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ABSTRACT BOOK

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2021 SS-ASPB ABSTRACTS

* denotes speaker

General Session (GS) 1 (Theme: Plant Responses to Abiotic Stresses I)

GS 1-1. Arabidopsis protein kinase GCN2 regulates cytosolic translation in response to chloroplastic reactive oxygen

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Cytosolic mRNA translation is subject to global and mRNA-specific control mechanisms in order to balance overall energy and metabolic resources with demand. Phosphorylation of eukaryotic translation initiation factor eIF2alpha is central to an immediate and reversible switch that represses translation globally. The GCN2 kinase (General Control Non-derepressible-2), is the only known kinase for eIF2alpha in plants. It can be activated by herbicides that inhibit amino acid biosynthesis. In the study presented here, we provide evidence of a novel signaling mechanism in Arabidopsis thaliana, where the GCN2-eIF2alpha paradigm extends the narrow definition of retrograde signaling by the chloroplast from transcriptional effects in the nucleus to translational regulation in the cytosol. Specifically, conditions that generate reactive oxygen species (ROS) in the chloroplast, such as dark-light transitions, high light, and the herbicide paraquat all rapidly activated the GCN2 kinase, whereas mitochondrial stress and ER stress did not. In addition, GCN2-dependent eIF2alpha phosphorylation was mitigated by photosynthetic inhibitors and ROS quenchers. Interestingly, gcn2 mutant plants were more sensitive to continuous high light as compared to wild-type in a root elongation assay, supporting the notion that high light and associated ROS signaling are mediated by the GCN2-eIF2alpha pathway. We describe here a genome-wide dataset on the translational defects in the GCN2 mutant. In the transcriptome of the gcn2 mutant responses to abiotic stress, and among them oxidative stress, as well as innate immune responses were sensitized. In keeping with a role for GCN2 in innate immunity, GCN2 kinase was also implicated in the priming by the fungal elicitor, chitin, of an antibacterial defense response. In conclusion, we provide evidence that GCN2–mediated eIF2alpha phosphorylation is a missing link in a non-canonical retrograde signaling pathway whereby the status of the photosynthetic machinery feeds back to the cytosolic protein synthesis apparatus.

GS 1-2. PROTECTION OF TELOMERES 1b regulates chromatin structure to prevent genomic oxidative damage during development in Arabidopsis thaliana

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Genome integrity requires an orchestrated response to environmental and cellular cues through highly conserved multifunctional proteins. Telomeres are the capping structures at the ends of chromosomes, indispensable for their role in genome integrity and cellular senescence. However, emerging studies indicate that telomere-associated factors may have a broader role in modulating the response to oxidative stress. PROTECTION OF TELOMERES 1 (POT1) is one of the most conserved telomeric proteins, essential for both chromosome end-protection and telomeric DNA replication. Arabidopsis thaliana harbors two highly divergent POT1 paralogs: POT1a which fulfills conserved telomeric roles, and POT1b. Here we explore the functions of POT1b. Unlike POT1a, POT1b is not a major player in telomere homeostasis. Instead, POT1b appears to play a unique role in redox biology. Plants null for POT1b were hypersensitive to chemical and environmental stresses, including temperature and salt; were sensitive to the absence of sucrose in the germination media; and, exhibited reduced fitness, as seen by defective pollen viability and lower seed biomass. To gain insight into the mechanism of POT1b, we combined transcriptomic, biochemical, genetic and cell biology assays. POT1b expresses in seeds, roots, gametophytes, and flowers; it is upregulated by oxidative stresses; and, it accumulates in the nucleus in clusters surrounding the nucleolus. Loss of POT1b leads to a global increase in endogenous reactive oxygen species. Within the nucleus we observed regions of abnormally relaxed chromatin during anaphase; further studies showed increased chromatin accessibility, decreased DNA methylation, increased transcription, and elevated DNA oxidation. All of these phenotypes were partially complemented by the expression of POT1b in the first generation, supporting the conclusion that POT1b functions as a regulator of the epigenome. We propose a model wherein POT1b regulates chromatin condensation and prevents genomic oxidative stress in tissues where endogenous reactive oxygen species are required for development.

GS 1-3. Cytokinin Response Factor 2 is involved in modulating cytokinin levels in response to salt stress

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Cytokinin has strong connections to development and a growing role in abiotic stress response. Here Cytokinin Response Factor 2 (CRF2) is examined for connections to salt (NaCl) stress response in relation to cytokinin levels. CRF2 promoter-GUS expression and physiological measurements of crf2 and CRF2OE indicate connections between CRF2 expression and salt stress response. Changes in response to salt stress in crf2 and WT were further examined for: Cytokinin levels (LC-MS/MS), Ionomics (ICP-OES), and Transcriptome expression (RNAseq). CRF2 mutants have few changes under non-stressed conditions, yet

many under salt stress. Cytokinin levels, increased in WT after salt stress, decreased in crf2, potentially from CRF2-regulation of cytokinin biosynthesis genes. Ion content increases in Na, K, Mn, Ca, Mg were found after salt stress in WT, while Ca and Mg increases are lacking in crf2. Many genes were transcriptional regulation by salt stress in both WT and crf2, yet interestingly ~1/3 of salt modified crf2 transcripts (2655) showed unique regulation. Two highly salt-induced CRF2-dependent genes (DDF2 and ELIP2) are connected to salt resistance during germination, linking CRF2 to that process. Overall connection identified between CRF2, cytokinin, and salt stress response will be presented.

GS 1-4. Identification and Expression of Abscisic Acid-Regulated Genes in US RIL Rice Population under Drought Conditions

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Rice is important staple food and drought is the limiting factor for yield. US is the third largest exporter of rice, and Arkansas is the top rice-producing state. A RIL population, derived from Kaybonnet (drought resistant/DR) and ZHE733 (drought sensitive), termed K/Z RILs was chosen. The abscisic acid (ABA) is important in signaling responses to drought. Under drought, ABA triggers stomatal closure to reduce transpiration leading to drought resistance. Roots can be screened for ABA sensitivity, which reflects their stress response. The objectives of this research were to evaluate the ABA response of the K/Z RIL population on root architectural traits related to drought resistance and to identify QTLs and candidate genes for root architectural traits related to ABA response. The RIL population of 198 lines were screened for drought stress in the field at R3 stage and for ABA sensitivity. The effect of drought stress in the field was quantified by calculating the filled grains per panicle number (FG). Drought stress effect in ABA sensitivity were quantified by measuring root architectural traits at V3 stage: root length (RL), root to shoot ratio (RSR), total root number (TRN), shallow root number (SRN), deep root number (DRN), and root fresh weight (RFW). Kaybonnet and 48 drought resistant lines under control display more FG, longer RL, higher RSR, more DRN, and heavier RFW compared to ZHE733 and 150 drought sensitive lines. Under exogeneous ABA, Kaybonnet and 48 drought resistant lines exhibited an ABA-sensitive phenotype, implying that they regulate osmotic stress tolerance via ABA-mediated cell signaling. A total of 147 QTLs and 510 candidate genes within the QTL regions were identified for ABA sensitivity. The RT-qPCR analysis of the candidate genes revealed that a high number of abscisic acid-regulated genes were up-regulated in Kaybonnet. This study provides information to develop drought resistant rice.

GS 1-5. NADP-dependent enzymes are involved in response to salt and dehydration stress in rice plants

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Abiotic stresses are the major environmental factors that limit the productivity of crop. The production of reactive oxygen species (ROS) in stressed plants is a common feature for a number of abiotic stresses. Reduced form of the coenzyme nicotinamide adenine dinucleotide phosphate (NADPH) is an essential reducing regent for the plant antioxidant system, which is important to ROS scavenging. NADPH is produced mainly by the two enzymes glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase in the oxidative pentose phosphate pathway. In this work, the effects of salt and dehydration stress on NADP-dependent enzymes in rice plants were examined. Enzyme activities of glucose-6-phosphate dehydrogenase in both leaves and roots of rice plants were significantly increased in response to salt and dehydration stress. Further, the enzymatic activity of phosphoenolpyruvate carboxylase in leaves of rice plants was affected by dehydration stress. Phosphoenolpyruvate carboxylase in cooperation with malate dehydrogenase and NADP-malic enzyme can produce NADPH. These results suggest the involvement of NADPH-enzymes in plant abiotic stress responses. The increased demands of NADPH in plants under abiotic stress can be furnished by enhanced activities of NADPH-producing enzymes.

GS 1-6. Arabidopsis DREB1a provides Salt and Drought Tolerance in Taipei 309

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Transgenic expression of Arabidopsis DREB1a is known to provide tolerance to abiotic stresses in diverse plant species including potato, chrysanthemum, rice, tobacco and peanuts. In this study, a comparative transcriptomic analysis for the cold stress of zero degree, and stress assays for salinity and drought was performed on the genetically engineered Taipei 309 rice lines expressing AtDREB1a under the AtRD29a promoter that is induced by abiotic stresses. These lines showed higher tolerance to 100 mM salinity and 25-40% water stress compared to their non-stressed controls without any morphological defects. To understand the genetic mechanisms underlying stress-tolerance associated with DREB1a expression, 10 days old seedlings of the single-copy transgenic lines were subjected to transcriptomic analysis on cold-induced and non-induced samples. The gene expression analysis by real time PCR showed 30-40 fold induction of AtDREB1a transcripts compared to the controls. The analysis revealed 2069 and 290 differentially expressed genes (DEGs) in the cold stress and controls, respectively, in comparison to the non-transgenic controls, indicating higher upregulation of DREB1a-targets under cold stress. Out of 2069 DEGs in the cold stress, 682 were upregulated and 1387 were downregulated. The gene set enrichment (GSEA) and KEGG analysis of the upregulated DEGs showed their involvement in MAPK and Plant Hormone signaling pathways in response to the cold stress. In the MAPK signaling pathways, DEGs were involved in the pathogen infection/attack and salt/drought/osmotic stress. In Plant Hormone signaling pathways, DEGs were involved in cysteine and methionine metabolism, carotenoid biosynthesis, alpha-linolenic acid metabolism and phenylalanine metabolism. The downstream target genes of AtDREB1a in the rice genome,

their DRE elemental analysis, and their experimental verification are being currently studied. This indicates that rice responds to the cold stress of zero degree through similar pathways, as has been observed in other cold studies of 4°C-12°C stress.

GS 1-7. Abiotic Stress Response in Shoot Apical Meristem: Identifying Gene Regulatory Networks that Link Shoot Meristem Development and Stress Responses

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The Arabidopsis homeobox gene SHOOTMERISTEMLESS (STM) is essential for formation of the shoot apical meristem and sustained activity. However, it is very little known about its role in proliferative activities of the meristems and the coordination between cell division and differentiation are maintained under environmental stress conditions. Indeed, by analyzing the downstream targets of STM, we have discovered a number of stress associated genes that are induced by different type of abiotic and biotic stresses. We use the term stress associated gene to refer to genes with at least one of the following attributes: genes whose mRNAs (or proteins) increase in abundance following imposition of a stress such as heat, drought, salt; genes with high sequence homology to genes implicated in stress responses in other systems; and genes in which mutations alter the plant's stress response. We describe three types of downstream stress-associated genes activated by STM – the stress responsive transcription factors (TFs), heat shock proteins, and a member of the Universal Stress Protein family. Those stress responsive TFs including the membrane tethered NTM-like, the AP2/ERF, and TCP transcription factors, We are working on characterizing the function of those stress-associated genes as well as identifying the downstream targets of these stress responsive TFs and determining what the biological role of these key regulators is in the shoot meristem development. Identification of those abiotic induced genes and their gene networks could help us understand the regulatory mechanisms that connect and integrate intrinsic developmental processes with extrinsic environmental signals during shoot apical meristem and lateral organ development in plants.

GS 1-8. NIP2;1: Arabidopsis thaliana Core Hypoxia Gene Essential For Lactic Acid Homeostasis and Hypoxia Survival

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Limitations of oxygen resulting from flooding or poor soil aeration are detrimental for plant growth and development. As a result, the plant undergoes a series of genetic, metabolic, physiological, and developmental changes as survival strategies to overcome hypoxia stress. Arabidopsis thaliana under hypoxia induces a set-up of core genes. NIP2;1 is one such core hypoxia gene that encodes a member of the "Nodulin 26-like Intrinsic Protein" (NIP) subgroup of the aquaporin superfamily. The previous biochemical study in Xenopus oocytes showed NIP2;1 as a lactic acid channel. In this study, using a T DNA insertion mutant of

NIP2;1, we investigated its physiological significance during hypoxia. NIP2;1 is constitutively expressed in the core tissues of roots, the anoxia core, even in normal growth conditions. Upon hypoxia, the NIP2;1 expression is upregulated significantly, reaching the maximum within two hours of the stress. The promote-GUS analysis showed NIP2;1 expression predominately in root tissues during hypoxia. Concordant to the gene expression, NIP2;1 protein expression is also upregulated in root tissue during the hypoxia. The protein is predominantly localized to the plasma membrane with some internal membrane localization at the later stage of the hypoxia. Unlike NIP2;1 mRNA, which showed a basal level of expression after returning to the normal conditions, the protein continued to express in reoxygenation conditions. The nip2;1 mutant showed poor survival and susceptibility to hypoxia stress. Further, the nip2;1 mutant displayed reduced lactic acid efflux activity, less media acidification, and higher accumulation of lactic acid in root tissues than the wild type under the hypoxia stress. The loss-of-function of the NIP2;1 also resulted in elevated expression of alcohol dehydrogenase (ADH) and alanine aminotransferase (AlaAT) during hypoxia. Overall, our study established NIP2;1 as an indispensable lactic acid channel for plant survival during hypoxia stress.

General Session (GS) 2A (Theme: Plant Responses to Abiotic Stresses II)

GS 2A-1. Comparative roles of proline, glycine betaine and compost on cowpea drought stress tolerance

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Water stress is one of the main abiotic stress factors that influence the growth and development of crop plants. The ameliorative effects of pre-sowing seed treatment with proline (P) and glycine betaine (GB) at 2.5, 5 and 10 mM as well as soil amendment with compost (2.5, 5 and 7.5 tons/ha) on leaf area, plant height, total number of leaves, shoot and root dry biomass, number of root nodules, effective nodules and fresh weight of nodules, P and GB contents was assessed in response to drought stress. The cowpea 1T07K-292-10 was exposed to water deficit for 10 or 20 days, at the vegetative (V) or reproductive (R) stages. Unstressed control plants were grown under well-watered conditions. The results showed that drought stress reduced biomass, growth and yield of cowpea and the vegetative stage of development is the most sensitive to water deficit in cowpea but with exogenous application of osmoprotectants as a pre-sowing seed treatment and soil compost application, cowpea showed a better ability to recover from increased stress at vegetative stage as both growth and yield under water stress condition were increased relative to untreated stressed plants. However, this study suggests that there is considerable variation in the effect of various levels of seed treatments and compost. Of various treatments, compost (C3) was more effective on growth parameters while, C2, G2, and P1 contributed to increase in nodulation, yield and yield components. it could be concluded that seed treatment with P, GB, or Compost improved the drought tolerance of cowpea

GS 2A-2. Genetics Analysis of Heat Stress Tolerance Contributing Grain Yield nd Quality in Rice

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Rice serves as one of the staple food crops for more than half of the world population. Rice production has been extremely affected by abiotic stresses in which heat stress, an important detrimental factor, affects grain yield and quality during the reproductive stage in rice. Declining grain yield and quality of rice is an emerging problem with high nighttime temperature (HNT) that has solely been the strong driving force to such declines, worldwide. However, our understanding of the genetic basis of the HNT stress phenomenon remains very limited. To dissect the complex stress tolerance and grain yield traits in rice, we established a controlled HNT stress screening system providing stable phenotypes on grain yield and quality leading to HNT stress tolerance in rice. To identify natural genetic variation for stress response and rice productivity, we screened a panel of the USDA rice mini-core collection (URMC) with well-adapted elite rice cultivars to Arkansas and dissect the natural genetic variation in the rice populations of the panel. Using global genetic variation in the panel, we conducted GWAS to identified putative candidate loci/SNPs for multiple productivity traits, affected by abiotic stresses of HNT and drought, which are being characterized in a genetic analysis using an advanced genetics approach predicting biological process and molecular functions. Moreover, integration of the GWAS putative loci/SNPs and previously reported genomic regions of QTLs of productivity traits under heat stress is being used to construct our understanding of the genetic basis of the heat stress phenomenon in rice.

GS 2A-3. Developing Improved Soybean Lines for Seed Composition, Quality, and Heat Tolerance in Mississippi

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High heat in the Early Soybean Production System (ESPS) is a major environmental stress factor resulting in yield reduction and poor seed quality, lowering seed composition constituents in heat sensitive soybeans, lowering market grade, and reducing the quality of soymeal. Therefore, identifying breeding lines with heat-tolerance and high seed quality, including seed protein and germination, is essential. The objective of this research was to phenotype seed quality traits, including seed protein, oil, fatty acids, and seed quality (germination and damage) in a previously developed recombinant inbred line (RIL) population segregating for heat tolerance (RILs derived from the cross DS25-1 x DT97-4290). A two-year field experiment was conducted in 2018 and 2019 in Stoneville, MS, using 200 RILs segregating for heat tolerance, with parental lines and heat-sensitive cultivars as controls. Mature seeds, harvested shortly after full maturity (R8 growth stage), were

phenotyped for seed composition constituents and mineral nutrition as well as seed quality traits. The experiment was not irrigated, in order to promote stress, and the design was a randomized complete block with two replicates. The results showed a wide range of seed composition constituents and seed quality components among the RILs. Although seed quality components are genetically controlled, the magnitude of distribution of seed composition constituents and quality components differed, depending on environmental factors, especially temperature and drought. Heat-tolerant breeding lines with high levels of protein and minerals can be used by public and private breeders to develop improved cultivars which, when adopted by Mississippi and US producers, will enable producers to more effectively compete nationally and internationally in soybean markets.

