TRANSFERASE REGULATES LEAF SENESCENCE: IDENTIFICATION OF HAC1 TARGETS AND REGULATORY ERF TRANSCRIPTION FACTORS

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Leaf senescence is the final stage of leaf development during which nitrogen and other nutrients are recycled to growing and storage organs. HAC1 (At1g79000) is a histone acetyl-transferase from the CREB Binding Protein (CBP) family. In older leaves, a significant decrease in chlorophyll levels in WT as compared to two *hac1* mutant lines implied HAC1 as playing a key role in epigenetic regulation of leaf senescence. Analysis of RNA-seq and H3K9ac ChIP-seq data, which were performed on leaves 12-14 of 49 day-old plants, resulted in a list of 44 genes which were both upregulated and gained acetyl levels in WT plants when compared to the two hac1 mutant alleles. Gene ontology analysis showed enrichment for defense biological process, which is expected for senescence associated genes. Motif enrichment analysis based on the ChIP-seq data determined a GCC-core element at the summits of H3K9ac peaks. The GCC-core is a known binding site for Ethylene Response Factors (ERF) family members. Med25 (At1g25540) is a subunit of the mediator transcriptional complex which binds to some ERFs and is a promising candidate to assess the documented relationship of HAC1 and ERFs in leaf senescence. Later bolting dates and higher chlorophyll levels of both *hac1* alleles and the *med25* mutant (as compared to WT) supported the participation of ERFs in the regulation of senescence through histone acetylation. To support this relationship, *erf5*, *erf6* and *erf72* mutants were selected as potential regulators of leaf senescence. These positive regulatory ERF genes were chosen based on their expression level and the expression fold-change during leaf senescence.

P-22: CROSS-SECTION OF POLYADENYLATION AND M6A RNA METHYLATION: THE ROLE OF CPSF30-YT521B

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Polyadenylation and N6-methyadenosine (m6A methylation) represent two of the most abundant post-transcriptional modifications of mRNA. In *Arabidopsis thaliana*, mRNA polyadenylation is mediated by a highly conserved complex of proteins including the 30-kD cleavage and polyadenylation specificity factor (CPSF30). The activities of CPSF30 were studied previously in a mutant without CPSF30 expression known as *oxt6*. There is an alternatively spliced 68-kD protein produced from the same gene termed CPSF30-YT521B, a YTH-domain containing protein. The YTH domain, like in this fusion protein and others, has been shown to be involved in m6A methylation. In a rat homolog, it was found that the YTH domain of YT521-B binds at higher affinity when a sequence of RNA is m6A methylated. Because of this, the YTH domain is known as an m6A reader. We studied the relationship of m6A methylation and polyadenylation in the *oxt6* mutant using m6A antibody enriched poly(A) tag sequencing which we have called ME-PAT-seq. With the m6A enriched dataset, we see minor differences in our suite of bioinformatics tests. Due to the different population of genes from the enrichment, there were more differences in differentially expressed genes and in the frequency poly(A) site usage or gene site switching. CPSF30-YT521B is a novel protein that represents a cross-road of polyadenylation and m6A RNA methylation that has not been previously studied. This study seeks to bridge that gap.

P-23: DEFINING THE ROLE OF THE *ARABIDOPSIS THALIANA ALTERNATIVE OXIDASE* GENE FAMILY IN MITOCHONDRIAL PRODUCTION OF REACTIVE OXYGEN SPECIES

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The mitochondrial electron transport chain (ETC) plays a central role in the bioenergetics of all eukaryotic cells. The ETC is also a major source of reactive oxygen species (ROS), a byproduct of electron transport. In humans, ROS-associated damage has been linked to aging and neurodegenerative disorders, and it is important to understand how ROS production is managed by different organisms. Plants have several "alternative" pathways for electron flow in their ETC which are not found in animals. This research focuses specifically on characterizing the function of the alternative oxidases (AOX), which have been proposed to help minimize the production of ROS in plants. To better understand the connections between AOX and ROS production, we generated a series of transgenic *Arabidopsis thaliana* plants using two RNA interference vectors designed to silence the entire AOX gene family (five genes). Real time RT-PCR analyses demonstrated significantly reduced levels of AOX transcripts in several transgenic plant lines, and *in vivo* respiratory assays showed significantly reduced AOX capacity in those same lines compared to wild-type. We then used these elite transgenic lines to gain insight into how decreasing expression of alternative oxidases affects ROS levels and ROS-associated damage in plants. Hydrogen peroxide, a type of ROS, was quantified in plant leaf extracts, and significantly higher levels were measured in AOX-silenced plants than in wild-type plants. Lipid peroxidation, a type of oxidative damage, similarly showed elevated levels in AOX-silenced plants. Overall, these findings suggest that AOX does play an important role in managing ROS levels and ROS-related damage in plants.

