
SAT 3/23
WESTERN REGIONAL
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CALIFORNIA STATE UNIVERSITY, LONG BEACH
Meng Chen
UCR (SPEAKER)
Wolfgang Busch
Salk Institute (SPEAKER)
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PHYTOAB

Western Regional ASPB Meeting 2024 at CSULB

Saturday, March 23, 2024

8:30 am breakfast at The Pointe, name tag pickup

(Gluten-free options available.)

9:00 am: Dr. Meng Chen, UC Riverside

Title: Shedding light on subnuclear membraneless organelles: Photobodies in phytochrome B signaling

9:45 am Aimee Uyehara, UC Riverside

Title: Division 'on the fly' – Preprophase-band-independent TANGLED1 recruitment

10:00 am Stephanie Martinez, UC Riverside

Title: KATANIN's role in cell elongation and division plane positioning in maize

10:15 am *Coffee Break and Networking Bingo*

10:45 am William Adams, University of Colorado at Boulder

Title: The Degree of Foliar Phenotypic Plasticity Reflects Ecotype Adaptation to Habitat Environmental Variability

11:00 am Michelle Smith, CSU, Long Beach

Title: Confirming the Role of the Zinc-Finger Homeodomain Transcription Factor HB34 and its Redundant Paralog, HB23, in the predicted BAG Gene Regulatory Network

11:15 am Kanwardeep Singh, Geneshifters LLC

Title: Controlled condition screening of wild germplasm and identification of candidate genes for ascochyta blight resistance

11:30 am Kevin Nguyen, Chapman University

Title: The Circadian Clock's Role in Alfalfa-Rhizobia Interactions: A Molecular Insight

11:45 am Barbara Demmig-Adams, University of Colorado at Boulder

Title: Zeaxanthin production in Lemnaceae as affected by light, nitrogen, CO_2, and the plant microbiome

BOX LUNCH, enjoy the day and hang up your poster.

(Gluten-free vegan options available.)

1:00 pm ASPB!

1:10 pm Dr. Wolfgang Busch, Plant Molecular and Cellular Biology Laboratory, Salk Institute

Title: Engineering Root Systems to Addressing Climate Change

2:00 pm S. Adeel Zafar, UC Riverside

Title: Deciphering regulatory networks controlling root xylem plasticity under drought

2:15 pm Courtney Cameron, San Diego State University

Title: Studies to Elucidate Plant Methanotroph Interactions

2:30 pm Alexander Borowsky, UC Riverside

Title: Gene network discovery and engineering to enhance rice root resilience

2:45 pm Jeoffrey George, UC Riverside

Title: Climate resilience in soybean: Root plasticity confers flooding tolerance

3:15 – 4:30 pm Poster Session and Refreshments

Poster Session refreshments sponsored by PhytoAB, Inc.

Shedding light on subnuclear membraneless organelles: Photobodies in phytochrome signaling

Meng Chen
Department of Botany and Plant Sciences, University of California, Riverside, CA, USA

Photobodies (PBs) are light- and temperature-sensory subnuclear membraneless organelles in plants, defined molecularly by the presence of the photoreceptor and thermosensor phytochrome B (phyB). PB formation is driven by the liquid-liquid phase separation (LLPS) of photoactivated phyB and correlates with phyB signaling, such as the light-dependent degradation of **p**hytochrome-**i**nteracting transcription **f**actors (PIFs). However, because phyB is diffusible between PBs and the surrounding nucleoplasm, one major challenge to characterizing PB functions has been the difficulty of uncoupling the signaling outputs of phyB in PBs and the surrounding nucleoplasm. As a result, the mechanism and function of PBs in phyB signaling remain enigmatic. Here, we show that phyB recruits PIF5 to PBs. Surprisingly, phyB exerts opposing roles in both degrading and stabilizing PIF5. Perturbing PB size by overproducing phyB provoked a biphasic PIF5 response: while a moderate increase in phyB enhanced PIF5 degradation, further elevating the phyB level stabilized PIF5 by retaining more of it in enlarged PBs. These results indicate that phyB stabilizes PIF5 in PBs to counteract PIF5 degradation in the nucleoplasm. Moreover, we established a robust Oligopaint fluorescence in situ hybridization (FISH) method in *Arabidopsis* to map the subnuclear locations of individual PBs. We show that phyB condensation occurs nonrandomly at twelve preferred nucleation sites, likely associated with specific chromatin loci that are discernible by chromocenter and nucleolar landmarks. Together, these new findings revealed a PB-mediated light and temperature sensing mechanism, in which environmentally-regulated nonrandom phyB condensation at distinct subnuclear nucleation sites allows the co-occurrence and competition of two antagonistic, phase-separated phyB signaling actions – PIF5 stabilization in PBs and PIF5 degradation in the surrounding nucleoplasm – to titrate environmental responses.

Engineering Root Systems to Address Climate Change

Wolfgang Busch
Salk Institute, La Jolla, CA

Climate change will soon profoundly and negatively affect the vast majority of our planet's biota, including most human beings. Despite the importance and urgency of addressing this problem, we still lack technologies to globally address the root cause of climate change – increased levels of CO_2 in the atmosphere. Since plants are central agents in the earth's carbon cycle, fixing atmospheric carbon that then mostly gets released when they decompose, engineering plant traits that affect the decomposition rate of plant derived carbon molecules can potentially lead to a large and globally significant drawdown of atmospheric CO_2. In particular, root systems and the rhizosphere are of interest for such approaches as soils are enormous carbon sinks. Since plants first colonized the earth's land surfaces, their carbon depositions have built up three times more carbon in the soil than is contained in the atmosphere. Specific root traits are important contributors to the accumulation and permanence of carbon in the soil. These include root depth, root biomass and the levels of refractory carbon compounds in root tissues. I will present our efforts in using natural variation, genome wide association mapping, chemical genetics and functional genomics approaches in the model plant *Arabidopsis thaliana* and several crop species to identify genetic and molecular mechanisms that regulate these traits and attempt to utilize this knowledge to enhance traits relating to carbon accumulation and permanence in soils.