GS 2A-4. Expression of ABA biosynthesis genes in response to high vapor pressure deficit in Sorghum bicolor.

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The ability of land plants to partially close their stomata in response to high vapor pressure deficit, called the limited transpiration trait, is a rare phenomenon in crop plants. The characteristic has been demonstrated in several crop species including Sorghum bicolor. Studies show that different genotypes within the sorghum species respond in three different ways to high vapor pressure deficit. While the transpirational responses have been shown to be under the control of the hormone abscisic acid, the molecular basis and regulatory mechanisms of abscisic acid metabolism are not clear. This study was conducted to determine the molecular basis for different transpirational responses to high vapor pressure deficit in three sorghum genotypes (SC1345, SC35 and Macia). Gene expression patterns of ten genes involved in the abscisic acid pathway and physiological responses were investigated after exposing plants to high vapor pressure deficit for 30 min and water deficit for 12 days respectively. There were marked differences in expression patterns for the ten genes across the three genotypes, coupled to different abscisic acid content. As expected, the NCED3 gene was upregulated in all three genotypes, and notably the BG1, ZEP and SDR genes were upregulated in SC35 and Macia, genotypes exhibiting the limited transpiration trait. The results suggest that there is natural variation in abscisic acid content within the species, and the differential expression patterns in the genes (BG1, ZEP, NCED and SDR) may be responsible for the different transpirational responses to high vapor pressure deficit.

GS 2A-5. Constitutive expression of the Inositol 5-Phosphatase gene alters plant development and enhances abiotic stress tolerance in creeping bentgrass

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InsP-5-Ptase from humans is involved in drought and other stress responses by regulating phosphoinositide signaling. Inositol-1,4,5-triphosphate (InsP3), a very important

component of phosphoinositide signaling, participates in plant response to the abiotic stress. The level of InsP3 declines sharply in transgenic dicotyledonous plants overexpressing inositol polyphosphate 5-phosphatase (InsP-5-Ptase), indicating an increase in the durability of InsP3 to better mediate stress response. To investigate how InsP-5-Ptase is involved in stress response in monocots, we have generated transgenic creeping bentgrass (Agrostis stolonifera L.), an important C3 cool-season turfgrass that constitutively expresses InsP-5-Ptase. Transgenic plants exhibit altered development. Preliminary data reveal that overexpression of InsP-5-Ptase gene leads to enhanced plant tolerance to drought stress associated with improved physiological parameters. Further characterization of the InsP-5-Ptase transgenic plants will allow a better understanding of InsP-5-Ptase-mediated plant stress response, providing information to develop novel biotechnology approaches for crop genetic improvement.

GS 2A-6. Improving plant productivity by accelerating recovery from photoprotection

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Photosynthesis is an important pathway for plants to accumulate organic matter and is critical for plant productivity. A photoprotection mechanism exists in plants called Non-photochemical Quenching (NPQ) which can prevent plants from photodamage at the expense of the photosynthesis efficiency while under excess light conditions, and dissipate extra energy by heat harmlessly. This project is focusing on the NPQ process, by accelerating plant recovery from NPQ to reduce the energy loss in photoprotection. The xanthophyll cycle, which is catalyzed by violaxanthin de-epoxidase (VDE) and zeaxanthin epoxidase (ZEP), is considered a major route for NPQ stimulation and relaxation. Photosystem II subunit S (PsbS) works as heat dissipation locus in the thylakoid membrane whose abundance is positively correlated with NPQ level. We have cloned these 3 genes from rice and introduced into creeping bentgrass, an important turfgrass, to generate transgenic plants. Preliminary experimental results showed that while overexpression of these genes suppresses plant development under normal condition, the suppression is mitigated under highlight condition. Transgenic plants also exhibit higher drought resistance than wild type controls. Further characterization of the transgenic plants would reveal whether or not the NPQ process would decline rapidly and reduce energy dissipation, ultimately increasing plant productivity.

GS 2A-7. Molecular physiological mechanisms of developing environmental resilient and high yielding plants

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Increasing crop yield while minimizing the adverse effects of environmental stress is goal of sustainable agriculture. High temperature, drought, nutrient limitation, diseases are threat to crop production and agricultural food security. We identified mechanism(s) regulating

both plant thermotolerance and disease resistance. Using virus-induced gene silencing (VIGS)-based genetic screening, we identified a thioredoxin-like 1 (TRXL1) gene involved in plant nonhost disease resistance and thermotolerance. TRXL1 is reduced, partly degraded via proteases and proteasome, and alters its chloroplast localization during heat stress. TRXL1 interacts with more than 400 proteins, including chaperonin CPN60A, caseinolytic protease (CLPC1), and NADP-dependent malate dehydrogenase (NADP-MDH). Chaperonin 60A (CPN60A) guards TRXL1 from degradation, whereas CLPC1 degrades TRXL1 during heat stress. TRXL1 regulates NADP-MDH activity, leading to an increase in malate level and inhibition of superoxide radical formation. We show that CPN60A and NADP-MDH positively regulate nonhost resistance, and CPN60A positively and CLPC1 negatively regulate thermotolerance. This study shows an antagonistic post-translational regulation of TRXL1 by CPN60A and CLPC1 and regulation of MDH by TRXL1, leading to plant disease resistance and thermotolerance. Furthermore, we have identified a chloroplastic factor that increases RuBisCO level, CO2 assimilation, starch content, plant biomass, yield and plant energy levels. We have also identified nuclear GTPase involved in regulation of stomatal transpiration via regulation of ABA and confers drought tolerance.

GS 2A-8. Early Detection of Chemical-Induced Temporal Stress Signatures in GMO and Non-GMO Maize

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Plants sense and respond to subtle environmental perturbations. The effects of small changes in the environment can be detected through the changes to plant physiology and biochemistry using molecular and imaging analytical techniques. Therefore, plants can be used as a sensor to detect anthropogenic changes in the environment, including the presence of unwanted chemicals. One chemical class of concern is engineered nanomaterials (ENMs) and, in particular, carbon nanostructures. This project investigates using plants to monitor the environment for the presence of carbon nanostructures. The whole plant effects of carbon nanostructures are examined in conventional maize with the use of phenotyping, mass spectrometry, RNA sequencing, and imaging technology. The approach employed monitors molecular signatures to enhance the ability to detect carbon nanostructures. One aspect of this project is determining the effects of carbon nanostructures on the composition of secondary plant metabolites. Secondary plant metabolites are phytochemicals that can be either non-volatile or volatile; both are currently being investigated. Another emphasis of this project is to identify the carbon nanostructure-induced molecular changes at the transcriptional and metabolite level. Currently, these changes cannot be monitored at a distance, however, a better understanding of the effects of carbon nanostructures on plant metabolism will provide new targets for monitoring and the opportunity to design targeted sensors. Imaging techniques and other carbon nanostructure detection techniques will later be explored. The end goal of this project is to use plants as indicators through monitoring phytochemicals to alert to the presence of carbon nanostructures in the environment.

GS 2A-9. Cold stress-induced changes in the regulation of specific lipid classes during the early stages of germination in upland cotton (Gossypium hirsutum L.)

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Specific classes of lipids are mobilized to support the major structural and metabolic events occurring in seeds during germination. In this study, we investigated the effects of cold stress on the regulation of specific lipid classes during the early germination stages of cotton. Lipid profiling was conducted using liquid chromatography with tandem mass spectrometry on intact seeds that were imbibed for 3 hours at 30°C (normal) and 12°C (cold-stress). Lipidomic data analysis identified a total of 7,971 lipid molecules belonging to six classes i.e. glycerophospholipids, sphingolipids, sterol lipids, glycerolipids, fatty acyls and prenol lipids. At normal temperature, a fold-change increase in all the detected glycerophospholipids and sphingolipids was observed in seeds after 3 hours of imbibition. Additionally, a fold-change decrease in diacylglycerols (DAGs) and monoacylglycerols (MAGs), as well as free fatty acyls was observed. In contrast, no significant fold-changes except an increase in PA were detected in the major lipid classes under cold stress. The rapid influx of water during imbibition triggers cell membrane reorganization from a porous, hexagonal II configuration to a more stable, lamellar structure. This transition requires the incorporation of phospholipids into the expanding membrane and the formation of microdomains by the sphingolipids which increase the biophysical ordering of membranes. The critical roles of glycerophospholipids and spingolipids during membrane reorganization may account for their increase in the seeds after 3 hours of imbibition. The reduction in DAGs and MAGs may be due to the requirement for glycerol-3-phosphate backbone of these lipids that are necessary for the synthesis of phospholipids. Under cold stress, the membranes are forced into a rigid configuration, preventing the incorporation of glycerophospholipids and the activity of spingolipids. The numerical reduction in phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol levels indicates a phospholipase D-mediated breakdown of these phospholipids, resulting in the significant fold-change increase in phosphatidic acid.

General Session (GS) 2B (Theme: Plant Omics)

GS 2B-1. New insights into CLAVATA-WUSCHEL signaling in the maize inflorescence meristem using single-cell RNA sequencing (scRNA-seq)

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CLAVATA (CLV)-WUSCHEL (WUS) pathway plays a crucial role in regulating stem cell maintenance. However, the molecular signatures associated with this pathway remain to be fully revealed in maize. Genetic studies of fasciated ear mutants have identified critical CLV-

WUS signaling components that control ear inflorescence meristem proliferation. Yet, understanding the underlying molecular mechanism requires cellular resolution insights. Our recent scRNA-seq profiling of the developing maize B73 ear (Xu et al., Dev Cell, 2021) shows this technology's power in identifying markers of diverse cell types and how scRNA-seq data may facilitate maize genetic studies. However, we did not detect CLV3 and WUS orthologs (ZmCLE7 and ZmWUS1) in that study due to their low representation. Thus, we finely dissected developing B73 ear tips (~ 600 μm), including inflorescence and initiating spikelet pair meristems, and further profiled ~10,000 single cells. The resulting scRNA-seq atlas identified a distinct ZmCLE7 marked stem-cell cluster and cell populations expressing ZmWUS1. Using scRNA-seq gene co-expression networks, we identified ~ 200 ZmCLE7- and ~ 60 ZmWUS1- co-expressed markers. These included many known maize meristem regulators, such as ZmLONELY GUY7 (ZmLOG7) and BARREN INFLORESCENCE4 (BIF4), and novel markers, such as a homolog of Arabidopsis AINTEGUMENTA-LIKE7 (AIL7). We also profiled ~9,000 ear tip cells from a fasciated ear double mutant, Zmcle7;fasciated ear3 (Zmcle7;fea3), that has extreme meristem proliferation. We detected mis-expression of ZmWUS1 in the epidermis cell cluster. The expression of ZmCLE7 was also significantly expanded in Zmcle7;fea3 clusters compared to wild type B73. We validated these results by mRNA in situ hybridization. Together, we generated valuable scRNA-seq resource of maize ear tips to identify candidate new regulators and provided cellular resolution insights into CLV-WUS signaling to maintain ear meristem.

GS 2B-2. Simultaneous genome-wide and targeted single-molecule analysis of chromatin accessibility and DNA methylation in plants using MAPit

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DNA methyltransferase accessibility protocol for individual templates (MAPit) localizes DNA-protein interactions by probing nuclei with exogenous methyltransferases. MAPit enables simultaneous determination of chromatin accessibility and DNA methylation in one assay, and provides a unique opportunity for high-throughput targeted analysis on chromatin accessibility in user-defined regions. In plants, the physiological architecture and multiple sites of endogenous cytosine methylation (CG/CHG/CHH) previously prevented the use of MAPit to map epigenetic responses. Here, we optimize the MAPit protocol for plant tissues and perform both whole-genome bisulfite sequencing (WGBS) and targeted sequencing of captured regions in Arabidopsis seedlings experiencing ambient and cold temperature. MAPit-WGBS identified differentially accessible regions (DARs) enriched with known cold-regulated motifs. The joint investigation of DNA methylation and chromatin accessibility afforded by MAPit revealed that CHH methylation patterns differed from that of CG and CHG within DARs in response to cold. Targeted assays visualized the shift of consecutive accessible regions in a small proportion of molecules and allowed co-localization of DNA methylation and chromatin accessibility in the same molecules. In conclusion, MAPit is a powerful tool for comprehensive analysis of epigenetic alterations that can be applied in both genome-wide and targeted manners in plants.

GS 2B-3. Analyzing the Effect of Heat and Cold-inducible CRISPR/Cas9 on Targeting Efficiency in the Rice Genome

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Targeted mutagenesis by CRISPR/Cas9 with reduced off-target effects is an important aspect of genome editing. In the current study, we compared the targeting efficiency of Cas9 expressed under constitutive (Ubi::Cas9), cold-shock (AtRD29a::Cas9), or heat-shock (HS::Cas9) promoter on Target of Rapamycin (TOR) gene. The TOR gene is known to regulate various anabolic processes such as cell cycle, ribosome biogenesis, and photosynthesis. We targeted the HEAT repeat regions (sg1) and kinase domain regions (sg2) of the gene. In Ubi::Cas9, 21 primary transgenics (T0) plants representing 9 lines were analyzed for the targeted mutations. The sg1 site was WT, while monoallelic or biallelic mutations were observed in 4 plants representing 3 lines in the sg2 site. The 33 T1 plants studied from three T0 plants, showed that 22 (66%) T1 inherited the mutations at sg2; but no de-novo mutations in the sg1 site were observed. In HS::Cas9 and AtRD29a::Cas9 lines, none of the 40 T0 plants which represents 17 lines exhibited mutations at room temperature in either sg1 or sg2. The expression analysis of a subset of these lines, showed 2-11x and 2-43x induced transcript levels of Cas9 in HS::Cas9 and AtRD29a::Cas9 lines respectively, indicating a proper regulation of the Cas9. The analysis of heat- or cold-induced mutations in these lines will be performed in the T1 progeny.

GS 2B-4. Assessing the Nucleotide-Level Impact of Spaceflight Stress using RNA-Sequencing Data

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Space is an exciting frontier that presents unique environmental stressors like microgravity and space radiation. It is difficult to study the impact of spaceflight on terrestrial life due to the limited resources of the International Space Station (ISS). NASA created Genelab, a public Omics database for spaceflight relevant data, which gives scientists access to data without needing a new experiment on the ISS. Transcription profiles are among the most common data type available on Genelab, and in the age of Next Generation Sequencing, this commonly means RNA-Sequencing data. A pipeline finding new information out of the public RNA-Seq data would be useful, especially in a case like this where the available data is extremely limited. We developed and utilized a pipeline analyzing Genelab's RNA-Sequencing data from Arabidopsis thaliana for sequence variants. RNA-Sequencing data is not the preferred method to call variants due to an associated high false discovery rate, however recent studies show it can be done with appropriate precautions. Our pipeline incorporates steps to combat factors leading to RNA Seq's high false variant discovery rate including 2-pass mapping methods and stringent filters. Our hypothesis is that space's environment will cause a higher number of variants to be called in the spaceflight A. thaliana samples compared to those on the ground. Preliminary results show A. thaliana samples from space tend to have higher variant counts than those from the ground, showing the damage spaceflight can have at the nucleotide level. Further, this demonstrates that our analysis pipeline can use RNA-Seq to acquire additional information on nucleotide sequence variation from abiotic stressors like microgravity and space radiation. Findings from this research can lead to a better understanding of what future precautions are needed to ensure the safety of those in space.

GS 2B-5. WRI1 and DGAT1 overexpression in soybean embryo alters oil composition and starch metabolism

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Soybean commercial value is directly linked to its seed quality, especially its oil content. The numerous applications for its oil generates a big demand for cultivars with increased levels of seed lipids. Strategies for oil optimization include both breeding and genetic engineering. In this work, we evaluate the behavior of transgenic soybean lines that overexpressed two genes known to control oil accumulation in oilseeds. WRI1, a master regulator in transcriptional control of oil biosynthesis, and DGAT1, considered a rate-limiting enzyme for triacylglycerol accumulation, were used to "push" and "pull" lipid production. Seed specific promoters were incorporated to restrict their expression only to embryo development. The biomass analysis of the transgenic mature seeds revealed that the total oil content was not increased but changes in the fatty acid composition and sucrose and starch content were observed. To have a better understanding of how the embryo metabolism was altered by the overexpression of these two genes, biomass and targeted metabolomics studies were performed at different points during seed development. The analysis showed that starch

content and intermediates of starch biosynthesis were increased in the transgenic lines. These results form new questions regarding the role of the transient accumulation of starch during soybean development and how it is linked to the oil production. The new insights obtained in this work will be valuable for researchers when designing new strategies for oil improvement in soybean.