P-24: NITRATE-INDUCED GLUTAREDOXINS DIRECTLY INTERACT WITH THE TGA1 AND TGA4 TRANSCRIPTION FACTORS, CONTROLLING PRIMARY ROOT GROWTH IN *ARABIDOPSIS THALIANA*

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Plant growth and development is often limited by nitrogen availability in the soil. We recently demonstrated that a group of class III glutaredoxin genes in *Arabidopsis thaliana* are strongly and specifically upregulated by nitrate, and act as negative regulators of primary root growth. Glutaredoxins are small disulfide oxidoreductase enzymes which can modify the structure and activity of target proteins. In order to gain a better mechanistic understanding of the glutaredoxin signaling pathway that controls primary root growth, we performed yeast two-hybrid assays using the *AtGRXS5* and *AtGRXS6* genes as baits. Both glutaredoxins displayed strong interactions with the transcription factors TGA1, TGA3, and TGA4. Notably, TGA1 and TGA4 had been previously identified as important regulators of nitrate response in Arabidopsis. Root growth assays performed on TGA transcription factor mutant lines demonstrated that the TGA1, TGA4, and TGA1/4 mutants all had significantly shorter primary roots than wild-type plants. In addition, preliminary bimolecular fluorescence complementation (BiFC) data supports the GRX:TGA1/4 interaction, and localizes it primarily in the nucleus. Collectively, these studies suggest that TGA1 and TGA4 act to promote primary root growth, and that these proteins are inactivated by a direct interaction with AtGRXS5 and AtGRXS6. Crosses between the TGA1/4 mutant (short root phenotype) and glutaredoxin-silenced transgenic lines (long root phenotype) are currently being analyzed, and will further clarify the biological significance of the GRX:TGA1/4 signaling interaction. More broadly, it is clear that glutaredoxins and TGA transcription factors play central roles in tailoring root system architecture to nitrate availability in the soil.

P-25: NANOBIOTECHNOLOGY APPROACH TO STUDY CHLOROPLAST GENERATED ROS REGULATION OF PLANT ABIOTIC STRESS GENE CLUSTERS

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Abiotic stresses have a negative impact on plant growth, development, and yield. A hallmark of abiotic stress is the generation of reactive oxygen species (ROS). ROS including superoxide and hydrogen peroxide (H_2O_2) have a dual role in plant cells. At high levels, ROS act as damaging molecules to plant cellular components whereas at low concentrations, molecules have been proposed to act as signaling molecules communicating organelles such as chloroplasts and nuclei (retrograde signaling). ROS are thought to have specific effects on plant responses depending on their subcellular origin. To better understand chloroplast generated ROS signaling, we use a novel approach based on functionalized nanoparticles to target specific subcellular organelles for inducing or reducing ROS levels. Herein, we describe a dual nanoparticle-based system to control ROS levels within specific leaf organelles of Arabidopsis thaliana plants to understand chloroplast H_2O_2retrograde signaling mechanisms and determine the abiotic stress genes markers affected by this ROS. Understanding the complex molecular crosstalk between the chloroplast and nucleus during abiotic stresses is essential for improving management and propagation of stress-tolerant crops.

P-26: USE OF A *tan1* MUTANT ENHANCER FOR FUNCTIONAL CHARACTERIZATION OF TANGLED1 AND AIR9 IN *ARABIDOPSIS THALIANA*

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TANGLED1 is a microtubule binding protein shown to play an important role in cell division orientation in maize. *tangled1* mutants in maize have a phragmoplast guidance defect. Despite forming structurally normal preprophase bands (PPB) and phragmoplasts, the phragmoplast fails to move to the division site originally established by the PPB. The study of TANGLED1 in *Arabidopsis thaliana* has been inhibited by a very weak *tan1* phenotype where the phragmoplast guidance defect is often minor or absent. AIR9 (auxin-induced in root cultures) is a microtubule binding protein that associates with the PPB and phragmoplast, and *Arabidopsis air9* mutants have no discernable phenotype. However, *Arabidopsis tan1 air9*double mutants possess a synergistic phenotype displaying altered cell file rotation, root growth, and division plane orientation. These mutant phenotypes were almost completely rescued by transforming the double mutant with full length TAN1 fused to yellow fluorescent protein (*TAN1-YFP*). Additionally, transformation with *TAN1-YFP* deletion constructs missing the first 126 amino acids (*TAN1-ΔI-YFP*) did not rescue the mutant phenotype, while transformation with *TAN1-YFP*missing amino acids 126-222 (*TAN1-ΔII-YFP*) rescued root growth and division plane orientation, but not cell file rotation. The synthetic phenotype of the *tan1 air9* double mutant sheds light on the roles of individual TAN1 regions, and will be a powerful tool for assessing the roles of TAN1 and AIR9 in division plane orientation.

P-27 GENETIC ANALYSIS OF TWO NEGATIVE REGULATORS OF LEAF SENESCENCE, WRKY54 AND WRKY58, IN *ARABIDOPSIS THALIANA*

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Leaf senescence is a degradation process in which the nutrients from the older leaves of a plant are recycled. The visible manifestation of leaf senescence is the yellowing of leaves due to the catabolism of chlorophyll. In the model plant, *Arabidopsis thaliana*, a network of transcription factor (TF) genes, many from the *WRKY* TF family, play key roles regulating leaf senescence. It has been previously shown that *wrky54*mutants show an acceleration of leaf senescence indicating that *WRKY54* is a negative regulator of leaf senescence. *WRKY58* was shown by our lab to also work as a negative regulator of leaf senescence. The additive effect of these two WRKY transcription genes will be tested by analyzing the senescence phenotypes of wild type, single and double mutants. The double and single mutants were selected using PCR analysis of genomic DNA. Senescence phenotypes to be measured include expression of senescence up-regulated genes and the rate of chlorophyll loss. If the genes are additive, the double mutants will show a faster rate of senescence than single mutant and wild type plants.