SHORT TALK ABSTRACTS

Division ‘on the fly’ – Preprophase-band-independent TANGLED1 recruitment

[1]Uyehara, Aimee N., [1,2]Diep, Beatrice N., [1]Allsman, Lindy A., [1]Gayer, Sarah G., [1]Martinez, Stephanie E., [1]Kim, Janice J., [1]Agarwal, Shreya, & Rasmussen, [1]Carolyn G.
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The orientation of cell division in plants is critical for proper growth and development. The preprophase band (PPB), a plant-specific cortical ring of microtubules and actin, predicts the future division site but disassembles before division is complete. Another microtubule structure called the phragmoplast promotes formation of the cell plate while expanding towards the location previously marked by the PPB. TANGLED1 (TAN1) is a microtubule binding protein and is among a small number of proteins that colocalize with the PPB and remain at the division site after PPB disassembly. Although the PPB has previously been shown to be required for TAN1 localization, here we show that 1) correct formation of the PPB predicts division orientation and 2) TAN1 can be recruited to the cortex through a PPB-independent mechanism in maize. Using live cell imaging in PPB defective mutants and chemically treated cells, we show that de novo TAN1 recruitment follows the phragmoplast and is partially dependent on actin. These experiments suggest cells can recruit TAN1 and possibly other division site proteins during these ‘on-the-fly’ divisions and provide insight into how cells determine the division site.

KATANIN's role in cell elongation and division plane positioning in maize

[1]Martinez, Stephanie E., [1]Choi, Audrey, [1]Allsman, Lindy A., [2]Lau, Kin H., [3]Wright, Amanda J., [4]Weil, Clifford, & [1]Rasmussen, Carolyn G
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Microtubule dynamics and organization influence cell shape and cell division plane orientation. One protein complex involved in mediating microtubule organization is KATANIN, a microtubule severing AAA ATPase hexameric complex composed of catalytic p60 and regulatory WD-40-containing p80 subunits. The p60 catalytic subunit is sufficient to sever microtubules through ATP hydrolysis. In *Zea mays* (maize), several katanin (p60) mutants have been identified, including a loss-of-function double mutant, *discordia3a-2 discordia3b* (*dcd3a-2 dcd3b*), and a semi-dominant mutant, *Clumped tassel 1* (*Clt1*), which may have disrupted ATP hydrolysis. To determine how these mutations influence KATANIN's microtubule severing function, in vivo microtubule severing was assessed. Results show reduced microtubule severing frequency in *dcd3a-2 dcd3b* and homozygous *Clt1* mutant plants, though not a total loss, suggesting other microtubule severing proteins may be functional in maize. As a result of the reduction in severing, *dcd3a-2 dcd3b* and *Clt1* mutants have less elongated cells than their wild-type siblings, leading to a shorter stature. Microtubule severing also influences proper cell division plane positioning. Abnormal asymmetric cell divisions were observed in 43.83% of *dcd3a-2 dcd3b* subsidiary cells, compared to 1.61% in wild-type siblings. Time lapse imaging of asymmetric divisions in *dcd3a-2 dcd3b* plants expressing YFP-TUBULIN show defective preprophase bands and subsequent misoriented expansion of the phragmoplast, leading to asymmetric division defects. Characterizing KATANIN function in maize will lead to a greater understanding of the impacts of microtubule dynamics and organization on plant growth and development.

The Degree of Foliar Phenotypic Plasticity Reflects Ecotype Adaptation to Habitat Environmental Variability

Adams, William W., III & Demmig-Adams, Barbara
Department of Ecology and Evolutionary Biology University of Colorado Boulder, CO 80309-0334

Arabidopsis thaliana ecotypes were grown experimentally under seven different growth regimes to assess phenotypic plasticity in leaf structure and function. There were no differences among ecotypes for plants grown under moderate conditions (400 µmol photons m–2 s–1; 25°C). However, when grown under pairs of extreme light intensities (100 vs 1000 µmol photons m–2 s–1) or temperatures (8 vs 35°C), ecotypes from sites of origin with large (versus less pronounced) ranges of daylength and temperature exhibited greater differences in photosynthetic capacity, leaf mass and thickness, phloem cells per vein, and water-use efficiency of CO_2 uptake. Moreover, ecotypes from the driest habitat exhibited greater phenotypic plasticity in vein density, ratio of water to sugar conduits in minor veins, and transpiration rate than ecotypes from the moistest habitat. Despite these differences in degree of phenotypic plasticity, common structure–function relationships existed across all ecotypes and growth conditions, with significant positive, linear correlations between (i) photosynthetic capacity and leaf dry mass per area, leaf thickness, and carbohydrate-export infrastructure (phloem features); (ii) transpiration rate and water-transport infrastructure (xylem features); (iii) the ratios of transpirational water loss to CO_2 fixation and of water to sugar conduits; (iv) sugar conduits and sugar-loading cells; and (v) water- and sugar-conducting cells. Additionally, the proportion of water to sugar conduits was greater for all ecotypes grown under warm-to-hot versus cold temperature. Thus, developmental acclimation to growth environment included leaf adjustments with common structural and functional relationships but pronounced differences in degree by ecotype that varied proportionally with habitat environmental conditions.

Confirming the Role of the Zinc-Finger Homeodomain Transcription Factor HB34 and its Redundant Paralog, HB23, in the predicted BAG Gene Regulatory Network

Smith, Michelle & Brusslan, Judy
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Bolting-associated genes (BAGs) are regulated by the transition from vegetative to reproductive state: the emergence of the inflorescence, termed bolting in Arabidopsis thaliana. BAGs are observed in the older rosette leaves at the time of inflorescence extension and are enriched in leaf senescence (LS) biological processes indicating that bolting promotes LS. During LS, a plant breaks down proteins, chlorophyll, and other cellular components seen as the yellowing of leaves. The plant then recycles nitrogen from the older leaves to the newer, growing tissues as a fitness advantage. Recently, a gene regulatory network (GRN) was developed (Hinckley et al., 2020) to summarize the direct interactions between BAGs and their targets. One of the main hubs in the GRN is the zinc-finger homeobox 34 transcription factor (HB34) which has the redundant paralog, HB23 with 67.3% identity and 72.8% similarity. Both HB34 and HB23 show increased gene expression in rosette leaves during inflorescence elongation. hb34 knockout and hb23 knockdown lines were crossed to produce hb23hb34 double mutants. Methods to quantify LS (chlorophyll quantification and NIT2 expression) were used to measure a significant delay in LS in hb23hb34 mutants. Additionally, one of the HB34 targets, ATGSTU7, is predicted to be up-regulated in the GRN and was found to be down-regulated in the double mutant, providing support for the proposed GRN. Upon dark-induced LS (starvation-induced), there was no change in the timing of chlorophyll loss in the double mutant compared to WT, inferring that HB34 and HB23 are specifically contributing to regulation in the developmental pathway for LS.