GS 2B-6. Dissecting the Genetic Architecture of Source-Sink Regulated Senescence in Maize

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Senescence is a complex developmental process regulated by a number of internal and external cues. The onset of premature senescence due to the absence of a strong sink, termed source-sink regulated senescence (SSRS), offers a unique opportunity to tease apart the role of sugar partitioning and signaling in senescence. To understand the genetic architecture and the underlying molecular mechanisms, we have completed a systems genetic analysis of SSRS in maize. Through characterization of the natural diversity for SSRS in a diversity panel and a biparental population, we have identified several genomic regions and the underlying candidate genes. To further confirm these findings, we performed time-course transcriptome and metabolic characterization of SSRS sensitive (B73) and SSRS resistance (Mo17) inbred lines. The metabolic analysis revealed that SSRS is associated with differential accumulation of sugars, cytokinin and abscisic acid activity, and hexokinase activity. We have validated two of the quantitative trait loci (QTL) in near-isogenic lines and fine mapping of these QTL is underway. Remarkably, natural diversity analysis resulted in the identification of a cathepsin B-like protease encoded by ccp4 as an important gene regulating SSRS. Further support to such a role was provided by the analysis of natural allelic variation of ccp4 in diverse maize inbred lines and by overexpression in the maize ccp4 in Arabidopsis. Confirmation of these findings through antibodies targeting CCP4 is currently underway. Finally, we have developed a novel clustering algorithm inspired by the features of hypothesis testing that allows the identification of significant genomic regions through the integration of the data from different omics systems. Characterization of the novel genes and pathways from this study will enhance the mechanistic understanding of senescence.

GS 2B-7. 'At-home' labs for Plant Molecular Biology

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The Covid 19 pandemic has caused significant change in how society functions, this includes how we teach the next generation vital skills for continuing on in the sciences. Although virtual simulations are excellent for teaching the basic premise of laboratory techniques,

they cannot replace hands-on experience. The required social distancing measures and need for several students to stream coursework left many of us in academia bewildered during the past year as to how to ensure our students get experiential hands-on learning in such an environment. A series of molecular biology labs were developed that could be done at-home or in the lab. The 'at-home' labs enabled streaming students to perform and analyze restriction digests, ligations, and loop-mediated isothermal amplification using pre-prepared molecular kits that were shipped to their homes or picked-up from the university. Kits included minimal equipment and supplies. These labs taught students critical molecular techniques and application of molecular theory. In conjunction with these at-home labs, students were tasked to develop a novel diagnostic testing strategy for Tomato Mosaic Virus (ToMV) using techniques acquired through the course. The combination of in-lab and at-home molecular techniques taught during the lab course provided students with a broad frame-work to design testing strategies applicable in both lab and field settings. ToMV was chosen due to importance of this virus in agriculture and similarities ToMV shares with Covid 19. Specifically, both ToMV and Covid 19 are positive single stranded RNA viruses with limited treatment and prevention options. These similarities highly engaged students and led student groups to design novel ToMV testing strategies.

GS 2B-8. A role of lipid transfer protein in plant defense revealed by redox proteomics

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Redox-based post-translational modifications (PTMs) involving protein cysteine residues as redox sensors are important to various physiological processes. However, little is known about redox-sensitive proteins in guard cells and their functions in stomatal immunity. In this study, we applied an integrative protein labeling method cysTMTRAQ and identified guard cell proteins that were altered by thiol redox PTMs in response to a bacterial flagellin peptide flg22. In total, eight, seven and 20 potential redox-responsive proteins were identified in guard cells treated with flg22 for 15, 30 and 60 min, respectively. The proteins fall into several functional groups including photosynthesis, lipid binding, oxidation-reduction, and defense. Among the proteins, a lipid transfer protein (LTP)-II was confirmed to be redox-responsive and involved in plant resistance to Pseudomonas syringe pv. tomato DC3000. This study not only creates an inventory of potential redox-sensitive proteins in flg22 signal transduction in guard cells, but also highlights the biological relevance of the lipid transfer protein in plant defense against bacterial pathogens.

General Session (GS) 2C (Theme: Plant Biotic interactions)

GS 2C-1. Poison ivy urushiol levels showed variable allometric tradeoffs and were not inducible by signals of microbial pathogenesis or insect herbivory

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Urushiol is the poison ivy (Toxicodendron radicans) natural product responsible for the characteristic "poison ivy rash" allergenic dermatitis symptoms on human skin. The anthropocentric obviousness of urushiol as an effective chemical defense is not mirrored by a corresponding aversive dermatological (or any other physiological) reaction in native/domesticated faunal species. This study posited that urushiol may be an inducible chemical defense against either microbial pathogens or insect herbivory. This was investigated by treating axenic poison ivy seedlings with the phytohormones Salicylic Acid (SA) or Methyl Jasmonic Acid (MeJA) as proxies for microbial pathogenesis or insect herbivory. First true leaves from axenic poison ivy seedlings treated with SA or MeJA showed comparable urushiol accumulation levels relative to ethanol treated control plants. The hypothesis of urushiol acting as a chemical defense against insect herbivores was tested more directly by measuring insect herbivory area on naturally recruited poison ivy leaves and their respective leaf urushiol levels. There was no significant correlation between insect herbivory levels and urushiol levels. Another topic of investigation was allometric relationships between urushiol levels and several plant biometrics. Poison ivy leaves showed significant differences in both C15- and C17-urushiol levels between individual plants. Overall, C15- and C17-urushiol levels were positively correlated in individual plants, but between plants there was a significant negative correlation of C15-urushiol levels with increasing C17-urushiol levels. Most, but not all, sampled vines did not indicate a metabolic cost to leaf area associated with foliar urushiol levels, suggesting leaf area was often buffered from the metabolic cost of urushiol production. However, two vines had significantly reduced leaf area associated with C15-urushiol levels, indicating the aforementioned buffering is not absolute. The hypervariability of steady state urushiol levels was not consistent with an effective chemical defense against vertebrate herbivores exerting purifying selection on the urushiol trait.

GS 2C-2. Investigate the crosstalk between plant regeneration and immunity

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Plants have a profound ability to regenerate after tissue damage. Regeneration of adventitious roots or shoots from leaf explants or stem cuttings lays a foundation to propagate valuable agricultural and horticultural crops. Plants must integrate physiological and environmental cues to complete this dramatic and sophisticated program of cell fate transition. Most studies on mechanisms of tissue regeneration are performed in sterile conditions. Thus, our knowledge about how biotic stresses influence tissue regeneration is very limited. Here, we systematically examined the wound-induced de novo root regeneration (DNRR) in mutants involved in plant immunity and found that endogenous salicylic acid (SA) suppressed DNRR. SA accumulation was rapidly induced by wounding, leading to an activation of a sector of SA response. Analysis of mutants involved in SA biosynthesis, metabolism, and signaling revealed that DNRR required a distinct SA pathway from the SA-mediated defense. Furthermore, SA did not interfere with auxin biosynthesis in response to wounding but inhibited the transport of auxin from distal tissue to the wounding site. In addition, we established an experimental system to study DNRR with microbes. We observed distinct requirements of SA signaling in the presence and absence of environmental microbes. We propose that the SA pathway integrates intrinsic and extrinsic signals to coordinates tissue regeneration.

GS 2C-3. PCB1, a membrane-localized calcium-binding protein modulates green peach aphid resistance in Arabidopsis

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Myzus persicae, more commonly known as green peach aphid (GPA), is a major pest affecting many crop species around the world and a vector of multiple viral diseases. PAD4-DEPENDENT CALCIUM-BINDING PROTEIN1 (PCB1) is a calmodulin-like protein-encoding gene that gets upregulated upon GPA infestation. The GPA-induced expression of PCB1 is PAD4 dependent. Constitutive overexpression of PCB1 restores resistance to the GPA in the pad4 mutant background. In contrast, constitutive overexpression of PAD4 is unable to restore resistance in the pcb1 mutant, thus indicating that PCB1 function is required for PAD4-conferred resistance to the GPA. Electrical penetration graph (EPG) analysis indicates that like PAD4, PCB1 is involved in limiting the GPA feeding from sieve elements. Expression of the bacterial UidA-encoded GUS reporter from the PCB1 promoter indicated that GPA feeding induces GUS expression in the phloem and surrounding cortex tissues. Furthermore, the deposition of callose in the sieve elements in response to GPA infestation was weaker in the pcb1 mutant, thus further implicating a role for PCB in promoting defenses against the GPA in the phloem. Out of about 50 calmodulin like genes annotated in Arabidopsis, only PCB1 and a closely related homologue of it have predicted N-terminal transmembrane domains. In agreement with this prediction PCB1-GFP fusion protein was found to be localized to plasma membrane. Radioactive 45Ca2+ overlay assay on purified PCB1 protein confirmed that it is a Ca2+-binding protein. Mutational analysis confirmed the requirement of EF hand domains in PCB1 in binding Ca2+. PCB1 binds calmodulin 1 (CaM1), a known regulator of plasma membrane bound cyclic nucleotide-gated calcium cannels. The localization of PCB1 to plasma membrane, its ability to bind Ca2+ and interact with CaM1,

prompts us to hypothesize that PCB1 has a role in GPA infestation-induced Ca2+ signaling possibly by modulating the activity of Ca2+ channels.

GS 2C-4. Peptidase-controlled rhythmic gene activity translates into functional patterns in systemic immunity

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Repetitive patterns in the spatial and temporal dynamics of signaling molecules can determine the transmission of information through biochemical networks and coordinate system-level functions of an organism. Our overarching aim is to identify molecular redox-modulated oscillatory systems and characterize their spatiotemporal dynamics as a way to unravel the mechanisms by which rapid immune responses at the site of pathogen infection are uncoupled from, and determine, the long-lasting systemic immune response and innate memory in the plant. Analyses of immune redox markers in Arabidopsis wild type and peptidase mutants (top1, top2, top1top2) with altered dynamics of oxidative bursts revealed remarkable synchronicity between local and systemic waves of ROS-sensitive mRNA accumulation in wild type, which was partially or entirely lost in the mutants. The peptidase-controlled pulses of redox gene expression correlated with functional patterns associated with establishing and implementing the plant's systemic immunity. A large-scale analysis of spatial and temporal patterns of gene expression covering over 700 defense-related genes. These experiments produced quantitative information on the targeted gene set and outlined gene activity patterns within a wide SAR signaling interval. Our results are consistent with a model whereby plants utilize redox-controlled biochemical pathways to generate temporal patterns of gene expression, such as oscillations and pulses. Moreover, a proteostasis-based mechanism regulates the amplitude and period of oscillations; signal synchronicity is required to establish the SAR state and may represent the core of the immune memory.

GS 2C-5. Investigate the molecular mechanisms by which plant growth-promoting bacteria, Azospirillum brasilense, mediate salt stress tolerance in rice

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Abiotic factors, such as salt stress, heat and drought stress, and nutrient deficiency, are a major concern for crop productivity. For instance, most of our food crops, such as rice and maize display severe yield losses (50-80%) under moderate to extreme salinity. Problems

associated with soil salinity are anticipated to worsen because of climate change. For improving crop performance under saline conditions, we need to implement sustainable agricultural strategies. One option is to take advantage of beneficial plant-microbe associations. Plants can form associations with different beneficial microbes including, arbuscular mycorrhiza, rhizobia bacteria, plant growth-promoting bacteria (PGPB). Several studies have suggested that PGPB improve plant growth via multiple mechanisms, including biological nitrogen fixation, hormone synthesis, protection against biotic and abiotic stresses, etc. Azospirillum brasilense is one of the most studied PGPB for the mitigation of salinity stress in different crops such as maize and wheat. However, not much is known about the molecular mechanisms by which A. brasilense mitigates salt stress. Recently, we optimized an experimental system where rice growth was improved in A. brasilense-inoculated plants compared to the uninoculated plants when these were grown under high salt concentration (200 mM NaCl). Currently, I am investigating the expression pattern of a salt-sensitive reporter gene (OsCam1-1) in rice plants inoculated with or without A. brasilense and grown under high salt concentration. In the future, I will perform an RNA-seq experiment to identify the transcriptomic responses in rice plants during A. brasilense-mediated salt stress tolerance. Overall, the results from this project will provide important insights into salt stress mitigation in rice by A. brasilense.

GS 2C-6. Multivariate RNASeq reveals the depth and breadth of the transcriptional response of Medicago truncatula to its rhizobial symbiont

Elise Schnabel, Yueyua Gao, William Poehlman, Suchitra Chavan, Rabia Elhawaz, Alex Feltus, and **Julia Frugoli*,** Clemson University, Clemson, SC

The legume rhizobia symbiosis, in which rhizobia inhabit the roots of legume plants and fix nitrogen from the atmosphere in exchange for carbon from photosynthesis, is an example of complex signaling between species from two different kingdoms of life over both space (soil, root, shoot) and time (rhizobial encounter to emerging nodules in our growth system is 72 hours). Understanding what is occurring at the transcript level in roots responding to rhizobia has been pursued through evolving technologies, but often these choose one or two points in time and growth conditions are not standardized, complicating the analysis and the comparison of data sets. Our analysis uses five time points, a narrow band of root tissue to separate nodulation signaling from growth signals, and comparisons with two Autoregulation of Nodulation mutants to uncover almost 2000 differentially expressed genes (DEGs) in early nodulation signaling. We examined their pattern of expression, as well as determining genes differentially expressed in the sunn4 and rdn1-2 AON mutants responding to rhizobia versus wild type plants responding to rhizobia at the same point in time. We also gained insight into the nature of the AON rhizobia response. This work is supported by NSF IOS 1444461 to Frugoli and Feltus.

GS 2C-7. A new approach to discovering genes inhibiting nodulation

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In response to rhizobial signals, legumes form nitrogen-fixing nodules in roots, allowing the plants to grow efficiently in N-deprived environments. Because nitrogen fixation is an energy-intensive process, plants use an autoregulatory feedback mechanism, triggered by nodule formation or external nitrogen supply, to control the number of nodules that form, called Autoregulation of Nodulation (AON). When legumes are exposed to rhizobia, only a portion of the lower root - the root hair maturation zone - is able to initiate nodule formation. Once AON has been triggered, this ability is lost. Understanding how this occurs is crucial to understanding the mechanism that enables nodule number optimization. We performed RNA-Seq analysis of the root hair maturation zone from Medicago truncatula wild type plants with and without AON triggered and the AON mutant sunn-4. We applied a differential expressed gene (DEG) analysis between +/- AON and between genotypes to identify candidate genes responsible for inhibiting nodulation. Several genes appear to be highly regulated in response to rhizobia. This work is supported by NSF IOS 1444461 to Frugoli and Feltus.

GS 2C-8. A toolbox of genetically engineered rhizobia for tracking infection in nitrogen fixing symbiosis in Medicago truncatula

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Leguminous crops can grow in nitrogen-depleted soils because of their ability to stablish a symbiotic association with nitrogen-fixing soli bacteria called rhizobia. This Symbiotic Nitrogen Fixation (SNF) process provides legumes with reduced nitrogen (N_2) in the bio-accessible form of ammonium (NH_4^+). A close relative to alfalfa, *Medicago truncatula*, is a broadly used genetic model to explore SNF. Subsequent to chemical signaling between *M. truncatula* and its symbiont, *Sinorhizobium meliloti*, specialized organs called root nodules develop on the root, providing a niche for rhizobia to fix N_2. SNF is a complex process requiring coordinated regulation of thousands of plants and bacterial genes. Several functional genomics resources have been developed in *M. truncatula* to investigate SNF including the genome sequence, transcriptomic datasets, and mutant collections. The tobacco retrotransposon (*Tnt1*)-insertion mutant population is a valuable resource for identifying new genes essential for SNF. Through forward genetic screening of the *Tnt1*-insertion mutant collection, a nodule-specific PLAT (Polycystin-1, Lipoxygenase, Alpha-Toxin) domain-encoding gene, *MtNPD1*, was identified and linked to a symbiotic defect. Nodules of *npd1* mutant are small and ineffective, where rhizobia fail to fix N_2 and quickly degrade. Importantly, the *npd1* mutant responds distinctly to different symbiotic partners, suggesting a putative role of MtNPD1 in host-strain specificity at the nitrogen fixation level (Pislariu CI, *et al*. 2019). To thoroughly analyze the Npd1-dependent host-strain compatibility, a biparental mating strategy was used to deploy different constitutively expressed reporters, including *hemA:LacZ* and four different fluorescent markers (GFP, YFP, CFP, and RFP), into twelve rhizobial strains. The genetically engineered rhizobial strains are being used in single inoculations and co-inoculations to better understand infection dynamics, rhizobial fate, and nitrogen fixation efficiency in the background of *npd1* mutant. Representative results will be presented.

GS 2C-9. Investigating the effects of BARELY ANY MERISTEM (BAM) genes on nodulation in Medicago truncatula

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The legume rhizobia symbiosis is negatively regulated by the host plant through the autoregulation of nodulation (AON) pathway. AON is a long-distance root-to-shoot-to-root pathway, enabling the plant to limit the number of nodules formed on roots. Central to the shoot response is a receptor complex involving a leucine-rich-repeat receptor-like kinase called MtSUNN. Mutations in SUNN, which has high sequence similarity to Arabidopsis CLAVATA1 (CLV1), result in a supernodulation phenotype. CLV1 controls stem cell populations in both the root and shoot, and in conjunction with other receptors including CLV2 and CRN, binds to CLE peptides as ligands. The CLV1 phenotype can be rescued by overexpression of members of the Arabidopsis BAM proteins, which also bind to CLE peptides and function as positive regulators of meristem development. In M. truncatula, the AON pathway includes a CLV1-like regulatory model: the SUNN receptor binds MtCLE peptides, and forms heterodimers with MtCLV2 and MtCRN. We reasoned that AON could also involve BAM proteins. To determine if any of the five MtBAMs function in AON, we have isolated mutants in each gene, tested their nodulation phenotypes, and are both overexpressing the genes in the sunn-1 mutant and making double mutants of each MtBAM with sunn-5 for phenotypic analysis. This work is supported by NSF IOS#1733470.