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P-28: EFFECTS OF INDOLE GLUCOSINOLATE BIOSYNTHESIS ON DEVELOPMENTAL LEAF SENESCENCE IN *ARABIDOPSIS THALIANA*

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Leaf senescence is the ultimate stage of leaf development in which nutrients are recycled and reallocated to newly developing organs. In a previous study, gene ontology analysis revealed an enrichment for the indole glucosinolate (IG) biosynthesis biological process among Senescence-Upregulated Genes (SURGs), which suggests IGs influence senescence. Indole glucosinolates (IG) are secondary metabolites present in the Brassicaceae plant family, and which possess protective properties against herbivory and fungal infections. We have shown that inhibition of IG synthesis at the beginning of the pathway results in premature senescence, however blocking this portion of the pathway can lead to loss of other metabolites as well. Recent data demonstrated that an IG transport double mutant *pen1/pen3*exhibited premature leaf senescence implicating the IG metabolites as playing a protective role.

This study hypothesizes that IGs play a protective role preventing premature leaf senescence. Furthermore, we intend to identify the IG metabolites that play this protective role. The IG biosynthetic pathway will be investigated through mutant lines for biosynthesis gene families *CYP81F* and *IGMT*, transport genes *PEN1* and *PEN3*, regulatory genes *MYB51* and *MYC2*, and signaling gene *MPK3*. The progression of senescence will be analyzed in homozygous mutant plants, and compared to *Arabidopsis thaliana* wild-type (WT) plants. Relative SURG expression and leaf chlorophyll content will be quantified at days 28 and 32 in leaves 3 and 4 to determine which lines demonstrate premature senescence.

Further investigation of the double mutant *pen1-1/pen3-1* and single mutants, *pen1-1* and *pen3-1,* and WT will be performed by quantifying the IG content in leaves. The single mutants do not display early senescence, and thus IG metabolites missing or reduced in the double mutants, but not in the single mutants, will be of interest. Currently, the triple mutant *pen1-1/pen3-1/sid2-1* is being constructed to determine whether premature senescence is dependent on Salicylic Acid signaling.

P-29: SHEDDING LIGHT ON THE ARABIDOPSIS DEFENSE RELATED MUTANT NDR1-1: OLD DOG, NEW TRICKS

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Multiple genetic components of the defense signaling pathways in plants overlap with the components of signaling cascades involved in various plant processes including plant development, and plant defense responses and hormone signaling. We analyzed *Arabidopsis thaliana* defense signaling and hormonal signaling pathway mutants in response to the soil borne pathogen, *Verticillium* spp., for insights into plant responses in Verticillium wilt-susceptible cultivars. This research enabled the discovery of the interplay between defense and gibberellic acid signaling that regulates growth and flowering time. Infection by two *Verticillium* spp. enhanced the early flowering phenotype of the widely studied *ndr1-1*mutant in *A. thaliana,* initially leading to accelerated growth and significantly increased disease severity relative to the wild type parent. Our findings using the *ndr1-1* mutant implicate *Arabidopsis NDR1* as a negative regulator of flowering in a gibberellic acid-dependent manner. It also provides evidence for a role of *NDR1* in *Verticillium*-mediated alteration in flowering time and growth response. Since *NDR1* function with respect to defense response is conserved in multiple plant species, our discovery highlights the importance of elucidating crosstalk between defense responses and hormonally regulated development prior to manipulating the pathways in crop plants.

P-30: CHEMICAL GENETICS DISSECTION OF INTERFERENCE BETWEEN PATHOGEN AND DROUGHT STRESS TOLERANCE SIGNALING IN PLANTS

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The plant hormone abscisic acid regulates adaptation to environmental stresses, particularly drought. How plants cope with multiple stresses, especially when challenged with pathogen infection and then drought, remains largely unknown. The tolerance mechanisms against the two stresses often negatively affect each other. However, the underlying mechanisms remain unknown. Using a chemical genetics approach that can address genetic redundancy and network robustness, a novel small molecule "DFPM" was identified that down-regulates abscisic acid signaling by activating plant immune responses (1-3). To dissect this interference signaling, an Arabidopsis thaliana reporter line harboring an ABA-inducible marker pRAB18:GFP was EMS mutagenized and screened for hyposensitive responses to DFPM. *rda* (***r***esistant to ***D***FPM inhibition of ***A***BA signaling) mutants were isolated and mapped to a putative receptor-like kinase. Further characterization of the functions of this receptor-like kinase in plant immune signaling and interference mechanisms with ABA signaling will be presented. This research will help understand how plants exposed to both pathogen and drought can coordinate effective tolerance responses which will be relevant for plant survival and crop yield.