Controlled condition screening of wild germplasm and identification of candidate genes for ascochyta blight resistance

***Rawale, Kanwardeep S.,** Gutierrez-Zamora, Gemini R., Venditto, Noah A., & Gill, Kulvinder S.
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Global chickpea production is restricted by ascochyta blight caused by the necrotrophic fungi *Ascochyta rabiei*. Developing locally adapted disease-resistant cultivars is an economically and environmentally sustainable approach to combat this disease. However, the lack of genetic variability in cultivated chickpeas and breeder-friendly markers poses a significant challenge to ascochyta blight-resistant breeding efforts in chickpeas. In this study, we screened the mini-core germplasm of *Cicer reticulatum* against a local pathotype of *Ascochyta rabiei*. A modified mini-dome screening approach resulted in the identification of five accessions showing a high level of resistance. The mean disease score of resistant accessions ranged between 1.75±0.3 and 2.88±0.4 compared to susceptible accessions, where the mean disease score ranged between 3.59±0.62 and 8.86±0.14. Genome-wide association analysis revealed a strong association on chromosome 5, explaining ~58% of the phenotypic variance. The underlying region contained two candidate genes (Cr_14190.1_v2 and Cr_14189.1_v2), characterization of which showed the presence of a DNA binding domain (cl28899 & cd18793) in Cr_14190.1_v2 and its orthologs in *C. arietinum*, whereas Cr_14190.1_v2 carried an additional N-terminal domain (cl31759). qPCR expression analysis in resistant and susceptible accessions revealed ~3 and ~110-fold higher transcript abundance for Cr_14189.1 and Cr_14190.1, respectively.

The Circadian Clock's Role in Alfalfa-Rhizobia Interactions: A Molecular Insight

Nguyen, Kevin & Atamian, Hagop
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Legumes mutualistically interact with the soil bacteria known as rhizobia that convert atmospheric nitrogen into usable forms like ammonia. Nothing is known about the role of the plant circadian clock, an internal biological timekeeper, in controlling this interaction. Alfalfa seedlings were entrained under 16 hours of light and 8 hours of dark cycles (16h Light/8h Dark) for two weeks. Following this entrainment, the alfalfa was moved to constant light for four days before inoculating with rhizobia at circadian time (CT) 0 (zero hours after lights on), CT4 (4 hours after lights on), CT8, CT12, and CT16 and were grown for an additional 2 weeks at 16h Light/8h Dark cycles. The efficiency of the alfalfa-rhizobia association was evaluated by counting the nodules developed on the alfalfa roots. Results showed that this interaction was most productive at early day (CT0 and CT4) and was regulated by the alfalfa's circadian clock. To gain insights into the molecular mechanisms underlying the circadian regulation of this interaction, the experiment was repeated with the time points CT0 and CT12. This time, the roots were collected 12 and 24 hours after inoculation at each time point, and the root transcriptome was sequenced using RNA-seq. Our analysis identified 1896 DEGs at 12 h and 411 DEGs at 24 h after inoculation at CT0, compared to 1413 DEGs at 12 h and 118 DEGs at 24 h after inoculation at CT12. The analysis identified specific enrichment for genes at CT0 involved in the immune system and hypoxia responses that could explain the observed differences in nodulation. This is the first comprehensive analysis investigating the molecular mechanisms underlying the circadian control of the agriculturally important plant-rhizobia mutualistic interaction.

Zeaxanthin production in Lemnaceae as affected by light, nitrogen, CO_2, and the plant microbiome.

Demmig-Adams, Barbara & Adams, William W. III
Department of Ecology and Evolutionary Biology, University of Colorado Boulder, Boulder, CO 80309-0334

The xanthophyll zeaxanthin has key roles in plant photoprotection as well as plant nutritional quality for the consumer. Zeaxanthin protects the human eye from damage by bright light and serves as an anti-inflammatory agent systemwide. Most plants exhibit a trade-off between (i) the degree to which they accumulate zeaxanthin and (ii) their productivity level (measured as photosynthetic activity and growth rate). In addition, zeaxanthin accumulated under exposure to high light levels is rapidly removed when plants are removed from high light. We show that floating aquatic plants in the Lemnaceae family (duckweeds) can be prompted to produce unusually high levels of zeaxanthin that are stable upon plant removal from high light – while at the same time maintaining exceptionally high growth rates. We describe plant growth conditions that can be used to optimize these outcomes, including light intensity, photoperiod, atmospheric CO_2 level, the level and type of nitrogen supply in the growth medium, and support from the plant microbiome. For example, the highest production of stable zeaxanthin in rapidly growing plants was achieved by growing plants under low light intensity (that still allowed unabated high growth rates in Lemnaceae) followed by an abrupt exposure to a very high light intensity (which did not cause the adverse effects often observed in terrestrial plants upon such a transition). The presence of the plant microbiome ameliorated downregulation of photosynthesis and carotenoid content under elevated CO_2 levels. Our findings identify Lemnaceae as promising plant models to study the effect of the growth environment on plant physiology, nutritional quality, plant-microbe interaction, and environmental footprint.

Deciphering regulatory networks controlling root xylem plasticity under drought

Zafar, Syed Adeel, Borowsky, Alex, & Bailey-Serres, Julia
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Increased resilience of rice to drought is increasingly needed in rainfed ecosystems. Many cereals respond to drought by promoting deep roots and limiting shallow roots to enhance access to moisture. We are interested in the plasticity of the xylem development, particularly xylem strand and diameter, which are important in root hydraulics. To address this at the systems level, we profiled ribosome-associated mRNAs (TRAP-seq) and chromatin accessibility (ATAC-seq) within the quiescent center (QC) and meristematic xylem cell populations, as defined by the domain of expression of the QHB/WOX5 promoter (pQHB) [1]. This was performed with root systems of plants cultivated under well-water (WW) and moderate water deficit (WD) conditions. In addition to identifying differentially regulated mRNAs, we identified hierarchical gene regulatory networks to predict players of root xylem plasticity under drought [1]. We are validating this network using CRISPR-Cas9 system, and defining the downstream targets of these regulators. To gain insight into the root xylem plasticity under drought, we have tracked xylem development in deep and shallow roots using pQHB:GUS-GFP lines under WW, WD, and recovery (WDR) conditions. We observe changes in metaxylem strands and lignin deposition in xylem cell walls under WD and WDR in shallow crown roots. These changes could be an adaptive strategy for water hydraulics or use efficiency under drought. We aim to resolve and manipulate transcription factor-target relationships that orchestrate the hormonal cues that confer beneficial xylem plasticity under water extremes. [1] Reynoso et al. (2022) Dev. Cell. 10.1016/j.devcel.2022.04.013. Funded by US NSF IOS-211980.