General Session (GS) 2D (Theme: Plant Development, Physiology & Metabolism)

GS 2D-1. Flavonoids play a role in resistance to accumulation of aflatoxin in corn

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Aspergillus flavus is a facultative pathogen capable of producing aflatoxins (AF), potent carcinogens that accumulate in corn kernels, peanuts, cottonseed and tree nuts. To understand resistance mechanisms in corn to AF accumulation we performed a high-throughput genomics study using an in vitro kernel screening assay with A. flavus 3357, resistant corn hybrid TZAR102 and susceptible corn hybrid Va35. We determined that corn genotype, fungal treatment and duration of infection significantly co-vary to influence the overall gene expression patterns. We performed gene ontology enrichment analysis on highly significant genes and found enrichment of pathways linked to fungal and microbial responses such as Pathogenesis-related (PR) proteins. To determine additional genes of interest using field and gene expression data, we linked genome-wide association analysis results with gene expression data, allowing us to detect significant expression quantitative trait loci (eQTL). Our results showed that resistance to aflatoxin contamination is associated

with specific flavonoid biosynthetic pathway genes. Additional experiments including functional genomics analyses and fungal bioassays to identify the role of flavonoids and their contribution to corn resistance to A. flavus growth and AF production will also be presented.

GS 2D-2. Exploring phosphate distribution in the Arabidopsis root reveals a developmental control of uptake, assimilation and sequestration

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Inorganic phosphate (Pi) is an essential macronutrient for plant metabolic activities, but its low availability in soil is a limiting factor for their growth and reproduction. Although the use of Pi-fertilizers can overcome this deficiency, this practice has both environmental and economic costs. Therefore, our understanding of how plants acquire, store, recycle, and distribute this essential nutrient is critical for growing crops with lessor no fertilizer use. Till date, this study has been limited by the inability to monitor Pi concentrations with sufficient spatial and temporal resolution. To address this limitation, we measured cytosolic Pi concentrations in different cells, tissues, and developmental zones of the Arabidopsis root using a genetically encoded FRET-based Pi sensor. Microinjection was used to calibrate Pi-dependent FRET signals for absolute quantification of Pi concentrations. Steady-state Pi concentrations varied between developmental root zones with the highest levels in the transition zone, whereas equivalent concentrations were measured in epidermis, cortex, and endodermis within each zone. Pi concentrations in all zones were reduced by Pi starvation with varied kinetics, but the overall developmental pattern persisted. Multiple processes could influence this concentration gradient, including uptake from the environment, assimilation to ATP, recycling via metabolism, and sequestration in organelles. We used cyanide treatment that blocks Pi assimilation to distinguish uptake from metabolic recycling in wild-type and vacuolar Pi uptake mutant, pht5;1. Spatial differences in these activities were observed between zones after cyanide treatment. However, only vacuolar sequestration was found to be responsible for the pattern of Pi distribution. These results highlight the complexity of Pi homeostasis at the cellular and subcellular levels, and provide the basis for future experiments to discern the role of the root Pi gradient on plant growth and development.

GS 2D-3. Substrate supply and laccase specificity drive lignin composition during the switch from G- to C-lignin accumulation in Cleome hassleriana

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Catechyl lignin (C-lignin) is a linear homopolymer of caffeyl alcohol (C-monolignol) that possesses unique properties as both a biomaterial and substrate for biological funneling to

small molecule precursors of chemicals. However, it appears to be limited to seed coats. Cleome hassleriana is emerging as a model system for studying C-lignin biosynthesis because the lignin synthesized in the seed coat switches from classical guaiacyl (G) lignin to C-lignin at around 13 days after pollination (DAP). Here, we coupled targeted metabolite profiling and isotopic precursor labeling experiments to examine the provision and utilization of monolignols in Cleome seed coat. We show that, whereas lack of synthesis of C-monolignol limits C-lignin formation prior to around 12 DAP, coniferyl alcohol (G-monolignol) is still synthesized and accumulated after 14 DAP, even though the classical route to its synthesis has been suppressed. However, this G-monolignol is not incorporated into lignin, because C-monolignol is a strong competitive inhibitor of the oxidization of G-monolinol by seed coat laccases. Among potential C-lignin related laccases, recombinant ChLAC8 has significant level of activity with C-monolignol, but cannot oxidize G-monolignol. Glutamin289 of ChLAC8 appears to be critical for binding of C-monolignol. Suppression of ChLAC8 expression led to significantly reduced C-lignin content in the seed coats of transgenic plants. Feeding of C-monolignol to the Arabidopsis thaliana caffeic acid O-methyltransferase (comt) mutant expressing ChLAC8 led to appearance of C-lignin with over 5% of total lignin. ChLAC8 possesses the unusual property of oxidizing C-monolignol but not G-monolignol and plays a critical role in initiating C-lignin polymerization. We propose that, during the period of C-lignin biosynthesis, the seed coat possesses a mechanism to maintain levels of coniferyl alcohol while blocking its formation and polymerization through the classical monolignol pathway. This coupled with laccase specificity, determines the metabolic fate of G- and C-monolignols.

GS 2D-4. Unravelling the role of pennycress (Thlaspi arvense L.) proteins in the modulation of neutral lipid droplet abundance

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The finite nature of crude oil-derived fuels coupled with their adverse effects on the environment means the search for alternative, renewable sources of energy that are more environmentally friendly is paramount. Pennycress (Thlaspi arvense L.) has been identified as a promising alternative crop for aviation fuel production. It is an annual winter Brassicaceae which produces seeds with high oil content (26-39%). The average yield of pennycress seeds is 1,500 kg ha–1, corresponding to 600–1200 L ha–1 of oil, which is higher than that of soybean and camelina. While pennycress benefits from the fully sequenced genome and research tools of the closely related model plant Arabidopsis thaliana, there are still significant challenges associated with establishing gene function that would make pennycress much more valuable as a bioenergy oilseed crop. Transcriptional analysis of 22 pennycress accessions resulted in the identification of potential gene candidates whose expression levels were correlated with seed oil yield. Here, we show that protein products of six of these candidate genes- a lipid transfer protein homolog (LTP6), a lipid droplet associated protein homolog (LDAP3), an annotated lipase (α/β hydrolase), a long-chain acyl-coA synthase protein (LACS1), an endomembrane regulatory protein (RABA3), and a lipid

storage and packaging protein (Oleosin)- mainly localize to lipid droplets when transiently expressed in Nicotiana benthamiana. The overexpression of coding sequences for these six proteins in N. benthamiana leaves resulted in a proliferation of cytoplasmic neutral lipids droplets. Analysis using GC-MS also indicated that the overexpression of these proteins increased the total neutral fatty acid content and somewhat altered the fatty acid composition of the infiltrated leaves. Our data point to possible roles of these six candidate proteins in the compartmentalization and/or stability of pennycress lipid droplets and represent interesting targets for genetic manipulation of pennycress seeds with increased oil content.

GS 2D-5. The power of engineering organelle movements in plants

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Organelle movements in plants mainly rely on Myosin class XI motor proteins and actin filament cytoskeleton. Other factors, such as organelle-organelle interactions and hydrodynamic flow, are also considered to contribute to organelle movements, but it is largely unknown how these multiple components affect different organelles' movements. We hypothesized that each organelle group has a different mechanistic signature underpinning their movements and, in turn, play a different role in creating the overall environment for organelle movements. In order to understand the movements of specific organelle groups, we generated 'Booster' and 'Anchor' constructs in agrobacterium binary vectors. Each construct was designed to enhance or suppress the targeted organelle's movements by expressing an organelle specific membrane marker with either the DUF593 domain from MyoB1, an adaptor protein for Myosin XI, or a microtubule binding domain, respectively. We targeted peroxisomes, endoplasmic reticulum (ER), and mitochondria separately to profile their movements in the leaf epidermal cells of Nicotiana benthamiana. In addition, we examined movements of other, non-targeted organelles including Golgi stacks in cells expressing Booster or Anchor constructs. In all three targeted organelles, we observed a significant increase of linear movements, with their corresponding Booster expression, both in terms of linear speeds and run lengths. Interestingly, both ER and mitochondria Boosters also enhanced linear movements of other non-targeted organelles, whereas peroxisome Boosters were selectively effective in increasing ER movements. By contrast, Anchor expression strikingly diminished the linear movements of targeted organelles, accompanied by morphological alterations especially in mitochondria and ER. No significant effects of Anchors on non-targeted organelles were found, except for a small reduction of ER movements by peroxisome Anchors. Collectively, these Booster and Anchor data suggest that organelle movements in plants are coordinated among different types of organelles although the degree of coordination differs between the organelle groups.

GS 2D-6. The correlation between the bract and floral symmetry evolution in angiosperms

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During reproductive growth, flowers usually develop from the axial position of a modified leaf-like structure, known as bract. Developmental studies indicated that the polarity established by the axis and bract is likely a structural requirement to influence the development of floral symmetry. Here, we investigate whether the presence of bract influences floral symmetry evolution using a phylogenetic approach. We extracted the character state information from floral diagrams representing phylogenetically diverse angiosperms with diverse flower morphologies from 1031 species representing 60 orders (95%), 223 families (52%). We analyzed these floral traits using six phylogenies based on published mega trees representing three different hypotheses of angiosperm evolution. The ancestral state reconstruction with a maximum-likelihood approach was used to analyze the evolutionary transition of 34 floral characteristics. Our analyses based on the six trees agreed with the fossils findings that bract is present in the most recent common ancestor (MRCA) of angiosperms, specifically a single abaxial one (98% - 99%), and lost many times independently across angiosperms. Importantly, we examined the correlation between bract location and floral symmetry, and found that the presence of floral zygomorphy is strongly correlated with the presence of a single abaxial bract ($p = 0.000$, ML in Mesquite). The transition rates between floral actinomorphy and zygomorphy specifically affected by the presence of bract based on a parameter restriction test using corHMM package in R. The regain of single abaxial bract is more common when flower is zygomorphy ($p = 0.029$), and floral zygomorphy evolve at a higher rate in the clades that possess the single abaxial bract (SAB) compared to the clade having no SAB (NSAB) (z-score = 4.27, $p = 9.591e-06$, ML in Mesquite). These results suggest that the bract and its location is likely key in promoting the origin and maintenance of floral zygomorphy during angiosperms evolution.

GS 2D-7. Evolution and function of floral symmetry genes in Solanaceae

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Comparative studies demonstrated that independent origins of floral zygomorphy are associated with the redeployment of CYCLOIDEA2 (CYC2)-like genes in core eudicots. It is, however, unclear whether parallel genetic mechanisms exist since there were an estimated 125 independent origins of floral zygomorphy in angiosperms. We have used a candidate gene approach to study the evolution, expression, and function of CYC2s in solanaceous species, with the intent to characterize the genetic basis of floral zygomorphy development in this family. Two major sequence types of CYC2 identified in Solanaceae, CYC2A and CYC2B, result from a gene duplication that occurred before the diversification of the family. In the zygomorphic-flowered species of Solanaceae, the CYC2A genes are expressed in androecium and gynoecium but with divergent spatial patterns. In contrast, CYC2B genes are expressed consistently in the dorsal region of the zygomorphic calyxes. Furthermore, RNA in situ

hybridization indicated that the CYC2As are expressed across the floral meristem at early stages and exclusively within the developing pollen sacs and ovules at later stages for both zygomorphic-flowered Schizanthus pinnatus and actinomorphic-flowered Solanum lycopersicum. Surprisingly, none of the CYC2 genes were found differentially expressed in the zygomorphic corollas of solanaceous species. Using virus-induced gene silencing (VIGS) to silence CYC2A and CYC2B simultaneously, we observed the mutant Solunum lycopersicum plants producing the pollens with significant short pollen tubes compared with the wild type. When both CYC2s are silenced in Nicotiana obtusifolia, the main morphological changes are the decrease of petal, sepal, and stamen from five to four. These findings suggest that CYC2-like genes likely play a role in meristem patterning, organ number regulation, and pollen development in Solanaceae. The molecular basis underlying the floral symmetry of Solanaceae is probably not CYC2 based, which suggests the convergence to floral zygomorphy with an unknown mechanism in this core eudicot clade.

GS 2D-8. The evolution of inflorescence and floral symmetry development in Solanaceae

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The inflorescences of Solanaceae are unique and complex, and this has led to long-standing disputes over floral symmetry, mainly due to different interpretations of inflorescence structure. The main disagreements stemmed from different interpretations of how the bract or bracteoles were arranged relative to the inflorescence axis and during the early flower initiation. Here we investigated the evolution of Solanaceae's inflorescences by analyzing inflorescence structures in the context of phylogeny using ancestral state reconstruction (ASR) to determine the evolutionary transitions between loosely arranged and tightly clustered inflorescences and between monochasial-like and dichasial-like cymes. Furthermore, we reconstructed two- and three-dimensional models for twelve solanaceous species representing both inflorescence and phylogenetic diversity in the family. Our results indicate that the most recent common ancestor (MRCA) of Solanaceae has a loosely arranged and monochasial-like cyme while tightly clustered inflorescences and dichasial-like cymes are derived. Compared to the known scorpioid cyme evolution process, Solanaceae achieved their scorpioid cyme-like inflorescences through an undescribed way of developing such inflorescences. Furthermore, we show that the development of floral zygomorphy in Solanaceae is also unique; the two leaf-like structures subtending the flower frequently have buds in the axillary position, which suggested that they are not the bract or bracteole directly subtending the flower. Together, we demonstrate that a better understanding of the morphological evolution of solanaceous inflorescence structure helps us clarify the floral development in this family.

GS 2D-9. Cottonseed extracts regulate cell viability and gene expression in human colon cancer cells

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Plant extracts have been used as medicinal remedy for various diseases. The objective of this study was to survey the effect of cottonseed extracts on cell viability and gene expression in human colon cancer cells. COLO 225 cells were treated with ethanol extracts from glanded and glandless cottonseed followed by MTT and qPCR assays. Cottonseed extracts showed minor effects on cell viability. qPCR assay analyzed 55 mRNA levels involved in several pathways including DGAT, GLUT, TTP, IL, gossypol-regulated genes and TTP-mediated mRNAs. BCL2 mRNA was selected as the internal reference for qPCR assay due to its stability among the 55 mRNAs analyzed. qPCR data showed minor effects of ethanol extracts from glanded seed coat and kernel and glandless seed coat on mRNA levels in the cells. However, glandless seed kernel extract significantly reduced mRNA levels of a number of genes involved in glucose transport, lipid biosynthesis and inflammation. The inhibitory effects of glandless kernel extract on gene expression may provide a useful opportunity for improving healthcare associated with colon cancer since it is safe to use without toxic gossypol contamination. This in turn may provide the potential of increasing cottonseed value by using ethanol extracts as a health intervention agent.

GS 2D-10. Evaluating continental-scale poison ivy leaf variability using citizen science data and COVID pandemic-motivated undergraduate researchers

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Poison ivy (Toxicodendron radicans (L) Kuntze) is a North American woody liana well known for leaf shape variability. Poison ivy is notorious because it produces small molecule natural products (urushiols) that cause allergenic contact dermatitis symptoms (aka poison ivy rash) that lasts for weeks. The main line of defense against poison ivy urushiol exposure is to avoid contact with the plant. This necessitates accurate identification of poison ivy, but accurate poison ivy plant identification is impaired by plasticity of leaf margin shape. The objective of this study was to quantify and compare poison ivy leaf shape complexity with another compound three-leaflet plant (Hog peanut) that shares the same environments. In the 2021 Spring semester four undergraduate researchers (UG) in the Jelesko lab were working on lab-based research projects. In mid-March Virginia Tech administration ordered that all UG-research must be transitioned to "on-line" research projects due to COVID-19 pandemic health safety concerns. Over the course of six weeks, the UG researchers pivoted to developing a matrix of poison ivy leaflet shape indices, and analyzed ~2,000 poison ivy and Hog peanut images from iNaturalist.org collected by citizen scientists. The leaf shape scores were aggregated into a composite leaf complexity score and checked for >80% inter-rater reliability. The mean poison ivy leaf complexity score was significantly greater than Hog peanut; moreover, the distribution of poison ivy leaf complexity scores was far more disperse than hog peanut. In addition, poison ivy leaf complexity scores showed statistically significant trends of increasing leaf complexity from North to South, and increasing

complexity from East to West. This study demonstrated the utility of publicly-available citizen science plant data (i.e. image, GPS coordinates, dates, etc.) that could be readily analyzed to yield continental-scale analyses of leaf morphological variability performed by UG researchers collaborating entirely through video conferencing media.