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P-31: COMPARISON OF SOLUBLE LEAF PROTEIN AND PIGMENT CONTENT IN TWO RICE CULTIVARS GROWN UNDER FREE-AIR CO_2 ENRICHMENT AND VARYING NITROGEN SUPPLY

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The demand for the production of rice is increasing with the growing global population, as it is currently the staple food for over 50% of the world. It is thus important to develop new rice varieties (*Oryza sativa* L.) that can thrive in the oncoming higher atmospheric [CO_2] in order to ensure food security. In this study, a standard *Japonica* cultivar grown in Japan, Koshihikari, was compared to the recently developed cultivar Takanari. Takanari has previously demonstrated a higher biomass and grain yield, which is linked to its ability to maintain high rates of photosynthesis in its upper leaves. We investigated the effect of elevated [CO_2] and nitrogen supply on soluble protein and pigment content in the leaves of Takanari and Koshihikari. The two rice varieties were grown in a paddy field under a combination of season-long free-air CO_2 enrichment (FACE, approximately 200 µmol mol^{-1} above ambient [CO_2]) and two N applications (0 and 8 g N/m^2). The total soluble protein and pigment content of the uppermost fully expanded leaves were quantified from samples collected at several growth stages. At every growth stage, elevated [CO_2] generally decreased the leaf total soluble protein content. During the critical grain-filling stage, the low N treatment significantly decreased protein content in Koshihikari plants grown under elevated [CO_2]; however, Takanari did not show any decrease. The chlorophyll content of the uppermost leaves of Takanari was between 50-90% higher than that of Koshihikari in all treatments at the heading and grain filling stages. We conclude that Takanari exhibits better tolerance of nitrogen limiting conditions under future [CO_2] than Koshihikari, making it a valuable genetic resource for breeding efforts.

P-32: SYNERGISTIC EFFECTS OF MIXED POPULATIONS OF *SINORHIZOBIUM MELILOTI* AND *BACILLUS SIMPLEX* ON ROOT INFECTION AND NODULATION

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Legume plants form mutualistic relationships with various rhizobacteria in the soil, which promote plant growth by improving nutrient acquisition, modulating plant hormone levels, and protecting the plant from biotic and abiotic stress. The rhizobacteria *Sinorhizobium meliloti* and *Bacillus simplex* are known to have symbiotic relationships with white sweet clover, *Melilotus alba*. *S. meliloti* is an alpha-Rhizobium species which forms a host-specific endosymbiosis with *Melilotus*, *Medicago*, and *Trigonella* species where the bacteria fix atmospheric nitrogen into ammonia that can be used by the plant. In exchange, the legume provides the bacteria with carbohydrates; it houses and protects the rhizobia in specialized structures on the root called nodules. The effect of *B. simplex* on plants is not as well-characterized; however, it has been shown to increase plant biomass, enhance resistance to fungal disease, alter lateral root architecture, and improve nodulation in pea. This study examined whether *B. simplex*could enhance infection and nodulation by *S. meliloti*. Furthermore, *B. simplex* was assessed for its ability to rescue various infection-deficient *S. meliloti* mutants. The *pilA* and *exoY* genes encode Type IV pili and exopolysaccharide biosynthesis, respectively; mutation of *pilA genes* delays root infection and mutation of *exoY* leads to fewer infection sites and abortive infection threads. *M. alba* roots were inoculated with individual strains (*S. meliloti* or *B. simplex*) or they were co-inoculated with *S. meliloti* and *B. simplex*. Plants co-inoculated with *S. meliloti*and *B. simplex* displayed greater biomass, more root nodules, and more infection sites than plants inoculated solely with an individual *S. meliloti* strain.

P-33: IDENTIFICATION OF WHITEFLY RESISTANCE LOCI IN ALFALFA USING GENOTYPE-BY-SEQUENCING

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Whiteflies (WF) are a significant *Hemipteran* pest found worldwide. An outbreak in the late 1980s saw *Bemisia tabaci* B. overtake the native counterpart (*B. tabaci* A.), causing widespread damage to southwestern US agriculture. WF feeding damages plants through feeding which compromises plant growth and development, vectoring viruses and spreading of sooty mold due to honeydew secretions. WFs have a large host range, develop insecticide resistance and have few natural predators that can successfully control outbreaks. Therefore, developing an integrated pest management program based on host plant resistance would be the most effective means of control. Alfalfa is a high-value crop that is heavily afflicted by WF feeding. However, alfalfa cultivars expressing WF resistance, manifested as nymph death, were identified for a germplasm pool. From this germplasm pool, three populations were developed: UC-1872 (susceptible), UC-2845 (resistant) and UC-2933 (highly resistant). DNAs from 100 plants (25 / population) were sequenced using genotype-by-sequencing (GBS). These analyses identified two candidate resistance genes: one on Chr. 8 and one on Chr. 3. The researcher's objectives are to: evaluate alfalfa-WF resistance to other species, validate alfalfa SNP association with resistance via high-throughput phenotypic screens, and identifying genes underlying resistance through RNA-seq and differential gene expression analysis. Over 80 genotypes of alfalfa are now phenotyped and resistant and susceptible genotypes have been identified for each population. Select lines are being used to create a mapping population. In addition, the researcher is comparing various WF behaviors (adult feeding choice, nymph development, egg deposition, and adult longevity) on resistant and susceptible lines to determine if resistance is "broad-based". Finally, time-course infestations correlated with WF development will be performed on two genotypes (one resistant and one susceptible). Samples will be used for RNA-seq, RT-PCR and metabolomics to further identify possible resistance genes.

P-34: DO CHANGES IN CYTOSINE METHYLATION AFFECT CMR-SURG *UGT78D1* EXPRESSION?