Studies to Elucidate Plant Methanotroph Interactions

Cameron, Courtney, Baldwin, Olivia, Bowman, Chynna, Delherbe, Nathalie*, Kalyuzhnaya, Marina G, & Waters, Elizabeth
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Methane is a potent greenhouse gas that contributes to global climate change, which has caused increased extreme and prolonged drought. Advancements in both methane mitigation and plant drought resilience are greatly needed. Here I describe studies examining the interactions between a methanotrophic bacterium, *Methylocaldum 0917*, and two species of plants in the Brassicaceae family, *Boechera depauperata* and *Arabidopsis thaliana*. *B. depauperata* is an endemic California perennial found in the Sierra Nevada Mountains and *A. thaliana* is a well-studied relative of *B. depauperata*. *Methylocaldum 0917* is a methane oxidizing bacteria isolated from arid soils in the Anza Borrego desert that uses methane as its sole carbon source. When Methylocaldum is applied to either plant species' roots, quantitative measures of plant resilience, photosystem activity and leaf area, are statistically higher than control plants. *B. depauperata* samples were collected for metabolomic and transcriptomic analysis. In the presence of *Methylocaldum 0917* and methane we find an increase in symbiosis promoting flavonoids and a decrease in various amino acids including tryptophan. We then see upregulation of genes involved in the tryptophan mediated IAA biosynthesis pathway. Scanning Electron Microscopy analysis shows evidence of close physical associations between plant roots and *Methylocaldum 0917*. Our data strongly indicates a mutualistic relationship between both Boechera and Arabidopsis plants and *Methylocaldum 0917* leading to higher plant drought tolerance. To build on these findings, we will be knocking out genes in the biosynthesis pathways of the upregulated flavonoids in *Arabidopsis thaliana* to identify essential pathways for the establishment of symbiosis between roots and Methylocaldum.

Gene network discovery and engineering to enhance rice root resilience

Borowsky, Alexander, Reynoso, Mauricio, & Bailey-Serres, Julia
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Understanding how roots modulate development and metabolism under varied irrigation or rainfall is crucial for development of climate resilient crops. However, root development and environmental response involves the complex orchestration of different genetic programs in different cell types. For example, in rice, a water- and airtight barrier of suberin in the exodermis is formed in response to drought and waterlogging. We established a toolbox of tagged rice lines to profile translating mRNAs and chromatin accessibility within specific cell populations. We used these tools to generate multi-omic profiles of rice root cell types in a range of environments: plates in the lab, controlled greenhouse stress and recovery conditions, and outdoors in a paddy. Through integration of chromatin and mRNA data, we resolve regulatory networks of genes involved in the drought-responsive deposition of suberin in the exodermis. Using this information, we are using engineering and synthetic biology approaches to manipulate the levels of suberin in the exodermis, both under stress and in well- watered conditions. Ultimately, we hope that these engineered plants will demonstrate enhanced tolerance to multiple stresses.

Climate resilience in soybean: Root plasticity confers flooding tolerance

[1]George, Jeoffrey, [2]Ye, Heng, [1]Hummel, Maureen, [2]Zhou, Lijuan, [2]Song, Li, [3]Wu, Chengjun, [4]Chen, Pengyin, [2]Nguyen, Henry T., & [1]Bailey-Serres, Julia,

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As climate changes, flooding intensifies disrupting crop production by depriving roots of oxygen and limiting nutrient acquisition. The identification of natural genetic variation in soybeans that can tolerate soil flooding (waterlogging) can provide a path to increased yield stability. A genome-wide association study and recombinant-inbred lines have identified genomic regions associated with flooding resilience revealing polymorphisms within a gene we have named *WATERLOGGING-TOLERANCE 1* (*WLT1*). Notably, introgression of the semi-dominant tolerant allele (*WLT1-1*) into a soybean elite line significantly increases yield under field conditions following 7 or 14 days of waterlogging. Mechanistically, WLT1-1 promotes a plastic root architecture with enhanced lateral and adventitious root development. Allelic polymorphisms are associated with reduced protein abundance of WLT1 in the tolerant allele. While polymorphism within the promoter does not influence transcript abundance or localization in the pericycle, waterlogging induces greater upregulation of *WLT1* in the sensitive allele (*wlt1-2*). However, *WLT1* allelic polymorphism within the 5’ untranslated region controls the level of translation of the coding sequence. WLT1 acts as a negative regulator of auxin abundance, with the tolerant allele promoting elevated auxin levels. We propose a model where the reduction of WLT1 protein abundance in the pericycle facilitates auxin-mediated root architectural changes during flooding stress. This work highlights the potential of utilizing natural genetic variation to develop flood-tolerant soybean varieties and provides valuable insights for future breeding efforts.


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POSTER ABSTRACTS

Drought distinctly alters plant and microbe symbiotic programs

Akmakjian, Garo & Bailey-Serres, Julia
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Plant-microbial interactions influence stress resilience to the host plant by altering plant growth and modifying soil chemical and physical properties. Plant symbiosis with arbuscular mycorrhizal fungi (AMF) promotes plant growth by directly providing mineral nutrients such as phosphate and water to the host in exchange for photosynthates provided to the symbiont to sustain the interaction. AM symbiosis has further been shown to improve plant drought resilience and yield outcomes; however, relatively little is known about how water restriction impacts the intimate association between AMF and their plant hosts. Furthermore, the biology of the fungal symbiont is challenging to study because AMF are obligate symbionts and thus “non-symbiotic” controls are not possible. We performed a transcriptomic experiment to analyze symbiotic progression in rice during drought. Remarkably, we found that despite the consistently observed benefit of AM symbiosis, water deficit strongly represses the symbiotic program in pre-colonized rice plants, accompanying a reduction in uptake for many nutrients, suggesting that plants de-prioritize energy-intensive processes unrelated to drought survival. We further leveraged our data to investigate the biology of the fungus and found that during drought the fungus enters a state of quiescence and reduced growth. We hypothesize that this strategy allows both organisms to spare energy and permit survival until water becomes re-available and that our dual-species analytical approach can further our understanding of the interplay between the two symbionts during complex environmental stressors

Hormone, medium, and light responses of European Pear cultivars in tissue culture

Anderson, Delaney, Sycalik, Jennifer, & Waite, Jessica
USDA ARS Tree Fruit Research Lab Wenatchee, WA

Tree fruit production faces major climate, resource use, and consumer preference challenges, requiring use of biotechnological methods that allow for better understanding of genes, hormonal regulation, and development of breeding tools. Development and use of biotechnological tools in pear employ several tissue culture (TC) techniques, which require hormone inputs for most cultivars. Each cultivar has highly varied responses to the hormone types and concentrations used for different techniques, such as micropropagation, regeneration, or rooting. Further testing cultivar responses to various hormones at differing concentrations, variable lighting conditions, and media types is time consuming, and understanding and comparing cultivar-specific responses could lead to more predicable cultivation in pear. Here we demonstrate phenotypic differences in callogenesis, adventitious shoot regeneration, and rooting responses of several cultivars to a variety of different hormone, media, and light inputs. We also show two different optimization protocols within the same cultivar (Bartlett), one hormone-based and one media-based, result in similar degrees of improvement adventitious shoot regeneration. Additionally, rooting responses are strongly impacted by hormone concentration, delivery method, and media type in Bartlett and OHxF 87 and 97 cultivars. Furthering tissue culture methodology by designing fractional factorial experiments will allow us to test for a wider range of cultivar-specific responses and develop future transcriptomic experiments to determine key genes involved in these responses.