Competitive Concurrent Session (CS) 3A

CS 3A-1. The role of the transcription factor lbd40 in Arabidopsis embryogenesis

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Somatic Embryogenesis (SE) is a process by which an embryo is derived from a single somatic cell or group of somatic cells that are regulated by key transcription factors (TF), including AGAMOUS-like 15 (AGL15). SE is a valuable means to generate transgenic plants to meet food demands or test gene function but is poorly understood. One of the intriguing proteins with which AGL15 interacts in yeast 2-hybrid assays is LBD40. LBD40 encodes a LATERAL ORGAN BOUNDARIES (LOB)-domain TF that is unique to plants, is specifically expressed during seed development and has a role in supporting SE. In planta protein interaction of AGL15 and LBD40 has now been documented using co-immunoprecipitation. Siliques and SE tissue with epitope-tagged transgenes were used for Chromatin-Immunoprecipitation (ChIP) which allows one to determine where TFs bind to DNA in vivo, a step necessary to understanding genes directly controlled by a TF. More than four hundred binding regions for LBD40 were found genome-wide in three biological replications of ChIP-sequencing. RNA-seq results of 7-8 days old seeds from a lbd40/41 mutant line compared to wild type seeds showed genes as significantly expressed and repressed targets. More than seven hundred genes had increased RNA (785 genes) in the mutant, while 2086 genes were downregulated (decreased RNA accumulation) in the mutant line compared to the wild type. The Gene Ontology (GO) enrichment analysis of these regulated genes showed an overrepresentation of biological processes that are associated with SE, further indicating the importance of LBD40 in SE.

CS 3A-2. ATL12, a putative ring-type e3 ubiquitin transferase that involved in chitin-induced, hormone and NADPH oxidase mediated defense response

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Plants, as sessile organisms, have evolved complex systems to respond to changes in environmental conditions. Chitin is a PAMP,which is found in the cell walls of fungi.The recognition of chitin by plants induces pattern triggered immunity (PTI), which plays a critical role in plant innate immunity.Previous studies have shown that Arabidopsis Toxicos en Levadura 12 (ATL12), encodes a putative RING-H2 finger protein that may have E3 ubiquitin ligase activity and is highly induced in response to fungal infection and chitin treatment. Here, our results suggest that loss of function mutants in atl12 are more

susceptible to the powdery mildew fungal pathogen infection while overexpression of ATL12 increased resistance to powdery mildew infection. Transient expression of a 35S::ATL12::GFP construct in tobacco leaves showed that the protein is localized to the plasma membrane. Histochemical staining of 35S::ATL12::GUS showed that the protein is localized in seeds, roots and flowers. qRT-PCR indicated that ATL12 is highly induced by chitin at early stages after chitin treatment and that ATL12 expression is related to MAPK cascade activation.Additionally, DAB staining indicated that mutanst of atl12 have less reactive oxygen species (ROS) generation after chitin treatment, while overexpression of ATL12 results in more ROS generation. RT-PCR indicated that the expression of respiratory burst oxidase homolog protein D/F (AtRBOHD/F) is decreased in the atl12 mutant, while the expression of ATL12 is not affected in atrbohd and atrbohf mutant, suggesting that chitin-induced ATL12 expression is related to NADPH oxidase AtRBOHD/Fdriven ROS production. Furthermore, the expression of ATL12 is up-regulated by treatment with salicylic acid (SA-) and jasmonic acid (JA-), which suggest a possible role for ATL12 in plant hormone-mediated defense responses. Taken together these results indicate that ATL12 is involved in cross-talk between the SA-, JA-,chitin-induced and NADPH oxidase-mediated defense response in Arabidopsis.

CS 3A-3. Quorum sensing in Chlamydomonas reinhardtii

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Quorum sensing (QS) is a process by which microorganisms couple phenotypic switching to population density by utilizing low-molecular weight signals, generically classified as autoinducers (AIs). While QS has been well-established across prokaryotes, More recently, there is evidence that unicellular eukaryotes such as yeast and fungi are also capable of this phenomenon. However, there are challenges associated with determining QS in eukaryotes. We have previously demonstrated that the model organism Chlamydomonas reinhardtii exhibits a QS phenotype linked to motility. Isolating and characterizing the chemical signal and receptors responsible for this shift presents unique challenges. Here, I discuss QS in this model organism, as well as discussing how to face these challenges using analytical, chemical, and pharmacological processes.

CS 3A-4. Investigating microbe-plant symbioses in space

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As humanity explores space and moves farther away from Earth, it will be increasingly crucial to be able to grow fresh food on-site. Experiments involving the growth of edible plants on the International Space Station (ISS) have been ongoing since 2014 with the implementation of the Vegetable Production System (Veggie). These experiments have shown the benefits, as well as the challenges of trying to grow food crops in the microgravity environment. Conditions aboard the ISS are inherently stressful on plants, which can result in growth inhibition and a weakened immune system, the latter leaving them more vulnerable to contamination. Despite efforts to sterilize seeds and sanitize plant growth systems, studies at NASA's Kennedy Space Center have identified a significant diversity of bacteria and fungi in both the phyllo- and rhizospheres. From these studies it is clear that a sterile approach does not adequately protect plants from contamination, which leaves astronauts constantly on the lookout to remove contaminated plants. Utilizing the available data on strains which have already survived aboard the ISS, isolated from the VEGGIE system, we have attempted to identify a subset of microbes which may have plant growth promoting functions or improved disease resistance. Here we present our initial efforts to identify beneficial microbes from this collection. Our ultimate goal is to design a beneficial microbiome for future space crop systems to be inoculated with to improve food production in space.

CS 3A-5. Spatial regulation of thermomorphogenesis by HY5 and PIF4

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Plants respond to high ambient temperature by implementing a suite of morphological changes collectively termed thermomorphogenesis. Here we show that the above and below ground tissue-response to high ambient temperature are mediated by distinct transcription factors. While the central hub transcription factor, PHYTOCHROME INTERACTING FACTOR 4 (PIF4) regulates the above ground tissue response, the below ground root elongation is primarily regulated by ELONGATED HYPOCOTYL 5 (HY5). Plants respond to high temperature by largely expressing distinct sets of genes in a tissue-specific manner. HY5 promotes root thermomorphogenesis via directly controlling the expression of many genes including the auxin and BR pathway genes. Strikingly, the above and below ground thermomorphogenesis is impaired in spaQ. Because SPA1 directly phosphorylates PIF4 and HY5, SPAs might control the stability of PIF4 and HY5 to regulate thermomorphogenesis in both tissues. These data collectively suggest that plants employ a distinct combination of SPA-PIF4-HY5 module to regulate tissue-specific thermomorphogenesis.

CS 3A-6. Understanding upland cotton (Gossypium hirsutum l.) resiliency to drought in semi-arid environments

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Cotton is one of the most important fiber crops for the textile industry and a leading cash crop. Texas ranks first in cotton production in the United States (US), accounting for 40

percent of the total production. Texas High Plains (THP) is the primary cotton-growing region in Texas, having a cold semi-arid climate. Ogallala Aquifer is the primary source of water for all the irrigated cropland in THP, but it is declining at an average rate of 0.97 feet per year in the high plains district since 2011, which is a major concern to agricultural sustainability. Hence, understanding resiliency mechanisms against drought, among the commercial cotton cultivars of regional, national and international importance, could be a solution to secure adequate water for agriculture, pursuing the slogan "more crop per drop". For this, a field experiment was conducted which included two water treatments, well-watered (subsurface irrigation and rainfall, 20.4 mm) and Dryland (rainfall, 0.016 mm) and four cultivars (FM1888, NG4777, DP1646) and L23 (Australian okra leaf type cultivar)]. Both DP1646 and L23 are known to be adapted to dryland conditions. Our results showed a similar photosynthetic rate in dryland compared to irrigated conditions across the growing season. Although both varieties exhibited a positive physiological acclimation to drought, we found contrasting responses regarding important agronomic traits, including yield. Our results highlight that DP1646 and L23 use different strategies to acclimate to limited soil water availability conditions, with and without compromising productivity respectively. Selected physiological and agronomic traits will be presented.

CS 3A-7. Genotype-dependent and heat-induced grain chalkiness in rice correlates with the expression patterns of starch biosynthesis genes

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To understand the molecular basis of environment-induced and genotype-dependent chalkiness, six rice genotypes showing variable chalk levels were subjected to gene expression analysis during reproductive stages. In the high chalk genotypes, the peak expressions of ADP-Glucose Pyrophosphorylase (AGPase) Large Subunit 4 (AGPL4) occurred in the stages before grain filling commenced, creating a temporal gap with the upregulation of Granule Bound Starch Synthase I (GBSSI) and Starch Synthase IIA (SSIIA). Whereas, in the low chalk genotypes, AGPL4 expression generally occurred in later stages, close to the upregulation of GBSSI and SSIIA. However, heat treatment altered the expression pattern and created a gap between the expression peaks of AGPL4, and GBSS1 and SSIIA. This change was accompanied by transformed granular morphology, increased protein content, and chalkiness in the grains. AGPL4 expression pattern may partially explain chalkiness as it contributes to the pool of ADP-Glucose for producing amylose and amylopectin, the major components of the starch. Down-regulation of AGPase during grain filling stages could result in a limited pool of ADP-Glucose leading to inefficient grain filling and air pockets that contribute to chalkiness. The study suggests a mechanism of grain chalkiness based on the coordination of the three starch biosynthesis genes in rice.

Competitive Concurrent Session (CS) 3B

CS 3B-1. Physiological basis of carbon partitioning in peanut under water-stress conditions

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In the Texas High Plains (THP), peanut (Arachis hypogaea L.) often competes with cotton (Gossypium spp L.) for the same croplands. Although the premium is higher for cotton compared to peanut, the THP is already experiencing a shortage of irrigation water as the Ogallala Aquifer is declining. With the new agricultural initiative of reducing the N inputs and greenhouse gas emissions from croplands, while improving soil health, peanut production will most likely increase. Hence, to understand the physiological basis of carbon partitioning and drought tolerance, the field experiment was conducted under the water-stress condition at the USDA-ARS research farm, Lubbock, Texas. An experiment was designed in split-plot design as irrigation treatment [dryland and irrigated] as main-plot and peanut cultivar [Georgia-09B, Lariat, and C7616] as sub-plot with four replications during 2020 growing season. The survey leaf gas exchange was measured using the infrared gas analyzer portable photosynthesis system, LI-6800. The partitioning of biomass into roots, shoots, and nuts was done at physiological maturity. The net assimilation rate, stomatal conductance, and internal CO2 concentration were 14%, 26%, and 26.3% higher in irrigated as compared to dryland treatment. Among cultivars, C7616 had a higher assimilation rate (13% and 7%), stomatal conductance (10.9% and 21.4%), and internal CO2 (4.7% and 3.9%) concentration than in Georgia-09B and Lariat, respectively. Also, carbon allocation towards root growth in dryland conditions was the greatest in C7616 (95% more) compared to Georgia-09B (27% less) and Lariat (5% more). Similarly, the difference in seed yield was lower in C7616 (35%) than Georgia-09B (390%) and Lariat (215%) when compared among the dryland and irrigated. This result indicates that C7616 has the best resiliency to the dryland conditions in the THP.

CS 3B-2. Investigating the molecular responses in rice roots during interaction with plant growth-promoting bacteria, Burkholderia unamae

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Major crops such as rice and maize can benefit from associations with different plant growth-promoting bacteria (PGPB). Studies have shown that these PGPB (e.g., Azospirillum, Herbaspirillum, Burkholderia) promote plant growth primarily via nitrogen fixation and phytohormone secretion. However, our current understanding of the underlying molecular mechanisms involved in these associations is limited. For instance, very little is known about the associations between plants and the symbiotic Burkholderia species, B. unamae, at a molecular level. Earlier, we set up an experimental system where PGPB such as Azospirillum brasilense and Herbaspirillum seropedicae could colonize rice roots and promote plant

growth. In this study, we used the same experimental system and show that B. unamae can promote growth and colonize the roots of rice plants. Next, using RNA sequencing, we identified the transcriptomic responses in rice roots, 1-day post-inoculation. We identified 1128 differentially expressed genes (DEGs) in rice roots. Several of these DEGs are involved in defense response, flavonoid synthesis, hormone signaling, and nitrate transport. We are currently validating the expression pattern of a few genes identified in our dataset via RT-PCR. Our findings will be an excellent resource for future studies investigating the genetic pathways controlling this plant-microbe association.

CS 3B-3. Vacuolar dynamics and function require endosomal NHX-type cation/H+ exchangers during cellular acclimation to salinity

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Soil and water salinity severely reduces plant growth and productivity. One plant salinity acclimation strategy is sequestration of salt in vacuoles where it is less toxic. Sequestration is mediated in part, by tonoplast localized NHX-type cation/H+ antiporters (NHXs) that couple the efflux of H+ out of the vacuole with the uptake of Na+ into the vacuole, resulting in vacuole alkalization. Vacuolar Na+ accumulation often leads to cellular osmotic adjustment, water absorption and vacuole expansion. Little is known about endomembrane dynamics and remodeling during salinity, but endomembrane trafficking is likely to play a key role in both salinity acclimation and vacuole expansion. We showed previously that NHX5 and NHX6 are cation/H+ exchangers that localize to the Golgi, trans-Golgi network and pre-vacuolar compartments, implicated in luminal pH homeostasis and critical for protein processing and trafficking. nhx5nhx6 double knockout mutants are highly sensitive to salinity making them useful tools for studying endomembrane dynamics and trafficking under salt. When grown under prolonged salinity, nhx5nhx6 root tips exhibit significantly higher numbers of dead cells as assessed by propidium iodide staining. Using live cell imaging and quantitative imaging of root cells loaded with fluorescent ion responsive dyes, we show that nhx5nhx6 plants have higher vacuolar pH that do not alkalinize further under salinity as seen in wildtype. Under salinity, nhx5nhx6 accumulate less Na+ in vacuoles and exhibit limited vacuole expansion typical of wildtype cells. Moreover, compared to wildtype, vacuolar dynamics and remodeling are significantly slower in nhx5nhx6 in both control and high salinity conditions. These results suggest that the trans-Golgi network localized NHXs could be important regulators of vacuole dynamics and may link endomembrane trafficking with vacuolar function under high salinity.

CS 3B-4. Targeting wheat genes associated with susceptibility to Fusarium graminearum for enhancing fhb resistance

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Fusarium head blight, which is a serious disease of wheat and small cereal grains caused by Fusarium graminearum (Fg), adversely affects grain yield and quality. Fg-infected grains accumulate mycotoxins. Fg can also infect Arabidopsis. 9-lipoxygenases (9-LOXs), which catalyze the first step in the synthesis of oxylipins, act as susceptibility factors in wheat and Arabidopsis interaction with Fg. Knock-down of 9-LOX encoding genes in Arabidopsis confers enhanced resistance against Fg, which can be complemented by the wheat Lpx3 gene. Also, knock-down of Lpx3 in wheat cv. Bobwhite by RNA-interference (RNAi) strategy conferred enhanced resistance against Fg. Fungal infection was largely confined to the inoculated spikelet of Lpx3-RNAi plants. To develop a non-GMO approach for FHB resistance, wheat TILLING lines with nonsense and/or missense Lpx3 variants in the hexaploid Cadenza and tetraploid Kronos were identified and characterized for their response to Fg. FHB disease severity and DON accumulation was reduced in some of the non-sense Lpx3 variants. The Fg-resistant TILLING lines have been backcrossed to wild type to clear out unwanted mutations at other loci. Simultaneously, crosses have been made to generate lines with non-sense mutations at multiple Lpx3 homeologs. Molecular approaches are being used to distinguish between mutants with SNPs and wild type. It is anticipated that knockdown of multiple Lpx3 homeolog(s) will confer higher levels of resistance to FHB.

CS 3B-5. DAR1, a putative o-fucosyltransferase, in plant systemic immunity

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Abietane diterpenoids accumulate in conifers (gymnosperms) as a component of oleoresin, which is valued as a starting material for pharmacologically active compounds. Although recent studies show that abietane diterpenoids are produced in angiosperms, their biological role in flowering plants is largely unknown. We identified the abietane diterpenoid Dehydroabietinal (DA) as a potent activator of systemic acquired resistance, which confers enhanced resistance against a variety of pathogens. To further understand the mechanism underlying signaling by abietane diterpenoids, we have identified dar mutants that are resistant to abietane diterpenoid. DAR1, which was identified in this screen, encodes a protein that is homologous to human protein O-fucosyltransferase 2. dar1-1 mutant plants are compromised in SAR. dar1-2, a T-DNA insertion mutant at the DAR1 locus showed similar phenotype as the missense mutant dar1-1. Plants expressing the GUS reporter from the DAR1 promoter indicate that the DAR1 promotor is active in the vasculatures of leaf tissue and root. DAR1 was found to be ER localized. Our experiments suggest that DAR1 is required for accumulation and long-distance transport of a SAR signal. We hypothesize that DAR1 is involved in the post-translational modification (O-fucosylation) of a protein that is essential for SAR. We further suggest that O-fucosylation likely promotes channeling of the DAR1 targeted protein through the ER/Golgi network.

CS 3B-6. Impact of fatty acid elongase-1 mutation on pennycress metabolism

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The aviation industry has expressed a growing interest in using alternative crops to produce renewable biofuel. Replacing traditional fossil fuels can help to reduce greenhouse gas emissions and combat global climate change. Thlaspi arvense, colloquially known as field pennycress, is a promising oilseed plant that can be grown in a double cropping system to produce renewable biofuel. Pennycress is widely undomesticated and there is abundant opportunity to improve its seed oil composition. Eliminating erucic acid in pennycress seed oil has been identified as a route to improve biofuel cold flow properties. Fatty Acid Elongase-1 (FAE-1) loss-of-function mutants were generated through CRISPR Cas-6 gene editing. The mutants fail to synthesize very long chain fatty acids including erucic acid. Understanding the impact of this mutation on plant metabolism is a key aspect of crop improvement. The overall hypothesis is that the elimination of FAE-1 alters the levels of metabolites involved in fatty acid elongation, specifically metabolites associated with the cytosolic Oxidative Pentose Phosphate Pathway (OPPP) and the Tricarboxylic Acid cycle (TCA). Indeed, the OPPP and the TCA cycle were previously shown to provide reductant and carbon for fatty acid elongation under the form of NADPH and citrate, respectively. To test this hypothesis, intracellular metabolites were extracted from developing pennycress embryos and leaf tissue. Then, extracted metabolites were quantified by liquid chromatography tandem mass spectrometry (LC-MS/MS). The results indicate that several intermediaries of central metabolism were significantly increased in mutant leaves and developing embryos. Pathway analysis revealed that seven pathways, including glycolysis/gluconeogenesis and the pentose phosphate pathway, were commonly impacted in both developing embryos and leaves. This study couples metabolomics with pathway analysis to describe fatty acid compositional changes in the promising oilseed crop pennycress.