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Leaf senescence is the final stage of leaf development in which nutrients are mobilized from older leaves to new growth and storage organs of the plant. Efficient leaf senescence is essential for high crop yields and plant fitness. 5-methyl cytosine methylation can activate or repress transcription depending on its location. Cytosine methylation in the promoter region is associated with the inhibition of mRNA expression; therefore, a decrease in promoter cytosine methylation is associated with activation of transcription. Dimethylation of histone H3 at lysine 9 (H3K9me2) is a histone modification associated with cytosine methylation in a positive feedback loop. A negative correlation between cytosine methylation and *UGT78D1* gene expression was previously observed as leaves age in *Arabidopsis thaliana*. For this reason, *UGT78D1* may be a cytosine methylation regulated-senescence up-regulated gene (CMR-SURG). Mutant lines *ros1-1* and *suvh4*will be used to verify the relationship between cytosine methylation, *UGT78D1* mRNA expression, and leaf senescence. ROS1-1 is a cytosine demethylase, therefore the *ros1-1* mutant will have increased cytosine methylation when compared to wild type. SUVH4 is responsible for histone H3 dimethylation at lysine 9 (K9), therefore the *suvh4* mutant will have decreased H3K9me2 and cytosine methylation when compared to wild type. *Arabidopsis thaliana* leaf 6 and 7 tissue will be collected at 28d, 35d, 42d, and 49d. Genomic DNA will be isolated and subject to sodium bisulfite treatment to measure cytosine methylation. RNA will be isolated to measure *UGT78D1*expression using *ACT*2 mRNA levels as a reference. We hypothesize that *suvh4* with low cytosine methylation will show higher *UGT78D1* expression while *ros1-1* with increased cytosine methylation will show

reduced *UGT78D1* expression. These results would support a cause and effect relationship between the loss of promoter cytosine methylation and mRNA induction during leaf senescence. This study will help further our molecular understanding of the regulation of senescence.

P-35: THE INVOLVEMENT OF THE TANGLED2 PROTEIN IN PLANT CELL DIVISION

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As with all other organisms, maize must undergo cell division to grow and develop correctly. Changes in the orientation of the division plane can result in altered plant growth and delayed development. TANGLED1 (TAN1) is a protein that aids in cell division by helping maintain proper division site information throughout mitosis. The *tan1* mutant in maize is much smaller in size when compared to the wild type sibling, has textured leaves, and cells with abnormal shapes. TANGLED2 (TAN2) is a protein similar in sequence to TAN1, however its role in cell division is unknown as no mutant exists for this gene. By analyzing the phenotype of *tan2* mutant plants, we hope to determine the impacts of the TAN2 gene and compare them to the functions of the TAN1 gene. CRIPSR-CAS9 was used to design guide RNAs to target the TAN1 and TAN2 genes, to generate mutant alleles in maize. Novel *tan1*and *tan2* mutant alleles were generated and characterized through PCR and sequencing. The newly identified *tan2*homozygous mutants will next be analyzed to assess its phenotype. Because of the similarities in the TAN1 and TAN2 genes, we hypothesize these two proteins share a similar function, therefore resulting in a similar division plane defect phenotype. Understanding both genes may help better aid in the studies of cell division and growth, leading to information for next generation crop improvement.

P-36: ANALYSIS OF COMPETITION BETWEEN *RHIZOBIUM LEGUMINOSARUM* AND *RHIZOBIUM RHIZOGENES* WITHIN THE RHIZOBIA-LEGUME MUTUALISM

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Rhizobia bacteria provide leguminous plants useable nitrogen through their capability to fix atmospheric nitrogen (N_2) into ammonia (NH_3). Specifically, rhizobia fix nitrogen in nodules, specific root structures, and receive carbohydrates from the plant in a reciprocally beneficial symbiosis. Nitrogen fixation is energetically costly to rhizobia as it uses resources that could be used towards growth and reproduction. Therefore, natural selection favors 'cheaters'; rhizobia living in nodules that fix less nitrogen. To maintain this symbiosis, plants must favor rhizobia that fix the most nitrogen and/or use sanctions to penalize 'cheaters'. I am investigating this relationship by isolating rhizobia and observing their interactions between each other and *Pisum sativum* (pea). Five bacterial strains were isolated from nodules of *Lupinus sp.* and *P. sativum*. 16S rDNA analysis identified four of the strains as *Rhizobium leguminosarum* and the other as *R. rhizogenes*. Nodulation assays showed that *R. rhizogenes* and one of the *R. leguminosarum* strains do not nodulate pea. Successful nodulating strains resulted in healthy green plants with pink nodules, however co-inoculation with nodulating and non-nodulating strains resulted in white nodules and yellow leaf coloration signifying stress. Furthermore, biofilm production was found to differ among strains of *R. leguminosarum*. All strains were resistant to ampicillin, and green fluorescent protein (GFP) and mCherry fluorescent markers were introduced into the bacteria. This fluorescence will be used to identify bacteria in co-inoculated pea nodules to observe occupancy patterns of the bacteria within the nodules of the pea plant. This study will increase our understanding of the interactions between agriculturally applied bacterial bio-fertilizers and naturally occurring soil rhizobia.