Transfer of the “stress metabolite” ectoine between soil bacteria and plants

[1]**Arcos Chavelas, Angelie**, [1]Robles, Jorge, [1]De La Torre, Cesar, [2]Araiza, Robyn, [1]Escobar, Matthew
[1]California State University San Marcos Department of Biological Sciences, 333. S. Twin Oaks Valley Rd., San Marcos, CA 92096 [2]California State University San Marcos Department of Chemistry, 333. S. Twin Oaks Valley Rd., San Marcos, CA 92096

Ectoine is a cyclic amino acid and compatible solute that is produced by many types of soil bacteria in response to osmotic stress. Recently we showed that tomato plants transport microbially-produced ectoine from the soil into their leaves and fruit. To investigate how plants take up ectoine from the soil, *Arabidopsis thaliana* Col-0 (wild-type) and six *A. thaliana* mutants lacking root-expressed amino acid transporters were grown in a sterile plant growth medium containing ectoine. After quantifying ectoine content by HPLC-MS, we found that shoot ectoine levels were significantly reduced in the lysine-histidine-like transporter 1 (LHT1) mutant (28% reduction, P = 0.002), suggesting that this transporter contributes to ectoine uptake. To complement this targeted approach, we are using RNA-sequencing to study how ectoine affects gene expression in *A. thaliana* seedlings, with the hope of identifying transporters and biochemical pathways that are regulated by ectoine. In the long term, we hope to uncover the physiological and ecological significance of ectoine in plant-microbe interactions.

Mutational Analysis of Candidate Phosphorylation Sites at the N-Terminus of Coiled-Coil Domain-containing Immune Receptors from Solanum

***Cruz, Ailyn, *Ochoa-Vazquez**, Sandoval, Carina, Tran, Tim, & Sacco, Melanie A.
Department of Biological Science, California State University Fullerton, California

In plant effector-triggered immunity, intracellular receptors with nucleotide-binding (NB) and leucine-rich repeat (LRR) domains recognize proteins from diverse pathogens to activate the hypersensitive response (HR). The potato immune receptor Rx recognizes the coat protein (CP) of Potato virus X (PVX), while the related tomato immune receptor Tm-2^2 recognizes the movement proteins (MP) of Tobacco mosaic virus (TMV). The coiled-coil (CC) domains of these related immune receptors have conserved N-terminal serine (S) and threonine (T) residues, suggesting a potential role for phosphorylation in regulation. To test this hypothesis, we substituted amino acids with alanine (A) to abolish potential phosphorylation or negatively-charged aspartate (D) as a phosphomimic into full-length immune receptors or an Rx CC domain construct that functions in trans with the NB-LRR fragment. Rx CC + NB-LRR co-expressed with PVX CP and wild-type Tm-2^2 co-expressed with TMV MP activated HR which was blocked in Rx CC mutants T7D and S16A and Tm-2^2 mutants T7D, S8D, and T7D/S8D. Rx CC mutant S8D showed earlier onset of a stronger HR. All other mutants tested presented a typical HR. Ongoing work is probing further into these sites by cloning the remaining single mutants of Rx T20A and Tm-2^2 S13A and S13D, plus various double mutant combinations and phosphorylation will be directly examined for wild-type and mutant Rx CC proteins biochemically. Phosphomimic inhibition for the conserved T7 residue of both immune receptors suggests phosphorylation negatively regulates activation of these CC-NB-LRR proteins at this site, while S16A mutation may indicate a role of positive regulation by phosphorylation.

Nonrandom phytochrome B condensation enables the biogenesis of distinct individual photobodies with diverse thermosensitivity in Arabidopsis.

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Photobodies (PBs) are membraneless subnuclear organelles that self-assemble via the liquid-liquid phase separation (LLPS) of the plant photoreceptor and thermosensor phytochrome B (phyB). PBs form via the condensation of the active form of phyB. As such, environmental light and temperature cues directly regulate the number and size of PBs, which is considered a critical mechanism in phyB signaling. However, whether phyB condensation occurs randomly in the nucleoplasm or nonrandomly at preferred nucleation sites remains ambiguous. Here, we established a robust fluorescence in situ hybridization (FISH) method using computationally designed unique oligos as probes (OligopaintFISH) to label individual PBs in Arabidopsis. We show that PBs are nonrandomly positioned at twelve distinct subnuclear locations that are discernible by chromocenter and nucleolar landmarks, suggesting that PBs are seeded at preferred nucleation sites likely associated with specific chromatin loci. Intriguingly, warm temperatures reduce PB number by inducing the disappearance of particular thermo-sensitive PBs, suggesting that individual PB seeding sites possess diverse temperature-responsive PHYB nucleation activities. These results reveal a nonrandom PB nucleation mechanism, which provides the framework for the biogenesis of diverse individual PBs via PhyB LLPS in vivo, and unveils that the spatiotemporal control of phyB condensation nucleation may represent a critical mechanism in plant light and temperature signaling.

Understanding AIR9 Function Through Native and Mitotic Promoter Expression In Arabidopsis

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Plant cell division is unique because it begins in the center of the cell, growing radially outward toward the cell cortex. Due to this complexity, there exist many structures to assure that the immature cell wall – called the cell plate – expands to the right location, such as the preprophase band (PPB). The PPB is an array of microtubules and microfilaments that predicts the site of division and cell wall construction during G2, like lights on a runway for a landing plane. Additionally, the PPB recruits proteins like TANGLED (TAN1) and AUXIN-INDUCED-IN-ROOTS9 (AIR9) to the division site. TAN1 and AIR9 seem to be functionally redundant proteins required in proper cell division. In the *tan1* and *air9* Arabidopsis single mutants, there exists only a subtle phenotype; however, in the *tan1 air9* double mutant, there exists a severe phenotype, with improper cell division (unguided cell plate expansion) and short-statured plants. Previous studies showed that constitutively-expressed *AIR9* rescues the *tan1 air9* phenotype. To further test AIR9's functional necessity in cell division, we are introducing multiple domains of YFP-AIR9 (Δ1, Δ15, full-length) with its native and mitotic (*KNOLLE*) promoters to *tan1 air9* double mutants. We expect to see the rescue of the *tan1 air9* double mutant phenotypes in Δ1, Δ15, and full-length AIR9. We hope that a better understanding of proteins critical to cell division, like TAN1 and AIR9, will provide us with better insight into cell division mechanisms in other organisms, such as in humans, which may have possible health applications.