CS 3B-7. Seedling age and healing chamber type on grafted tomato

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Tomato production has been characterized by infections, mainly during the rainy seasons. Over time, researchers have adopted as one of the primary management strategies the use of agro-synthetic chemicals and the planting of resistant varieties. Vegetable grafting has therefore become an immediate tool used to overcome the challenges of soil-borne diseases, pests, and harsh weather conditions affecting tomato production, especially in Ghana. The objective of the study was to assess the influence of healing-chamber-type (wooden and Styrofoam box) on tomato graft-take and to determine the influence of seedling age on tomato graft-take. Treatments used for the seedling age experiment were four-week-old S. lycopersicum and S. macrocarpon seedlings, five-week-old S. lycopersicum and S. macrocarpon seedlings, six-week-old S. lycopersicum and S. macrocarpon seedlings, and non-grafted S. lycopersicum seedlings. A Completely Randomised Design (CRD) was used for this experiment. Seeds of both S. lycopersicum 'Pectomech' and S. macrocarpon were sown

at the same time in 72-cell seed trays, filled with FERTIPLUS® (Universal Potting Soil, comprising of partially decomposed sphagnum moss). Seedlings were fertilized twice a week using NPK 19-19-19 at 5 g/L until were ready for grafting. The success of tomato (Solanum lycopersicum 'Pectomech')/ Solanum macrocarpon grafts after 14 days was higher in the wooden chamber (84.2%) compared to graft success in the styrofoam chamber (80.0%). Misting twice a day in addition to the partial opening of the wooden chamber enhanced graft-take. Four-week and five-week-old seedlings had a graft success of (92.5%) and (85%) respectively, as compared to the six-week-old seedlings (73.6%). Grafting four and five-week-old seedlings thus increases graft-take and subsequent survival over time.

Competitive Concurrent Session (CS) 4A

CS 4A-1. Greenhouse evaluation of six solanum species as potential rootstocks against fusarium wilt disease on tomato

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Fusarium wilt of tomato caused by Fusarium oxysporum f. sp. lycopersici, a soil-borne pathogen poses serious threats to food security in Ghana. This organism can cause total yield and crop losses if not managed effectively. Grafting of tomato scions onto resistant rootstocks is effective in controlling Fusarium wilt in tomato. This study was conducted to evaluate the resistance of six Solanum species (Solanum torvum, Solanum macrocarpon, Solanum melongena, Capsicum annum "Legon 18", Solanum lycopersicum "Mongal F1" and Solanum lycopersicum "Petomech") as potential rootstocks against F. oxysporum f. sp. lycopersici inoculum (4.4x106 conidia/mL) in greenhouse environment. The experiment consisted of a 2x6 factorial, arranged in a completely randomized design (CRD), and replicated three times. Additionally, gene- specific primers were used to screen for I, I2 and I3 Fusarium resistant genes in the Solanum species. Solanum macrocarpon, S. torvum and S. lycopersicum (Mongal F1) were resistant to 4.4x106 conidia/mL of F. oxysporum f. sp. lycopersici, C. annum (Legon 18) and S. melongena were moderately susceptible whiles Solanum lycopersicum (Petomech) was highly susceptible to 4.4x106 conidia/mL of F. oxysporum f. sp. lycopersici with the highest disease severity (4), percentage wilt incidence (100%) and infection percentage (64.36%). Moreover, plant growth and yield of the Solanum species were affected by the inoculum differently based on their physiological make-up. The I3 gene specific primer, AkCDS4-F/R amplified the I3 gene at 800 bp in S. macrocarpon and S. melongena. However, the 'I' and I2 genes were not amplified in any of the Solanum species using their specific primers TFusF1/TFusrr1, TFusF1/TFusrr2, SSR67F/R and SSRD-F1/R1. The identification of resistant rootstocks (S. macrocarpon, S. torvum, C. annum, and S. melongena) will therefore be useful in managing Fusarium wilt disease through grafting of tomato in the country.

CS 4A-2. Studying physiological and molecular regulation of stomatal behavior and its relationships to water use efficiency in alfalfa

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Alfalfa (Medicago sativa), being a highly nutritious legume plant has always been one of the top choices for the forage production. Its production, however, has high cost of irrigation in many dry and warm areas such as California and Arizona. Thus, a reduction of irrigation by using the higher water use efficiency (WUE) varieties can help the growers in reducing the cost and is critical for sustainable agriculture production. In a previous study, we identified an alfalfa genotype with higher WUE under drought conditions. Our recent studies suggested that a higher stomatal sensitivity to ABA may contribute to a greater WUE in this genotype. As a first step to understand the underlying mechanism of a higher stomatal sensitivity to ABA, we are examining the expression of ABA receptor genes in alfalfa. One of the families of ABA receptors, Pyrobactin resistant like (PYL) in Arabidopsis is found to play important roles in drought responses. We hypothesized that PYLs in alfalfa could be involved in regulating stomatal conductance and hence play an essential role in WUE of the plants. The current study involves identifying the homologs of PYL family in alfalfa and analyzing the change in gene expression levels during drought stress. By comparing with a genotype with lower WUE, our goal is to identify candidate genes that are correlated with better WUE in alfalfa.

CS 4A-3. elucidating the role of the pro-survival to pro-death molecular switch in the ire1a signaling pathway in Arabidopsis thaliana

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In eukaryotic cells, biotic and abiotic stress causes disruptions in the function of endoplasmic reticulum (ER), which subsequently induces unfolded protein response (UPR). The most conserved UPR sensor amongst eukaryotes is Inositol-Requiring Enzyme 1 (IRE1), which mitigates ER stress by up-regulating the cellular pro-survival pathway. However, under extreme or chronic conditions, the cells undergoing UPR will ultimately "switch" from the pro-survival to pro-death signaling to avoid unfavorable circumstances for the plant as a whole. In plants, the molecular mechanisms underpinning the attenuation of pro-survival branch of IRE1a signaling remain largely undefined. In Arabidopsis, upon ER stress IRE1a directly cleaves bZIP60 (basic leucine zipper 60 transcription factor) mRNA, leading to the production of an active transcription factor that activates cytoprotective genes. Throughout this study, the plant bacterial pathogen Pseudomonas syringae pv. tomato (Pst DC3000) was used as a biotic inducer for ER stress in an attempt to uncover regulatory mechanisms governing the regulation of bZIP60 expression during immune response. Our findings suggest that a novel Arabidopsis thaliana microRNA miR5658 can target bZIP60 for degradation. Quantification of miR5658 reveals that its induction coincides with bZIP60

mRNA suppression, suggesting that it might participate in regulating bZIP60. Reporter-based assays reveal that the miR-binding sequence of bZIP60 is required for its degradation, and T-DNA mutants that do not produce miR5658 display enhanced bZIP60 transcript stability. We also demonstrate that the central plant immune regulator NPR1 (Non-Expressor of PR genes 1) plays an important role in regulating miR5658. Taken together, these results suggest that upon cell death-triggering stimuli, NPR1, via miR5658, may target bZIP60 mRNA to turn off the IRE1a-mediated pro-survival pathway and thus, tip the scales of cell fate.

CS 4A-4. How plants respond to high potassium stress: insights from Arabidopsis and its extremophyte relative Schrenkiella parvula

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Schrenkiella parvula, a close relative of Arabidopsis thaliana and Brassica crops, grows on the shores of a hypersaline lake, Tuz in Turkey. Its native soils are high in multiple salts including Na+ and K+. In this study, we investigated the underlying genomic basis for high K+ tolerance using physiological, ionomic, transcriptomic, and metabolomic approaches between stress-adapted S. parvula and the stress-sensitive A. thaliana treated with KCl. Under high K+, root system architecture changes significantly compared to control conditions, and the phenotypic response in A. thaliana is more pronounced than the effect observed for S. parvula. Notably, A. thaliana was unable to maintain the macro and micro-nutrient homeostasis while S. parvula remained unaffected with increased accumulation of K+. We conducted co-expression network analysis to identify genetic mechanisms that may lead to differential ionomic responses. The S. parvula transcriptome responds to high K+ stress by adjusting only a few genes in selected pathways, whereas A. thaliana shows a large number of genes significantly changed. Clusters of co-expressed ortholog pairs showed the "stress-preparedness" in S. parvula and those clusters were enriched in response to salt and transport functions. Stress-initiated nitrogen reallocation in the root could be the mechanism of tolerance to high K+ in S. parvula suggested by the increased total nitrogen accumulation in root contrary to that in the A. thaliana. Concurrently, at the metabolome level, S. parvula roots and shoots accumulated a higher level of protective osmolytes and antioxidants including proline, myo-inositol, and their precursors, and amino acids in line with the transcriptomic responses. In conclusion, the ionomic, transcriptomic, and metabolomic profiles supported the physiological responses to high K+ stress in each plant where one was stress adapted and the other was stress sensitive.

CS 4A-5. Arabidopsis bax inhibitor 1 (atbi-1) interacts with AtIRE1A to execute its pro-survival function

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Unfolded Protein Response (UPR) is a universal, integrated signaling network governed by an evolutionarily conserved stress sensor IRE1 that responds to endoplasmic reticulum (ER)

stress and orchestrates cellular survival or death decisions. Here, we elucidated the intricate relationship between Arabidopsis Bax Inhibitor-1 (AtBI-1) and AtIRE1A during pathogen-induced acute ER stress. Our comprehensive biochemical study revealed that the C-terminal domain of AtBI-1 associates with the kinase domain of AtIRE1A, and two phosphoacceptor residues are essential to maintain this interaction. Furthermore, we showed the direct contribution of AtBI-1 in AtIRE1A-mediated AtbZIP60 splicing. Using higher-order mutants, bacterial infections, and biochemical analyses, we determined that AtBI-1 and AtIRE1A act in concert to positively contribute towards disease resistance and suppression of cell death. In summary, our study provided insights into a novel molecular, genetic, and biochemical interplay between two evolutionarily conserved ER-associated proteins in the AtIRE1-AtbZIP60 arm of cytoprotective UPR signaling pathway upon bacterial pathogen infection.

CS 4A-6. CRISPR-mediated gene editing of lipase and lipoxygenase in rice to improve storage life

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Oxidation of free fatty acids in the bran layer of rice (Oryza sativa) contributes to reduced shelf life of brown rice products. Lipase enzymes release free fatty acids from lipids; lipoxygenases act on such fatty acids to produce rancid odor molecules. Several lipases and lipoxygenases expressed in seed tissue are ideal targets for gene editing to mitigate their contributions to brown rice quality reductions. These include lipoxygenase-3 (LOX3) and a novel rice lipase (L2). LOX3 has been mutated with TALEN-based gene editing and silenced by RNA interference, but targeting of LOX3 by CRISPR gene editing or of L2 via mutation or silencing has not been reported. LOX3 and L2 were individually and simultaneously targeted for mutation by CRISPR-Cas9 at two locations per gene in the model cultivar Nipponbare. Multiple plants with mutations at the target sites were produced, but lack of fertile seed limited which lines could be analyzed; no single-knockout lines of LOX3 progressed. Productive Cas9-free progeny with homozygous mutations in L2, or both LOX3 and L2, were selected for bulk grain production. Wild-type and gene-edited grain was harvested at maturity, dried, and dehulled for storage as brown rice under accelerated aging conditions (37°C, 70% relative humidity) for 30 days. Samples were taken at 0, 1, 2, 3, 5, 10, 20, and 30 days, processed into flour, and tested for lipoxygenase and lipase activity, free fatty acid content, and conjugated diene formation. Previous research has shown limited effects of LOX3 mutations on brown rice rancidity. With simultaneous knockout of L2, it is hoped that a synergistic effect will result in more drastic reductions in oxidation product formation.

CS 4A-7. Identification and characterization of new nitrogen-fixation symbiotic genes in the Medicago truncatula tnt1-insertion mutant collection

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Most legumes have the ability to utilize atmospheric nitrogen fixed within specialized root organs (nodules) harboring nitrogen-fixing bacteria (rhizobia). Nitrogen is very important for plant growth and development, as a building block for chlorophylls, proteins, and nucleic acids. Plants acquire nitrogen as ammonium, or other reduced forms from soil, from applied fertilizer nitrogen, or, in the case of most legumes, via symbiosis with rhizobia. Research on symbiotic associations between model legumes and their symbionts revealed that tight regulation of thousands of plant and bacterial genes is required for efficient symbiotic nitrogen fixation (SNF)1-4. For the past 20 years, the Medicago truncatula-Sinorhizobium meliloti symbiosis has been used as genetic model to uncover molecular mechanisms of SNF. Less than 200 host genes have been characterized to various degrees, and more need to be discovered to better understand SNF5. To uncover new symbiosis-associated genes using a forward genetics approach, we screened 10 M. truncatula tobacco retrotransposon (Tnt1)-insertion lines under symbiotic conditions. We identified plants with mutant phenotypes in all lines. To identify disrupted candidate genes, we subjected genomic DNA from mutants to genome capture sequencing, to retrieve flanking sequence tags (FSTs). In total, this sequencing approach yielded 666 tagged genes, from which 78 genes are expressed in nodules and other organs. Using transcriptomic data from the Medicago truncatula Gene Expression Atlas and Symbimics2,4, we further narrowed down this list to 22 candidate genes, which are either nodule-specific or strongly induced during SNF. Results from phenotypic and co-segregations analyses will be presented.

Competitive Concurrent Session (CS) 4B

CS 4B-1. Effect of systemic acquired resistance on guard cell immunity

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After localized invasion by bacterial pathogens, systemic acquired resistance (SAR) is induced in uninfected plant tissues, resulting in enhanced defense against a broad range of pathogens. Although SAR requires mobilization of signaling molecules via the plant vasculature, the specific mechanisms remain elusive. The lipid transfer protein-defective in

induced resistance 1-1 (DIR1-1) was identified in Arabidopsis thaliana by screening for mutants that were defective in SAR. Since then, the structure and lipid-binding properties of DIR1 have been determined, showing that its barrel structure can bind two, long-chain fatty-acid molecules. Several SAR mobile signals, including dehydroabietinal (DA), azelaic acid (AzA), and glycerol-3-phosphate (G3P) are dependent on DIR1 for transport to systemic leaves. Closure of stomata, controlled by guard cells, is a local response to bacteria. Here we demonstrate that stomatal response to pathogens is altered in systemic leaves by SAR, and this guard cell SAR defense requires DIR1. Using a multi-omics approach, we have determined potential SAR signaling mechanisms specific for guard cells in systemic leaves by profiling metabolite, lipid, and protein differences between guard cells in wild type and dir1-1 mutant during SAR. We identified two 18C fatty actyls and two 16C wax esters as putative SAR-related molecules dependent on DIR1. Proteins and metabolites related to amino acid biosynthesis and response to stimulus were altered in guard cells of dir1-1 compared to wild type. Identification of guard cell-specific SAR-related molecules will lead to new avenues of genetic modifications/molecular breeding for disease resistant plants.

CS 4B-2. Metabolic signatures of KIN recognition in a model plant

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Plants alter their growth, defensive strategies, gene expression, and nutrient acquisition profiles based on their neighbors' proximity and identity. Such interactions and the associated variations in functional traits are an important element in initiating and maintaining ecological communities. Kin recognition (KR), the ability to discriminate the relatedness, genetic or otherwise, of another member of the same species allows plants to modify their growth in response to their neighbor's identity. The discovery that the model plant Arabidopsis thaliana is capable of KR provides an incredible resource for determining the signal mechanisms and phenotypic responses associated with this phenomenon. In the present study, we used Gas Chromatography-Mass Spectrometry (GC-MS) to investigate the changes in the metabolic profile of A. thaliana under nutrient scarcity and its influence on KR responses. Plants under nutrient scarcity conditions present adaptive strategies and phenotypic responses that are commonly observable based on the mechanisms defined by their metabolic pathways. Our findings impact our broader understanding of the KR phenomenon, as well as some elucidation of the strategies used by A. thaliana during KR. The metabolomics data will be presented as a tool for understanding the regulatory networks involved in the physiology of plant competition. Understanding the interactions that drive neighbor/kin recognition in plants is crucial to the future of agriculture in urban farming and space agriculture to develop techniques to reduce wasteful competition and improve yields in crops.