P-37: SEARCHING FOR A DEHYDROCOSTUS LACTONE RECEPTOR IN THE PARASITIC WEED *OROBANCHE CUMANA*

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Parasitic weeds in the Orobanchaceae cause millions of dollars in crop losses each year. A key adaptation of these parasites is the ability to germinate in the presence of strigolactones, a class of hormones released by plants into the rhizosphere to communicate with symbiotic fungi. We are interested in understanding how parasitic plants detect strigolactones and strigolactone-like signals from their hosts, as it may allow us to design new strategies to combat infestations. Here, we focused on the parasite Orobanche cumana because of its unique ability to germinate in the presence of both strigolactone and dehydrocostus lactone (DCL), a strigolactone-like molecule released by its host, sunflower. A rapidly evolving clade of strigolactone receptors, KAI2d, emerged in parasitic weeds after duplication of the karrikin receptor-encoding gene KAI2. Parasitic weeds typically have several copies of KAI2d, which may enable the detection of different strigolactones. We used a yeast two-hybrid (Y2H) assay to test seven O. cumana KAI2d proteins for the ability to interact with a downstream target ortholog from Arabidopsis, AtSMAX1, in the presence of rac-GR24 (a synthetic strigolactone analog) and DCL. rac-GR24 seemed to inhibit the interaction between OcKAI2d and AtSMAX1. DCL enhanced the interaction between KAI2d5b and AtSMAX1. Due to the nature of the Y2H assay, further experiments must be done to determine whether KAI2d5b functions as a DCL receptor. Once we have locked down the receptors for rac-GR24 and DCL, we will then be able to screen for molecules that inhibit their activation, and investigate what determines the KAI2d protein's specificity in detecting different strigolactones and strigolactone-like molecules.

P-38: THE ROLE OF MANGANESE ON LITTER DECOMPOSITION ALONG AN OXIC-ANOXIC INTERFACE

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Soils play an important role in carbon cycling, releasing three times more CO_2 emissions into the atmosphere than anthropogenic factors through forest floor litter decomposition. Studies have shown that manganese concentration is positively correlated to the rate of litter decomposition. Mn^{2+} is found in fallen leaves and leaf litter, specifically within Photosystem II. Using extracellular enzymes such as oxidase and peroxidases, fungi can oxidize Mn^{2+} to Mn^{3+}, a soluble and potent oxidant that decomposes lignin. At present, the environmental factors influencing the rates of Mn^{3+} formation and its use in litter decomposition in soils are not known. Here we examined the oxidation potential and the forms of Mn along a soil moisture gradient. We hypothesized that Mn^{3+} concentrations would be greatest along oxic-anoxic interfaces. To test this hypothesis, we found that soils with intermediate moisture, characterized by clear oxic-anoxic transitions in the A horizon, had the greatest oxidative potential. Our results also showed that this soil layer had the highest concentrations of pyrophosphate extractable Mn^{3+}. Furthermore, the largest quantities of extractable soil organic carbon were generated in this horizon, indicating greater decomposition. Our results suggest that the potential for Mn^{3+} formation is most pronounced in the suboxic zone, where enhanced decomposition may be responsible for the production of soluble compounds. As precipitation and temperature patterns change in New England, soil moisture is going to change as well. How climate change alters the natural Mn cycle will have to be taken into consideration if we want to estimate the soil carbon balance and predict the release of CO_2 in the future.

P-39: CHARACTERIZATION OF INCREASED *PANTOEA STEWARTII* RESISTANCE IN MAIZE *pan1* MUTANTS

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Pantoea stewartii is a gram negative bacterium that is the etiological agent of Stewart's wilt, the most agronomically significant bacterial disease of maize and sweet corn in the Midwest and Northeast of the USA. It is a hemibiotrophic vascular pathogen that is vectored by the corn flea beetle, which introduces the bacterium into both the intercellular spaces of the leaves, where it causes water-soaked lesions (WSL), and the vasculature. *P. stewartii* preferentially colonizes the xylem, leading to systemic spread throughout the plant and characteristic wilting symptoms. PAN1 is an enzymatically inactive leucine-rich repeat receptor-like kinase originally described as a regulator of stomata development. We discovered that maize *pan1* null mutants, which have stomatal defects in juvenile leaves but display normal growth and morphology, show dramatically increased resistance to *P. stewartii*. We found that *pan1* mutants are more resistant to Stewart's Wilt disease by impairing *P. stewartii* xylem colonization and spread, but not its ability to cause WSL. Our results suggest that an enhanced vascular defense response that involves the accumulation of host-derived material in xylem vessels to prevent pathogen spread can be a key factor that contributes to *P. stewartii* resistance in *pan1* mutants.

P-40: EXPRESSION ANALYSIS OF HEAT STRESS INDUCIBLE GENES IN *PAULOWNIA ELONGATA*

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Global Warming and climate change are the leading cause of decreased global crop productivity and can affect food security. Selection and development of heat tolerant crops require a deeper understanding of genetic and molecular mechanisms underlying the heat stress-related defense pathways. *Paulownia elongata* is a fast growing hardwood tree present in different parts of the world. Thus they are exposed to a wide variety of extreme temperatures. The primary objective of our study is to analyze and understand the ability of these trees to handle the heat stress. A transcriptomic analysis was done using the RNA-sequencing approach on the leaves of control and heat stressed *P.elongata*. A total of 2438 genes were identified to have differential gene expressions under heat stress. Heat stress kills the plants mainly by decreasing its photosynthetic ability. Most of the photosynthetic enzymes are heat-susceptible and so are easily affected. Most of the genes that were upregulated in *P.elongata* during heat stress were directly or indirectly involved in supporting photosynthesis during stress. This includes (i) the heat shock protein (HSP) family such as HSP 101,21, 22,23.6 etc. that protect photosystems and act on all phases of heat tolerance response such as induction, regulation and recovery, (ii) Magnesium chelatase a chloroplast enzyme that catalyzes the photosynthetic pathway and (iii) PPDK a photosynthetic enzyme regulating the C4 cycle in plants. The top 20 genes with differential gene expression was validated using real-time quantitative polymerase chain reaction (q-PCR). Our results can be a genomic resource to screen and breed the heat tolerant plant genotypes. Some of these genes can also be cloned and expressed to develop heat tolerant crops.