Photobody formation spatially segregates two opposing phytochrome B signaling actions to titrate plant environmental responses.

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Photobodies (PBs) are subnuclear membraneless organelles defined molecularly by the presence of the plant photoreceptor and thermosensor phytochrome B (phyB). Photoactivation of phyB triggers the nuclear accumulation and the subsequent liquid-liquid phase separation (LLPS) of phyB into discrete subnuclear PBs. PhyB regulates all aspects of plant development and growth by inhibiting the accumulation and activity of a family of transcription factors called PHYTOCHROME INTERACTING FACTORs (PIFs). However, the precise function of PBs in the light signaling events of PIF regulation remains ambiguous. Because phyB can diffuse between PBs and the surrounding nucleoplasm, a major challenge in dissecting the function of PBs has been the difficulty uncoupling the functional outputs of the two subnuclear compartments. The previous approaches of disrupting PB formation using loss-of-function mutants in phyB or phyB signaling could potentially disrupt phyB signaling in both the PB compartments and the surrounding nucleoplasm. Therefore, we had not been able to unequivocally define the specific signaling function of PBs. To circumvent this obstacle to characterizing the function of PBs, here we perturbed PB size by increasing phyB abundance. Theoretically, overproduction of PHYB during LLPS is expected to result only in the growth of PBs without changing the concentration of PHYB in either compartment. We found that phyB recruits PIF5 to PBs and, surprisingly, that phyB exerts opposing roles in degrading and stabilizing PIF5. Intriguingly, perturbing PB size by overproducing phyB provoked a biphasic PIF5 response: while a moderate increase in phyB enhanced PIF5 degradation, further elevating the phyB level stabilized PIF5 by retaining more of it in enlarged PBs. These results support a model in which phyB condensation stabilizes PIF5 in PBs to counteract PIF5 degradation in the surrounding nucleoplasm, thereby enabling an environmentally sensitive counterbalancing mechanism to titrate nucleoplasmic PIF5 and its transcriptional output. This PB-enabled signaling mechanism provides a framework for regulating a plethora of phyB-interacting signaling molecules in diverse plant environmental responses.

Analyzing Salt Stress Response in Maize Katanin Mutants

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Salt stress is a large and increasingly growing problem in agriculture. The widespread use of irrigation leads to increased levels of salinity in the soil which can be detrimental to plant growth and development. From a cellular perspective, microtubules–tubulin polymers which are crucial for several essential processes such as mitosis and cell elongation–undergo rapid depolymerization after salt treatment and later form a new array (Wang et. al, 2007). A fundamental enzyme known as KATANIN is responsible for severing microtubules at crossover sites to maintain organization. Recent studies provide evidence that KATANIN may play a role in salt stress response in *Arabidopsis thaliana*. Arabidopsis *katanin* mutants exhibited a temporal response in which they had an initially higher survival rate in high concentrations of salt, then an increased sensitivity and lower survival rate than the wild type at more mature developmental stages (Yang et. al, 2019). It is still unknown how salt stress response is mediated by KATANIN in maize. We are examining how katanin mutants respond to salt by comparing relative root growth rates of non-mutant and *katanin* mutants when grown under 0 or 100 mM NaCl. Preliminary root length data revealed similar growth rates between *katanin* mutants in 0 mM and 100 mM NaCl, suggesting salt tolerance, but additional replicates are needed to confirm these results. We will quantify katanin microtubule density and anisotropy under salt stress. Understanding the mechanisms behind salt stress response in maize is beneficial for closing the knowledge gap around a significant agricultural problem and may provide targets for crop improvement.

Towards an assessment of the ability of *Dunaliella salina* to produce value-added compounds when subjected to abiotic stress

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Dunaliella salina is a halophilic green alga found in many hypersaline lakes and salt evaporation ponds. This alga can adapt to intense light by producing high intracellular concentrations of carotenoids such as β-carotene and can adapt to changes in osmotic pressure by controlling their intracellular glycerol concentration. *D. salina* is grown commercially to produce β-carotene and other carotenoids for dietary purposes, and methods have been proposed to harvest glycerol from the algae for commercial use, for example in cosmetics. *D. salina* is also able to sequester and detoxify arsenic and may have potential use as a bioremediation agent. The goal of this project is to determine how abiotic stress from low temperatures, prolonged darkness, and arsenic exposure affect *D. salina*'s physiology and gene expression. Hemocytometry, UV-Vis spectroscopy, and GC-MS are being used to compare cell density, pigment production, and emission of volatile organic compounds respectively between stress groups. In preliminary research, cultures exposed to low temperatures experienced reductions in cell density and pigment production, and increased production of n-hexane and 2-methyl-1-nonene. Cultures kept in prolonged darkness appear to produce higher concentrations of pigments without much reduction in cell density. Cultures exposed to a low concentration of sodium arsenite experienced little change in cell density, slightly increased pigment production, and were found to emit arsenobetaine. Because *D. salina* produces higher concentrations of value-added compounds when subjected to abiotic stress, learning how low temperatures, prolonged darkness, and arsenic exposure affect the algae may lead to the development of more effective cultivation methods.

Investigating the Mode of Action (MOA) of Nematocidal Chalcones in *C. elegans*: A Lifespan and CRISPR/Cas9 Study

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Plant parasitic nematodes (PPNs) are roundworms, which infect crops causing a huge crop loss for farmers. Methyl bromide, once an effective nematicide, is banned due to its environmental impact. Identifying a nematicide that is efficient, eco-friendly, and secure in its control is vital. Dr. Calderón Urrea's team identified chalcones as promising eco-friendly alternatives, but their mode of action (MOA) remains elusive. To uncover the chalcones MOA, our lab has isolated various EMS-induced mutant lines of the wild-type N2 and VC2010 strain of *C. elegans*, which are resistant to Chalcones 17 and 30; these mutant lines were called RT-Ch17.1.2, Ch17.6.b, Ch17.7.b, Ch17.10.a, and Ch30.3.b. After Whole genome sequencing, and bioinformatics analysis on the RT-Ch17.1.2 line, we identified 6 Single Nucleotide Polymorphisms (SNPs) within intron regions of the PIF1 helicase and MRPL15 large ribosomal protein genes. We have targeted the mrpl-15 gene by using CRIPSR-based mutagenesis and CRISPR/Cas9-mediated mutagenesis confirmed the role of MRPL15 in Chalcone 17 resistance using three mutant lines (MRPL 15.2 V1, V2, V3), demonstrating the characteristic CRISPR/Cas9 footprint. We also investigated potential cross-resistance by exposing Chalcone 17 mutants to Chalcone 30 and vice versa using Lifespan assay. Interestingly, no cross-resistance was observed, suggesting that Chalcones 17 and 30 might target distinct pathways within *C. elegans*. We targeted the *pif-1* gene using CRISPR/Cas9 and generated 6 mutant lines (PIF 1.7 V2, V3, PIF 1.8 V1, PIF 1.9 V2, PIF 1.10 V2, V4). Currently, we are exploring the possibility of altered gene expression as a mechanism of resistance. By examining expression differences in *PIF1* and *MRPL15* genes upon chalcone treatment in these mutants, we aim to decipher the intricate MOA of these promising nematicides. Finding chalcone's intended target will help elucidate the molecular mechanism of chalcone action.