CS 4B-3. Abscisic acid signaling pathway dynamics

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Osmotic stress is a major abiotic stressor that significantly limits plant productivity. Elucidating the osmotic stress signaling responses in plants is important in improving plant growth and productivity. ABA is a stress phytohormone that is involved in osmotic stress response enabling the plant to withstand stress. The ABA signaling pathway consists of a series of proteins that are involved in a signaling cascade leading to ABA-mediated responses like gene expression or stomatal closure. Though the signaling pathway has been elucidated the dynamic behavior of the pathway across a period of time has not been studied. Using dynamic modeling and luciferase reporter gene assays on the activity of RD29A gene promoter an ABA-induced promoter, we seek to study the dynamics in the ABA signaling pathway over a 24-hour period. Preliminary results from dynamic modeling show the activity of RD29A promoter is sigmoidal with an increase in activity at the beginning 0-10 hours, and eventually levels off on achieving equilibrium 15-24 hours. However, studies with RD29A-LUC transgenic Arabidopsis which contains a firefly luciferase reporter gene, show that the activity of the promoter is transient, with a peak around 5-8 hours and activity declines after 12 hours. This shows that there must be additional molecules in the signaling pathway that regulate its dynamics over the course of time. We hypothesize that this molecule is phosphatidic acid (PA) PA is a membrane phospholipid and termed as a second messenger in osmotic stress signaling where it is transiently produced on the onset of osmotic stress or application of ABA. Our hypothesis is that PA which is not included in the established ABA signaling pathway plays a role in the dynamic behavior of the pathway. At the conference, we will present preliminary results on this.

CS 4B-4. Spatiotemporal genetic variation in two Zostera marina meadows in North Carolina

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Seagrasses are one of the most productive marine ecosystems in the world, providing numerous ecosystem services. High genetic diversity of seagrass meadows can increase their resistance to many environmental disturbances; however, differences in dispersal and life history can result in different levels of diversity in seagrass populations. As seagrasses decline globally, it is important to further our understanding of these ecosystems to improve conservation efforts. Two *Zostera marina* populations were sampled in North Carolina, one at Morgan Island (MI) and one at Phillips Island (PI), separated by 15 kilometers. MI is a perennial population, whereas PI is an annual population. Genetic diversity and structure were assessed within and between the two islands utilizing 9 microsatellite markers. Questions being asked are: 1) What are the patterns of genetic diversity, structure and kinship over time and space in these *Z. marina* populations? 2) Are these spatiotemporal patterns related to differences in dispersal or life history? 3) What are the implications of these patterns for meadow management? Shoots were collected at MI & PI in 2007, 2008, 2016 & 2017 during February, March, May & June, yielding 293 individuals among 11 subpopulations. Departure from Hardy-Weinberg equilibrium, evidence of linkage

disequilibrium, observed and expected heterozygosity, kinship & inbreeding coefficients, fixation index (F_{ST}), clonality and allelic richness were calculated. Spatial & temporal genetic analyses using AMOVA were also performed. Preliminary results with F_{ST} values found low-moderate genetic differentiation overall. Inbreeding was generally low yet heterozygosity and clonality were high, suggesting a high level of sexual reproduction and unrestricted dispersal between sites. AMOVA revealed a significant temporal effect, but little spatial structure. This research is one of the few that explores temporal genetic variation in seagrasses and hopes to shed light on the links between genetic diversity and seagrass health in the face of climate change.

CS 4B-5. Morphological variability, genetic diversity, and identification of salt tolerant breeding lines from multi-parental rice population developed in U.S. rice backgrounds

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Salinity is a major environmental constraint affecting rice production worldwide and threatening future food security. To cope with this problem, it is necessary to develop salt-tolerant rice varieties with good agronomic traits that can maintain high yield. In this study, we developed two different rice populations utilizing multiple salt-tolerant donors, Pokkali, FL478, and Hasawi in US rice backgrounds Mermentau, Jupiter, Cheniere, and Dular and named [(M/P//M/H and C/F//C/D (J)] respectively. The result from the initial screening of 180 introgression lines (ILs) in sand-culture showed significant variation and correlations among salt injury score (SIS), chlorophyll content, root length, shoot length, and root-shoot length ratio. Thirty-six ILs selected from the sand culture screening were further validated for salinity tolerance via hydroponics screening system and identified tolerant to moderately tolerant lines with SIS 3.5-5.8. Our results showed that shoot Na+, root Na+, shoot Na+/K+, and root Na+/K+ were positively correlated with SIS and significantly negatively correlated with shoot K+ and root K+ concentration, indicating increased uptake of K+ contributing toward salinity tolerance of those ILs. Phenotypic clustering based on 16 morphophysiological traits grouped the selected ILs with tolerant parents and recurrent susceptible parents were grouped separately suggesting the tolerant phenotype of ILs. Genetic diversity study using 100 polymorphic SSR markers indicated a low level of genetic diversity among genotypes. The genetic diversity of markers varies from 0.0868 to 0.6077 and polymorphism information content (PIC) among markers ranged from 0.0830 to 0.5511. In addition, genotypic clustering revealed that salt tolerance donors were clustered separately and recurrent parents were clustered with ILs, suggesting the genetic background of the ILs similar with recurrent parents. The salt-tolerant ILs identified in this study will be tested for agronomical traits and yield for future release and will be used for molecular genetic study for salt tolerance.

CS 4B-6. Understanding Arabidopsis gravitropism from two aspects: the microgravity effect and the role of membrane contact sites (MCSS)

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Plants use gravitropism to orient direction of growth and maximize access to resources. Amyloplasts inside statocytes sediment towards the direction of gravity and this is considered the first event for gravity perception. Amyloplasts in Arabidopsis zigzag1 (zig1) endodermis are trapped among fragmented vacuoles and the mutant displays agravitropic growth in shoot. Application of wortmannin (WM), an inhibitor of phosphatidylinositol 3-kinase induce vacuole fusion in zig1 and enhance amyloplast sedimentation. The first goal of our work is to study the effect of microgravity on vacuole fusion and organelle distribution in cell. Fluorescent protein tagged vacuoles, endoplasmic reticulum (ER) and plastids will be visualized for plants grown in the International Space Station (ISS) and on earth. Effect of WM induced vacuole fusion on Col-0 and zig1 between ISS and ground control plants will be compared. Several fixation protocols were tested to ensure fluorescent protein signal and cellular preservation during the spaceflight assay. Our studies will demonstrate feasibility of chemical treatments for plant cell biology experiments in space. As ER aggregated at junction of fragmented vacuoles and hyper-gravity stimulation failed to sediment amyloplasts in zig1, we hypothesized that membrane contact sites (MCSs) among vacuoles, ER and amyloplasts influence statolith sedimentation and regulate gravitropism. The Vesicle-Associated Membrane Protein-Associated Protein-27 (VAP27) and Lipid transfer proteins Anchored at Membrane contact sites (LAM) families are under investigation as they are predicted to mediate endomembrane contacts among ER and different organelles. Insertion mutants of VAP27 and LAM were tested for gravitropism defects. Mutants vap27-2, lam4 showed enhanced shoot gravitropic response while vap27-1 vap27-3 double mutants showed delayed shoot gravitropism. VAP27-2 fused with fluorescent protein localized at ER, apparent EPCS (ER-Plasma membrane contact sites) and membranes of chloroplasts and amyloplasts, which is consistent with a putative role in MCSs tethering. Current experiments include the analyses of VAP27-2 over-expression and double mutants with genes involved in gravitropism.

CS 4B-7. Integration of proteomics and metabolomics approaches to elucidate the glyphosate-induced stress response in Amaranthus palmeri

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Manifestation of plant response to suboptimal environmental conditions is regulated at all levels of central dogma – DNA, RNA, proteins, and metabolites. Evolution of resistance to herbicides in weeds through several interrelated mechanisms is an example of plant response to stress at multiple (genetic, metabolic, cellular) levels. Glyphosate is the most widely used herbicide worldwide that inhibits EPSPS enzyme in the shikimate pathway, thereby blocking the synthesis of aromatic amino acids, which results in protein starvation

and eventual plant death. To date, 51 weed species have evolved resistance to glyphosate through various mechanisms (EPSPS gene duplication, target-site mutation, reduced translocation, etc.). Amaranthus palmeri is the most important glyphosate-resistant weed species since it can cause yield losses of more than 50% in row-crop production systems of the southern US. In this study, we aimed at elucidating the glyphosate-induced stress response in A. palmeri at a multi-omics level by capturing the protein and metabolite profiles of plants in response to glyphosate treatment. We integrated global metabolomics (GC and LC-MS) and proteomics approaches to capture the glyphosate-induced cellular perturbations in A. palmeri. 3104 proteins and 114 metabolites were identified in the study, of which 151 proteins and 20 metabolites were significantly affected by the glyphosate treatment. Interestingly, there was no significant change in the abundance of proteins involved in the shikimate pathway except phospho-2-dehydro-3-deoxyheptonate aldolase, which acts upstream of the EPSPS enzyme, however the metabolites of shikimate pathway (shikimic acid and dehydroshikimic acid) accumulated in the glyphosate treatment. The integrated pathway enrichment analysis of the proteins and metabolites resulted in significant enrichment of ten pathways including, amino acid biosynthesis and secondary metabolite biosynthesis pathways. The results show that the glyphosate treatment affected cell regulation at both protein and metabolite levels. This is a pioneer study to investigate herbicide physiology in weeds at a multi-omics level.

Competitive Concurrent Session (CS) 5A

CS 5A-1. A dual recombination system for transgene containment in perennial grasses

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Biotechnology approach is an excellent choice for modifying perennial grasses like switchgrass and turfgrass, engineering new traits that are difficult to obtain by traditional breeding. However, transgene escape into the nature environment could bring about unforeseeable consequences. We have developed an integrated strategy that combines dual site-specific recombination system and total sterility induction mechanism for transgene containment and marker gene removal in switchgrass and turfgrass. This should generate transgenic line that is self-contained for desirable transgene but free of undesirable foreign DNAs, upon hybridization of two parental transgenic lines designed with dual recombination system. In the first line, a FLO/LFY RNAi expression cassette is separated from an upstream promoter by the Cre recombinase target site loxP-flanked phiC31 recombinase, hygromycin resistance (hyg) and Cas9 endonuclease genes. Another line contains an active herbicide resistance gene bar, recombinase gene Cre and FLO/LFY homolog gene guide RNA (sgRNA), and an inactive stress-regulating gene, AVP1. When the two transgenic lines are cross-pollinated, the phiC31 recombinase in the hybrids would excise the phiC31 target site att-flanked bar, activating Cre target site loxP-flanked Cre and FLO/LFY sgRNA, and consequently removal of itself and the sgRNA. This will activate AVP1 leading to enhanced plant stress tolerance. Cre will also excise the loxP-flanked phiC31, hyg and Cas9, activating

FLO/LFY RNAi, leading to total sterility. Additionally, Cas9/sgRNA-mediated gene editing will be active in the hybrids, ensuring FLO/LFY lockout for total sterility.

CS 5A-2. Effects of salicylic acid on de novo root regeneration on detached Arabidopsis leaves

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When plant tissues are detached or wounded, they can regenerate new roots, shoots or become a new plant. De novo root regeneration (DNRR) is one type of such process in which adventitious roots are regenerated from a detached leaf. Unlike tissue culture procedures where exogenous plant hormones, such as auxin and cytokinin, are added to induce regeneration, DNRR completely relies on endogenous hormones. Most published studies on DNRR are done in sterile conditions. The precise roles of biotic stress pathways in root regeneration are unclear. Here we show that salicylic acid (SA), a phytohormone involved in activation of plant defenses, represses root regeneration ability in Arabidopsis thaliana. Mutants with low SA levels have an increased number and rate of adventitious roots formed from leaf explants. Interestingly, NPR1, a central regulator of SA-mediated defense response, is not required for SA-mediated suppression of DNRR. SA suppresses the expression of WOX11, a key gene required for cell fate transition during DNRR. In addition, SA may suppress the auxin accumulation or response at the wounding site. These findings indicate that pathogen-induced signals and activation of plant defenses can decrease the root regeneration ability. Through these findings, we can further understand the specific role of SA during root regeneration and hopefully apply these discoveries to economically important plants.

CS 5A-3. Characterization of soybean root endophytes with protective activity against the soil-borne fungal pathogen Xylaria sp.

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Plant roots associate with a microbial community composed of a diversity of bacteria and fungi. The root microbiome communities associate with the root surface or penetrate the superficial layers of root tissues. It is of high agronomic interest to characterize root microbiomes and identify microbes with beneficial effects on plant defense to pathogens. Soybean taproot decline (TRP) is an emerging soil-borne disease caused by fungi classified as Xylaria sp. , challenging to control and with devastating effects on yield. Our group has initiated a study of the soybean root microbiome with two main goals: (i) perform a comparative study of root microbiomes from healthy and TRD-affected soybean, and (ii) identify microorganisms with anti-Xylaria and anti-TRD properties. Here, I will present the results of our work on the second goal, namely assembling a library of soybean root

endophytes and screening for strains with beneficial activities. We used healthy soybean roots from plants collected in the Mississippi Delta to extract the endophytic fraction of the root microbiome using published protocols (Kaplan et al., Lebeis et al.). To isolate endophytes, serial dilutions of the endophytic bacterial cell extract were plated on diverse agar media. Approximately 250 isolates were obtained, out of which over half have been tested using an in vitro dual-culture method to identify isolates with anti-Xylaria activity. Isolates which significantly inhibited Xylaria growth in vitro were selected for in planta assays to identify those with anti-TRD activity. To date, we identified a group of bacterial isolates which significantly inhibit or abolish the development of TRD in soybean. At the completion of library screening, isolates with strong anti-TRD activity will be tested for synergistic activity. Our long-term goals are to discover and deploy microbiome-based management techniques that lead to improved soybean health and yield.

CS 5A-4. Optimization of lipid accumulation in the Chlamydomonas reinhardtii strain CC5373-STA6 under nitrogen and sulfur deprivation

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The use of microalgae in biodiesel production is of increasing interest due to the capability of enhancing the algae's triacylgerol (TAG) biosynthesis. Research in this field focuses on optimizing stress conditions conducive to synthesis and accumulation of microalgal TAGs. Nitrogen starvation causes algal cells to accumulate starch and increase lipid content. Sulfur deprivation inactivates photosynthetic activity and increases H2 production. Chlamydomonas reinhardtii starchless mutant cc5373-sta6, has become of interest due to the overaccumulation of lipid bodies caused by inhibition of starch biosynthesis. To our knowledge, no studies have reported on depriving both nitrogen and sulfur from the C. reinhardtii strain cc5373-sta6 as a stress condition for lipid production. We hypothesized that by removing both nitrogen and sulfur from cc5373-sta6's Tris-acetate-phosphate (TAP) growth media lipid bodies will overaccumulate within the cell. The purpose of this study was to compare the effects of nutrient deprivation conditions on cc5373-sta6 via chemical and microscopic analysis to establish optimal lipid accumulation in the microalgae. The objectives of this study are to (1) determine the effects of nitrogen and sulfur starvation on cc5373-sta6's physiology by conducting spectrophotometric chemical analyses (cell density, chlorophyll content, and biomass determination) and (2) determine lipid accumulation via confocal microscopy. Chemical analyses for nutrient deprived cells displayed increased cell density, increased biomass accumulation and decreased chlorophyll content suggesting accumulation of lipids. Confocal imaging showed nutrient deprived cells to accumulate large lipid droplets compared to cells grown in TAP media. Cells deprived of sulfur showed an increased number of lipid bodies per cell, whereas cells deprived of nitrogen showed a larger lipid body size. This study will report the effects nitrogen and sulfur deprivation have on the physiological integrity and lipid accumulation of cc5373-sta6. This study will add to the knowledge on the potential of cc5373-sta6 as a renewable source for biofuel production.

CS 5A-5. Characterization and cloning of an albino mutant of maize by bulk-segregant whole genome re-sequencing.

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Chloroplasts and mitochondria are essential for plant growth and development. We have identified in a maize stock an apparent spontaneous recessive seedling lethal mutant manifesting albino or yellowish-white plantlet phenotypes in the field and greenhouse. We performed bulk-segregant whole genome resequencing of pools comprising of ~100 homozygous recessive mutant and ~200 WT individuals segregating in progeny of three back-crosses into the reference genome B73 background. The workflow entailed Illumina NovaSeq genomic DNA library sequencing to ~80X genome depth coverage, GATK to identify de novo SNPs/Indel variant alleles and QTLseqr to fine map the interval. The mutation was mapped to short arm of chromosome 9 and fine mapped to an interval of ~10 Mbp containing 31 genes. A top candidate with a two nt deletion in the N-terminus of the first exon was identified to be a key enzyme involved in protein synthesis which is dual targeted to both chloroplast and mitochondria. Reads abundances mapping to chloroplast and mitochondria genome revealed 6.3% and 2.8% mutant reads vs 0.8% and 1.3% WT sibling reads mapped to the chloroplast and the mitochondria genome, respectively. Orthogonal/independent tests of our interpretation of the causal mutation entailed transcriptome analysis showed the gene to be significantly downregulated in the albino seedlings. Moreover, the transcript levels of chloroplast and the mitochondria genes were also significantly affected in the mutant. Two homologous genes identified by forward genetics in Arabidopsis have been annotated as having recessive embryo lethal/albino phenotypes. One of the conserved functions of a C4 plant like maize is to increase and decrease the size of the chloroplast compartment in the mesophyll and bundle sheath cells in the leaves. Understanding the underlying mechanism of chloroplast development and the ability of the C4 plants to manipulate chloroplasts from this study will further help engineer C3 plants into C4 photosynthesis.