P-41: ANIONIC CERIUM OXIDE NANOPARTICLES PROTECT PLANTS FROM ABIOTIC STRESSES BY ENGINEERING ROS-MEDIATED PHYSIOLOGICAL RESPONSES

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Plant abiotic stress leads to accumulation of reactive oxygen species (ROS) and a consequent decrease in photosynthetic performance. We demonstrate that a plant nanobionics approach of localizing negatively charged, sub-11 nm, spherical cerium oxide nanoparticles (nanoceria) inside chloroplasts *in vivo* augments ROS scavenging and photosynthesis of *Arabidopsis thaliana* plants under excess light, heat, dark chilling, and salinity stress. Poly (acrylic acid) nanoceria (PNC) with a hydrodynamic diameter (10.3 nm) - lower than the maximum plant cell wall porosity - and negative zeta potential (-16.9 mV) exhibit significantly higher colocalization (46 %) with chloroplasts in leaf mesophyll cells than aminated nanoceria (ANC) (27 %) of similar size (12.6 nm) but positive charge (9.7 mV). Nanoceria are transported into chloroplasts via non-endocytic pathways, influenced by the electrochemical gradient of the plasma membrane potential. PNC with a low Ce3+/Ce4+ ratio (PNC1, 35.0 %) reduce leaf ROS levels by 52 %, including superoxide anion and hydroxyl radicals, for the latter ROS there is no known plant enzyme scavenger. Plants embedded with these PNC that were exposed to abiotic stress exhibit an increase up to 19 % in quantum yield of photosystem II, 67 % in carbon assimilation rates, and 61 % in Rubisco carboxylation rates relative to plants without nanoparticles. In contrast, PNC with high Ce^{3+}/Ce^{4+} ratio (PNC2, 60.8 %) increase overall leaf ROS levels and do not protect photosynthesis from oxidative damage during abiotic stress. Under salinity stress, PNC1-Leaves infiltrated plant leaves showed one fold higher ($P < 0.05$) cytosolic K^+ intensity signals in leaf mesophyll cells relative to controls. Non-invasive microelectrode ion flux electrophysiological measurements indicate that PNC1-Leaves have about three folds lower NaCl-induced K^+ efflux from leaf mesophyll compared to controls. The ROS-activated nonselective cation channels in the plasma membrane of leaf mesophyll cells were identified as the main •OH–induced K^+ efflux channels. Overall, this study demonstrates that anionic, spherical, sub-11 nm PNC with low Ce^{3+}/Ce^{4+} ratio can act as a tool to study the impact of ROS mediated physiological processes including photosynthesis and mesophyll K^+ retention, and to protect plants from abiotic stresses.

P-42: MAIZE TERPENE SYNTHASES 6 AND 11 ARE REQUIRED FOR ZEALEXIN PRODUCTION AND PROTECTION AGAINST MULTIPLE PATHOGENS

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Acidic terpenoid phytoalexins, zealexins and kauralexins, are dominant inducible antimicrobial metabolites in maize (*Zea mays*) following the combined pressures of herbivory and microbial infection. Zealexins were first isolated and identified through efforts to characterize stalk rot (*Fusarium graminearum*)-induced defenses. Zealexins are predicted to be synthesized de novo by terpene synthases from farnesyl diphosphate precursors into the rarely encountered β-macrocarpene olefin and modified by a series of oxidative reactions mediated by cytochrome P450 enzymes. Zealexin accumulation is associated with increased expression of the terpene synthases *Tps6* and *Tps11*, which have been demonstrated *in vitro* to catalyze production of β-macrocarpene. Despite proposed relationships, it remains to be empirically demonstrated that the endogenous biosynthesis of zealexins requires the exclusive activity of Tps6/11. Moreover, while select zealexins demonstrate significant in vitro antimicrobial activity, a combination of genetic and biochemical evidence causally linking zealexins to pathogen resistance *in vivo* is required. Using CRISPR we created *tps6* and *tps11* double mutants with frame shift mutations yielding non-functional copies of both tandemly-arrayed genes. Recent analyses utilizing liquid chromatography mass spectrometry (LC/MS) established that maize *tps6/tps11* double mutants plants lack pathogen-induced zealexins. In contrast, synthesis of diterpenoid defenses, such as the *ent*-15-kaurene-derived kauralexins, remained unchanged. Plant-pathogen bioassays revealed impaired disease resistance of *tps6/11* double mutants compared to wild type Tps6/11 plants, as measured through increased symptoms and colonization following necrotrophic fungi (*Fusarium graminearum*) and also xylem-dwelling bacteria (*Pantoea stewartii*)

challenge. Twelve years ago, Tps6/11 were demonstrated to be among the most strongly elicited transcripts in maize following pathogen attack and remain dominant inducible markers in a majority of studies. We now provide conclusive genetic, biochemical and physiological evidence that maize zealexin production requires Tps6/11 and mediates broad-spectrum defense against both fungal and bacterial pathogens.