Unravelling the Evolution of Baobab (*A. digitata*): Comparative Genomics with a Chromosome-Level Precision

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Baobab, known colloquially as the 'tree of life,' is a long-lived tree endemic to Africa and holds economic, ecological, and cultural value. However, our knowledge of its genomic features, evolutionary history, and diversity is limited, rendering it an orphan plant. Here, we report the first chromosome-level reference genome sequence for the baobab, *Adansonia digitata*, with an estimated size of 750 Mb, along with sequences of 25 trees collected across Africa. Baobab chromosomes have remained remarkably stable since the Mesozoic Era, with predominant 158 bp repeat arrays in the centromere. A study of transposable elements (TE) shows a unique pattern: DNA transposons make up 33% of the genome, while long terminal repeat retrotransposons only make up 10%. Comparative genome analysis reveals that the baobab shared whole-genome duplications (WGDs) with cotton and bombax. Additionally, baobab underwent a specific genomic event around 4 million years ago (autotetraploidy). Population structure analysis showed latitude-driven diversity. Gene expansions, indicative of strategies for adaptations to dry environments, were also noted. This work lays the foundation for both resource development and protection policies related to baobab.

Sequencing the Transcriptome of Medicinal Herb *Borago officinalis* to Assess Drought Tolerance Mechanisms

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Borago officinalis is an annual herb primarily used for medicinal purposes. Its seed oil contains multiple fatty acids which have been known to benefit various disorders in the respiratory system, heart, arthritis, and skin diseases. An uprising topic in this plant is how it reacts to drought stress, which is currently understood through its chemical and physiological activity. The molecular mechanisms of how *B. officinalis* responds to drought still need to be understood. Understanding these molecular mechanisms improves our knowledge on how the medicinal herb can be optimized and protected despite drought conditions.

Two Class E bZIP Transcription Factors Promote Bolting-Associated Leaf Senescence and May Regulate Response to Abiotic Stressors

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bZIP transcription factors (TFs) regulate development and response to stress and phytohormones (Shen et al., 2007). They are characterized by a basic region/leucine-rich zipper domain used to homo- or hetero-dimerize to initiate transcriptional regulation. In the Arabidopsis thaliana genome, there are 75 bZIP genes separated into 10 binding domain classes. bZIP34 and bZIP61 genes are the sole members of class E and were identified as bolting-associated genes (BAGs, Hinckley and Brusslan 2020). Class E bZIPs have a conserved proline residue in their zipper domain, preventing homodimerization. bZIP34 and bZIP61 were placed in a gene regulatory network (GRN) predicted to regulate bolting-associated leaf senescence (LS). Their role as LS and bolting-associated genes (BAGs) is not well characterized; however, they are predicted to regulate transcription of downstream targets HB34, NLP3, WOX2 and WRKY45. The two can form heterodimers with Class I member, bZIP51, alluding to one mechanism of how they might regulate expression in the BAG network. We isolated two double knockout (DM) bzip34bzip61 lines and measured the expression of a well-known LS marker NIT2 at time points T0, T4, and T12 days after bolting in rosette leaves (4 & 5). DM plants showed a delayed LS phenotype when compared to WT. Expression of predicted target genes HB34, NLP3, WOX2, and WRKY45 will be measured. Genes in the predicted bolting-associated GRN overlap with genes involved in response to abiotic stressors. For example, we have shown NIT2 is upregulated in response to salt stress. Therefore, we hypothesize there is a link between bolting-associated LS and abiotic stress response. Current research involves fine-tuning various abiotic stress protocols, including salt and drought, to measure DM sensitivity to such stressors.

Molecular and physiological responses of turmeric plants to heat stress

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Turmeric (*Curcuma longa*) is an economically important horticultural crop that is used in the pharmaceutical and food industries. Its worldwide market was valued at over four billion US dollars in 2023 and is expected to grow past seven billion US dollars by 2033. However, owing to turmeric's preference for warm climates, its commercial crops are at an elevated risk of suffering from excessive heat stress in times of weather anomalies, which are projected to become more frequent due to climate change. While there are plenty of reports available on the potential therapeutic applications of various turmeric-derived products, investigations into the effects of heat stress on live turmeric plants are scarce. Our study aims to establish a multifaceted understanding of the major effects that prolonged heat stress inflicts on turmeric crops. The experimental group was exposed to 42˚C ambient heat in periods synchronized with the day/night cycle for seven days, during which time carbon dioxide and water vapor concentrations for each individual plant were continuously measured, and atmospheric samples were taken for gas chromatography-mass spectrometry (GC-MS) analysis. At the end of the seven-day period, leaf tissue samples were taken for RNA extraction. These samples were then sent out for RNA sequencing, resulting in a minimum of 76.8 million valid reads per sample. De novo transcriptome assembly predicted 10104 genes of which 4223 are expressed differentially. Preliminary analysis indicates the predictable activation of heat shock proteins taking place as well as suppression of photosynthesis, but also, perhaps surprisingly, overexpression of genes associated with other sources of stress such as a pathogen or a toxin. Our research will assist agronomists in the cultivation and breeding of climate-resilient thermotolerant turmeric plants.

Engineering Rice Plants with Increased Suberin to Improve Drought Tolerance

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As the world faces global climate change, droughts becoming more frequent and extreme are raising concerns for crop production efficiency. Roots are important for plant responses to water deficit. Suberin, a waxy polymer in the root of plants, is hypothesized to be an aid in fighting water loss for plants. My goal is to engineer rice plants that have increased suberization in the exodermal cell layer of their roots and test the plants to see if they demonstrate improved drought tolerance. To do this, I generated transgenic rice plants that have different promoters driving regulators of suberin biosynthesis. I cloned promoters expressed in the exodermis using Golden Gate cloning, and assembled a plasmid that carries my promoter(s) fused to a transcription factor coding sequence. I transformed these assembled plant expression vectors into rice. I regenerated plants from callus and am screening the next generation for homozygous seed, and confirming promoter expression patterns using GUS staining. Finally, I am working to develop a marker-free visual reporter line which we can re-transform to accelerate our phenotypic assessment of transgenic plants. These experiments could validate a new method to increase drought tolerance and improve crop production in the face of climate change.