P-43: DEFENSE HORMONE TRANSCRIPTIONAL PROFILING REVEALS NOVEL COMPLEXITIES IN CASSAVA

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Outbreaks of superabundant whitefly populations throughout Eastern and Central Africa in recent years have dramatically increased the pressures of whitefly feeding and virus transmission on their host, cassava. Indeed, whitefly-transmitted viral diseases such as Cassava Mosaic Virus (CMV) and Cassava Brown Streak Virus (CBSV) continue to decimate African cassava yields, threatening the food security of millions. Considerable cassava yield losses due to such pests and pathogens evidences the need for both virus- and whitefly-resistant cassava lines for distribution to African small shareholder farmers. However, basic knowledge of the defense programs of non-model crops like cassava is lacking, limiting the characterization of cassava resistance mechanisms to whiteflies, viruses, and other pests/pathogens of cassava. Here, a collaborative effort between the African Cassava Whitefly Project (ACWP) groups at UCR and CIAT has generated the first defense-hormone-responsive transcriptomes in cassava. We have characterized the responses of the whitefly-susceptible cassava genotype Col2246 to the two major defense hormones, salicylic acid (SA) and jasmonic acid (JA), over a 24 h time course. Interestingly, comparison of the well-known SA and JA responses in Arabidopsis to those in the highly heterozygous tetraploid cassava has revealed marked complexities of these responses specific to cassava. These results provide evidence that defense programs in Arabidopsis may not always mirror those in crop species, and provide a baseline for characterizing the defense responses of cassava to yield-limiting pathogens and pests.

P-44: DEVELOPMENT AND STRUCTURE-FUNCTION RELATIONSHIPS OF THE ADULT MAIZE LEAF CUTICLE

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The cuticle is the outer physical barrier of plants, establishing an important interaction interface with the environment. This hydrophobic layer consists of the lipid polymer cutin embedded with and covered by waxes, providing protection against environmental stresses like desiccation, UV radiation, and pathogen attack. Thickness, structure, and chemical composition of the cuticle vary widely among plant species, and even within a species, depend on organ identity, developmental stage, and growth conditions. The functional contribution of the maize cuticle to abiotic and biotic stress responses have been rarely studied so far. Moreover, the cuticle's impact on the adult plant, agronomically the most important growth phase, is largely unknown. We are characterizing the biogenesis of the adult leaf cuticle and its genetic basis, and aim to elucidate its impact on important agricultural traits. A first part of the project is the characterization of cuticle maturation along the adult leaf developmental gradient as measured by cuticle permeability and resistance to water loss. Changes in cuticle composition, analyzed by GC-MS, are mapped to the developmental gradient of the adult maize leaf. In a collaborative effort we are conducting an epidermal-specific transcriptomic analysis, which will be related to the compositional changes along the leaf. A second part of the project tries to identify the relationship of cuticle structure, composition and function of different epidermal cell types of the adult maize leaf. Ultrastructural data show distinct alterations in cuticular organization dependent on the cell type, which we want to relate to differences in cuticle composition to identify crucial components of cuticular function in these epidermal cell types.

P-45: CYCLING DOF FACTOR 1 USES TOPLESS TO CONCERT MORNING REPRESSION OF PHOTOPERIODIC FLOWERING GENES IN ARABIDOPSIS

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CYCLING DOF FACTOR 1 (CDF1) and its homologs play an important role in the floral transition by repressing the expression of floral activator genes such as *CONSTANS (CO)* and *FLOWERING LOCUS T (FT)* in Arabidopsis. The day-length specific removal of CDF1-dependent repression is a critical mechanism in photoperiodic flowering. However, the mechanism by which CDF1 represses *CO* and *FT* transcription was unknown. We present that Arabidopsis CDF proteins contain non-EAR motif-like conserved domains required for interaction with the TOPLESS (TPL) co-repressor protein. This TPL interaction confers a repressive function on CDF1, as mutations of the N-terminal TPL binding domain largely impair the ability of CDF1 protein to repress its targets. TPL proteins are present on specific regions of the *CO* and *FT* promoters where CDF1 binds during the morning. In addition, TPL binding increases when *CDF1* expression is elevated, suggesting that TPL is recruited to these promoters in a time-dependent fashion by CDFs. We show a reduction of TPL activity induced by expressing a dominant negative version of *TPL (tpl-1)* in phloem companion cells results in early flowering and a decreased sensitivity to photoperiod in a manner similar to a *cdf* loss-of-function mutant. Our results indicate that the mechanism of CDF1 repression is through the formation of a CDF-TPL transcriptional complex, which reduces the expression levels of *CO* and *FT* during the morning for seasonal flowering.

P-46: ORCHIDS – A MODEL FOR STUDYING BACTERIAL ENDOPHYTES DURING EMBRYOGENESIS
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P-47: THE SEARCH FOR LEUCYL AMINOPEPTIDASE SUBSTRATES
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P-48: AN INVESTIGATION INTO THE COMPOSITION OF ACTIVE MAIZE RNA EDITING COMPLEXES
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