Investigating Roles for the Tomato 14-3-3 Protein Family in Defense Responses Mediated by the Immune Receptor Tm-2^2

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Effector-triggered immunity (ETI) in plants often results in the hypersensitive response (HR), a type of programmed cell death, following the recognition of pathogens by host immune receptors. While the mechanism of ETI remains to be fully understood, the highly conserved 14-3-3 protein family has been implicated in disease resistance against bacterial and fungal pathogens. The tomato genome encodes twelve 14-3-3 isoforms that are referred to as TFT1 – TFT12. We are studying the highly durable tomato immune receptor Tm-2^2, which confers resistance to tomato and tobacco mosaic viruses (ToMV and TMV, respectively) by recognition of the virus' movement proteins (MPs). Our lab previously observed a novel interaction in *N. benthamiana* between 14-3-3 proteins and the coiled-coil domain of Tm-2^2. Treatment of *N. benthamiana* leaves with 5-Aminoimidazole-4-carboxamide 1-β-D- ribofuranoside (AICAR), a chemical inhibitor of 14-3-3 protein function, blocked HR cell death responses normally caused by co-expression of TMV MP and Tm-2^2 in the absence of AICAR. To further probe the potential role for the 14-3-3 family in Tm-2^2 function, we cloned members of the tomato 14-3-3 family into binary expression vectors to co-express TFTs with Tm-2^2 and TMV MP, both as wild-type proteins and as dominant-negative mutants generated by two amino acid substitutions. Overexpression of wild-type TFTs generally enhanced Tm-2^2-mediated HR, while dominant-negative mutant overexpression inhibited HR development. Findings from this study will provide a greater understanding of 14-3-3 protein involvement in disease resistance and Tm-2^2 function, which can be valuable to further efforts for the development of durably resistant crops.

Arsenic-reducing water management strategies may cause lower stomatal conductance and increased leaf temperature in rice plants.

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Arsenic absorption by lowland rice plants into the grain presents a food safety risk. Water management strategies (i.e., short-term paddy drainage) have shown potential in mitigating arsenic uptake but may disrupt plant physiological function and growth. To date, no previous studies have reported adverse physiological side effects caused by these drainage treatments. Our objective was to further investigate whether this method of arsenic reduction could induce physiological stress in rice plants. The rice variety 'Koshihikari' was grown in a rice paddy in Tsukuba City in eastern Japan. Two control subplots were maintained with continuous flooding. The remaining two subplots were flooded for three days and drained for four days weekly (3F-4D treatment), starting three weeks prior to heading, and ending three weeks after. Stomatal conductance (gsw) and leaf temperature of the upper and lower canopy were measured over three diurnal time points (morning, midday, and afternoon) per day over the period from two weeks before heading to two weeks after heading. This past summer's climate at the field site was notably hot and dry. Post heading, during the last two days of the paddy draining cycle, midday gsw was as much as 37% lower in the 3F-4D subplots than the control subplots. At the same time, leaf temperature in the 3F-4D plots were up to 1°C higher than in the control plots. Lower stomatal conductance may limit photosynthesis and carbon assimilation. However, biomass and yield showed no significant differences between the treatments. In a warming world, farmers may need to be careful when using paddy drainage to reduce arsenic due to a potentially higher risk of water and heat stress, leading to lower grain yield and quality.

Investigation of Differentially Expressed Genes in Extreme Resistance versus Hypersensitive Response Elicited by Poleroviruses in *Nicotiana glutinosa*

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Plants defend themselves against infectious pathogens through inducible defenses operating at the molecular level. Two inducible responses to pathogens are termed extreme resistance (ER), in which the pathogen is eliminated without cell death, and the hypersensitive response (HR), which limits the pathogen to the infected area by local programmed cell death. We are interested in identifying determinants of the plant response using the model system of infection of *Nicotiana glutinosa* by viruses of the Polerovirus genus. *N. glutinosa* accession TW61 exhibits ER when infected by Turnip yellows virus (TuYV), but presents with HR when infected by Potato leaf roll virus (PLRV). In contrast, *N. glutinosa* accession TW59 exhibits HR when infected by either polerovirus. An RNA-seq experiment was conducted at a timepoint of visible HR onset and candidate genes were selected for further validation of differential expression (DE) through reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Our ongoing experiments are examining plants agroinfiltrated with the infectious binary vector clones of TuYV and PLRV used for RNA-seq, or with clones for expression of the proteins $P0^{Tu}$and $P0^{PL}$ that have been shown to function as the elicitors of defense responses, with preliminary data demonstrating differential regulation of some candidates. One candidate gene encoding a transcription factor has been selected for functional analysis in development of HR by overexpression and silencing. Progress toward resolving the mechanisms of determining how plants commit to elimination of virus infection with or without concomitant programmed cell death will be discussed.

Syntaxin PEN1 and Tetraspanins (TET3 and TET8) Play Opposing Roles in the Regulation of Leaf Senescence in Arabidopsis

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Extracellular vesicles (EVs) are membrane-bound exosomes that are secreted into the apoplast, and likely function during defense against biotrophic fungi. Two distinct populations of EVs have been described: PEN1-associated and TET8-associated. PEN1 is a syntaxin known to facilitate vesicle-mediated membrane fusion events while TET8 is a tetraspanin, part of a widely conserved family that forms tetraspanin-enriched microdomains. Our lab became interested in whether EVs affect leaf senescence when we observed early senescence in the *pen1* single and *pen1pen3* double mutant. Both PEN1 and PEN3 are abundant in EV proteomes. We noted that TET8 is more abundant in the apoplast of early senescing *pen1* and *pen1pen3* mutants and in older WT plants. The increase in apoplast TET8 was shown to occur post-transcriptionally. In addition, apoplast TET8 was more abundant in early leaf senescence *myb59* mutant and less abundant in delayed leaf senescence mutant *Atnap*. Genetic analysis showed a significant delay in leaf senescence in *tet3tet8* double mutants suggesting that these two highly-expressed tetraspanin paralogs operate additively and are positive regulators of leaf senescence. This is opposite of the effect of *pen1* and *pen1pen3* mutants that show early senescence and suggest PEN1 to be a negative regulator of leaf senescence. Our work provides initial support that PEN1-associated EVs and TET8-associated EVs may have opposite effects on leaf senescence.

Note: The TET8 antibody used in this study was purchased from PhytoAB, Inc.

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