CS 5A-6. Novel structural characteristics of oil biosynthesis regulator protein WRINKLED2 in avocado

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Plants synthesize and store oil, mostly triacylglycerol (TAG), in various storage tissues that serves as a source of carbon and energy. In most tissues, fatty acid biosynthesis is transcriptionally controlled by WRINKLED1 (WRI1), a member of the APETALA2 (AP2) class of transcription factors. Among the four Arabidopsis WRI1 paralogs, only WRI2 is nonfunctional and failed to complement wri1-1 mutant seeds. In avocado (Persea americana), however, fruit mesocarp with 60-70% DW oil showed high expression levels of WRI2, along with WRI1 and WRI3. The role of alternate transcription factors to WRI1 in seed or in non-seed tissues is poorly understood. Hence, we conducted structural analyses to

elucidate distinct features of avocado WRI paralogs compared to their orthologs in seed tissues. Comprehensive comparative in silico analyses of WRI1 paralogs from Arabidopsis (dicot), maize (monocot), and avocado, which is basal angiosperm revealed distinct features associated with their function. Our analysis showed the presence of only one AP2 domain in all WRI2 orthologs, compared to two AP2 in others. The highly conserved N-terminal region and the less conserved C-terminal regions made up the primary structure of the proteins, with amino acid composition bias characteristic of intrinsically disordered proteins. Additionally, the avocado WRI2 showed a high proportion of random coil secondary structure, although it lacks a C-terminal intrinsically disordered region. Also, both WRI1 and WRI2 have distinct predicted phosphorylation target sites compared to their orthologs, whereas WRI2 lacks a PEST motif. Finally, through transient expression assays, we demonstrated that both avocado WRI1 and WRI2 are functional and drive TAG accumulation in Nicotiana benthamiana leaves. Our study showed that avocado WRI2 is structurally different and is functional, unlike its ortholog in Arabidopsis. This study provides us with new targets to enhance oil biosynthesis in various plant tissues.

Competitive Concurrent Session (CS) 5B

CS 5B-1. Genetic dissection of agronomic and yield component traits under reproductive stage salinity stress in rice (Oryza sativa L.)

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Salinity is one of the major environmental constraints severely limiting worldwide crop production, resulting in approximately 27 billion US dollars loss annually. Rice cultivars are fundamentally susceptible to salinity stress beyond 3 dSm-1 Tolerance of rice varieties to salinity stress varies across different developmental phases, with seedling and reproductive stage being most vulnerable to salinity stress. Extensive knowledge regarding the QTLs and genes addressing various salt tolerance mechanisms at the seedling stage have already been gathered. However, little emphasis has been given to the identification of QTLs responsible for tolerance at the reproductive stage. Therefore, a double-cross (Jupiter/TCCP//Jupiter/FL478) rice population was screened for salt stress at flowering stage to determine the major-effect QTLs responsible for reproductive stage salt tolerance mechanisms. Ninety-two lines along with parents were subjected to 8 dSm-1 saline stress at the advanced booting stage to identify superior lines showing higher grain yield and yield component traits in comparison with the recurrent parent Jupiter. Significant difference was observed among all the lines for different agronomic and yield-related traits. Spikelet sterility, an important indicator of reproductive stage tolerance, ranged from 28 % to 88 %. About seven lines showed spikelet sterility comparable to a donor parent (TCCP). Using the mean phenotypic values of all traits, QTL analysis will be performed using a high-density SNP map obtained from genotype by sequencing (GBS) method. Overall, our study will provide important insight regarding the reproductive stage salt-tolerant QTLs which will, in turn,

accelerate the development of salt-tolerant rice varieties through marker-assisted QTL pyramiding.

CS 5B-2. Transcriptome analysis on the interaction of upland cotton (Gossypium hirsutum L.) chromosome substitution line, CS-B15SH, with 2,4-dichlorophenoxyacetic acid (2,4-D) as herbicide

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Upland cotton, Gossypium hirsutum L., is a natural source of fiber and a major row crop in the US with an estimated $7 billion raw product value in 2019. However, it is extremely sensitive to broadleaf herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D), used to control weeds and reduce effects on yield. The evolution of herbicide resistance and the limited variety of characterized herbicide resistance genes available for plant improvement necessitate the identification and development of novel sources of herbicide tolerance in cotton germplasm. Here we report a study of the cotton chromosome substitution line, CS-B15sh, developed in the genetic background of G. hirsutum L. Texas Marker 1 (TM-1) with introgression on the short arm of chromosome 15 from G. barbadense L., and its interaction with 2,4-D herbicide. Cotton seedlings were screened for injury after 2,4-D application at 1x, the field rate (1.12 kg ae ha-1), in the greenhouse. Uptake and translocation of 14C-labeled 2,4-D was measured in CS-B15sh and TM-1 plants to determine whether altered movement of 2,4-D is involved in the tolerance mechanism. Differential expression of genes in 2,4-D treated and non-treated leaf tissues in CS-B15sh and TM-1 was assessed using RNA sequencing. Greenhouse screening revealed that CS-B15sh plants averaged 41% lower injury compared to TM-1 at 21 days after application. After 24 hours of [14C]2,4-D treatment, CS-B15sh plants showed restricted movement of [14C]2,4-D within the treated leaf in contrast to TM-1 where 2,4-D showed greater movement to distal leaves. Transcriptome analyses revealed differential expression of genes between treated CS-B15sh and TM-1 plants in several components of the 2,4-D/auxin response pathway, including ubiquitin E3 ligase, PB1|AUX/IAA, ARF transcription factors, and F box proteins of the SCFTIR1/AFB complex. Progress on understanding the genetics and molecular mechanism of tolerance to 2,4-D in CS-B15sh plants will be discussed.

CS 5B-3. The integration of time of day and transcript abundance in sorghum in chilling stress

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Transcriptional changes are an early response to stress or environmental changes. Identifying transcripts that change in response to stress can provide important insights into how a plant perceives and responds to stress. Therefore, it is a widely-used practice to perform RNA-Seq as a first step to compare treatment and control plants and identify differentially expressed genes. However, the expression levels of transcription factors are regulated by the circadian clock. The time of day an experiment is done can have significant effects on the differentially expressed genes identified. The same response monitored at two times of day can show very different results, and it may give a more accurate picture to capture the response at multiple time points. However, transcription itself may be under the control of the circadian clock. If global transcription is altered either by time of day or the stress that the plant is exposed to, the standard approach of normalizing the total reads or counts per million can lead to the wrong interpretation of the data. A spike-in control is an external RNA with known sequence and quantity for calibrating RNA-Seq data. Adding spike-in controls can eliminate the bias from global transcriptional changes and allow accurate data interpretation. Here we evaluated how the time of day the plant response was examined and the RNA normalization approach affected the observed response of sorghum to early-season chilling stress. We found that using external RNA spikes as a normalization factor helped remove within-sample variations but captured variations between samples from different times of the day and chilling stress treatment. The differential expression analysis indicated an increase in the number of differentially expressed genes in the dataset normalized by external spikes. Our results suggested that normalizing RNA-Seq data with external RNA spikes provided a better insight into RNA-Seq data interpretation.

CS 5B-4. Characterization of changes in lipid profile during development of the moss Physcomitrium patens

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Lipids are the main constituents of the cell membrane and maintain their fluidity. Plants undergo various lipid changes during stress conditions. The moss Physcomitrium patens is an early land plant with a unique ability to tolerate stress like cold and dehydration. Moss undergoes the transition from gametophyte (vegetative stage) to sporophyte (reproductive stage) only under cold temperatures. Developmental stages play a diverse role in moss lifecycle but there is a scarce study in lipids. Thus, we wanted to study the changes in lipid content and composition at various developmental stages. To this extent, using LC-MS/MS analyses, we identified and quantified the major and minor lipid classes and their acyl composition of protonema, gametophyte (early, mid and late), and sporophyte tissues. Also, we compared moss with Arabidopsis, Selaginella, and mouse lipid data. Galactolipids are predominant plastidic lipids and thus most abundant in the moss vegetative tissues but not in sporophytes. Phosphatidylcholine (PC) was the abundant phospholipid during moss life cycle. Sporophyte tissues were distinct from gametophyte and protonema and also other vascular plants with high amounts of phosphatidic acid (PA). Plants accumulate PA in response to stress which indicates that the temperature cue necessary for sporophyte formation is associated with a spike in PA. Also, PC was abundant in sporophyte, indicating its's role in stability while transitioning into the sporophyte. In comparing the acyl

composition of the various lipid classes, we identified that in addition to 34C and 36C lipids, moss also contain 38C and 40C lipids, which are not represented in vascular plants. We predict that the occurrence of long-chain, highly unsaturated lipids might contribute to the dynamic nature of the membrane and stability under stress. Overall, it aids our understanding of how early land plants coped through harsh environmental conditions during their transition from marine to land habitat.

CS 5B-5. SPL10 activates age-dependent resistance to bacterial pathogen Pseudomonas syringae

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Under constant exposure of pathogens, plant immunity is critical for plants to survive. Many plant species gain resistance along with one or several developmental process(es). This termed as age-related resistance (ARR). The genetic programming of plant age-related resistance is poorly understood. To dissect the mechanism of ARR, we used Arabidopsis-Psedomonas Syringae pv. tomato DC3000 (Pto DC3000) as a model system. In Arabidopsis, microRNA156 masters vegetative phase change by inhibiting members in the SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) gene family. We discovered that miR156 suppressed resistance to Pto DC3000 in juvenile phase. A subset of SPLs, SPL2, 10 and 11, promoted resistance in adult phase of Arabidopsis. Furthermore, the age-related resistance to Pto DC3000 was correlated with high amplitude induction of PR2 gene expressions, a salicylic acid (SA) marker gene. The high amplitude induction of PR2 expression was dependent on SPL2, 10 and 11 only in adult but not juvenile phase. A subgroup of SA biosynthesis genes was shown to be SPL10-binding candidates in a literature-based search. Interestingly, the expression of those SA genes can be enhanced in inducible overexpressing SPL10 and/or be reduced in loss-of function spl2/10/11 mutant lines. All together, we propose that SPL2, 10, 11 promote age-related resistance potentially through strengthening the SA biosynthesis. Our research will bring insights regarding how the miR156-SPL pathway coordinates disease resistance with developmental timing. As the function of miR156 in regulating juvenile-adult transition is conserved in all land plants. The proposed mechanism may unlock the potential to engineer age-related resistance in many plant species.

CS 5B-6. Functional characterization of avocado PaPDAT1 and PaDGAT1 by complementation of corresponding Arabidopsis mutants

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The rapid increase in the world's demand for vegetable oils in the form of triacylglycerols (TAG) due to its nutritional and industrial value has driven the research towards enhanced production of oil. In higher plants, phospholipid: diacylglycerol acyltransferase1 (PDAT1) and acyl coA: diacylglycerol acyltransferase1 (DGAT1) catalyze the terminal step in TAG synthesis and are highly conserved. We identified and characterized PDAT1 and DGAT1 in

avocado (Persea americana), which accumulates 70%-80% TAG in the mesocarp, a non-seed tissue. Transient expression of avocado PDAT1 and DGAT1 (hereafter, PaPDAT1 and PaDGAT1, respectively) in the leaves of Nicotiana benthamiana led to an increase in oil content with a preference for oleic acid, C18:1. We hypothesize that both PaPDAT1 and PaDGAT1, although predominantly expressed in non-seed tissues, are capable of contributing to oil accumulation in seeds. To test this, Agrobacterium transformants with PaPDAT1 and PaDGAT1 cloned in pCAMBIA with dsRed were generated. Subsequently, Agrobacterium-mediated transformation of Arabidopsis Atdgat1-/- and Atpdat1-/- mutant lines was carried out by floral dipping method. A successful functional complementation of Arabidopsis mutants by PaPDAT1 and PaDGAT1 will be determined by genotyping T1 expressing the genes and T2/3 lines to get homozygous lines. The homozygous transgenic seeds will be evaluated for TAG content and fatty acid composition relative to wild type seeds. The transgenic seed morphology, germination rate as well as expression level of PaPDAT1 and PaDGAT1 at different stages of seed development will be studied. These data will allow us to determine if PaPDAT1 and PaDGAT1 increase the oil content in seeds and are effective in generating oleic acid rich TAG. This study is aimed to serve as a proof-of concept to generate heart healthy canola oil.

Competitive Concurrent Session (CS) 7A

CS 7A-1. differences in heat requirements and abscisic acid levels may explain contrasting bloom dates among apple cultivars

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Apple production in temperate regions is continuously imperiled by spring frosts. Economic losses caused by frost damage can be enormous, and the risk of such losses is projected to rise due to global climate change. Induction of bloom delay has been suggested as a potential frost avoidance strategy. However, applicable practices toward this goal are largely lacking, primarily due to the complexity of mechanisms governing dormancy and blooming in deciduous woody perennials like pome fruits. Cultivars differing in bloom time provide a suitable platform for investigating the regulatory mechanisms of these phenological processes. In this study, we investigated the hormonal regulation of bud dormancy and blooming in two apple cultivars, 'Honeycrisp' and 'Cripps Pink', which represent late- and early-blooming apple cultivars, respectively. Our results indicated that the two cultivars showed similar chilling requirements but differed significantly in the number of growing degree hours (GDHs) required for bud burst and flowering. Abscisic acid (ABA) levels were remarkably elevated in the dormant buds of both cultivars during endodormancy, but they were significantly higher in 'Honeycrisp' buds, especially during the transition from endo- to eco-dormancy. Through investigating ABA biosynthetic and catabolic genes, we identified two biosynthetic genes, NCED1 and NCED2, and one catabolic gene CYP707A 2.3, whose differential transcript levels can explain the distinctive ABA patterns in the two apple

cultivars. Our data also showed higher cytokinin levels in the buds of 'Cripps Pink' compared to 'Honeycrisp', shortly before the time of budburst. The results of this study indicate that differences in bloom times among apple cultivars can be explained, at least partially, by the differential heat requirements and ABA levels during eco-dormancy. Future investigations, especially at the omics levels would provide a better understanding of molecular networks linking ABA hemostasis with bud dormancy and bloom regulation in apple.

CS 7A-2. Hydrogen cyanamide alters carbohydrate metabolism in southern highbush blueberry

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Hydrogen cyanamide (HC) is a plant growth regulator used to overcome insufficient chill accumulation in deciduous fruit trees and small fruits. When spray timing and concentration are ideal, HC expedites bud break and enhances yields. Despite its popularity, little is known about how this plant growth regulator expedites fruit production. We studied phenological and gas exchange responses to HC application in southern highbush blueberry (Vaccinium corymbosum interspecific hybrids, cv. 'Optimus') in separate growth chamber and field experiments. Plants were forced into dormancy in a growth chamber using short days and low temperatures. Subsequently, dormant plants received 0, 50, 100, 200, or 400 hours of chilling at 7 °C. After chilling, plants were sprayed with 0.63% HC solution with 0.5% non-ionic surfactant and placed in a greenhouse. A datalogger was used to measure heat accumulation. Gas exchange and non-structural carbohydrate measurements were made at periodic intervals as plants emerged from dormancy and started growing. Simultaneously, HC-treated branches from field-grown 'Optimus' plants were also collected at different phenological stages over the 2020-2021 growing season and analyzed for soluble non-structural carbohydrate concentrations and starch concentrations. HC application expedited vegetative bud break, leading to 1.5 cm2 leaves per cm cane 12 days earlier in the 400-hour treatment compared to the control. HC application and leaf development affected gas exchange. Net photosynthesis was approximately 3-fold higher in the 400-hour treatment compared to the control 28 days after HC application. Non-structural soluble carbohydrate and starch measurements are ongoing. Preliminary results suggest that HC affects the carbohydrate balance of deciduous fruit crops and this leads to accelerated fruit development.

CS 7A-3. Evaluation of systemic acquired resistance (SAR) inducers for management of downy mildew (Hyaloperonospora parasitica) on baby leaf kale (Brassica oleracea 'Lacinata')

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Kale is considered a rich source of important minerals and has risen in dietary popularity in recent years, particularly baby leaf kale. Downy mildew (DM), caused by the oomycete Hyaloperonospora parasitica is the most important economic disease of kale. The objective

of this research was to compare the efficacy of compounds demonstrating Systemic Acquired Resistance (SAR) inducing properties for DM management. Kale was direct-seeded in a commercial spring mix production field in twenty-two rows with a 2-in. row spacing on top of 8-in. raised beds formed on 6-ft centers. The experiment consisted of a randomized complete block design with four replications of five treatments; an untreated check, Actigard WDG (0.25 oz/A), LifeGard WP (3.0 oz/A), salicylic acid (2.0 oz/A), and K-Phite SL (48 fl oz/A). A total of three foliar applications were made at 6-day intervals, initiated at the cotyledon stage. Downy mildew was visually assessed at two random locations per experimental unit 22 and 26 days after planting and yield was measured by randomly harvesting 20 plants from each experimental unit, obtaining an aggregate fresh weight and then obtaining the marketable weight of symptomless leaves. Based upon a comparison of disease severities, all fungicides provided for significant reductions in downy mildew severity. Actigard and K-Phite were significantly better than LifeGard and salicylic acid at both ratings and salicylic acid was significantly better than LifeGard at the second rating. Actigard and K-Phite produced significantly higher total fresh biomass and marketable biomass than the other SAR treatments and the check. With SAR treatments providing a near minimum 50% DM control, this mode of action could prove promising in a disease management program looking to utilize low-risk chemistry. However, results do not suggest that SARs can be used alone to produce the low disease tolerances demanded by the commercial spring mix industry.

CS 7A-4. Evaluation of various rates of potassium phosphite for management of downy mildew on baby leaf kale

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Florida ranks third among states in production of leafy spring mix, with baby leaf kale being an important component. Downy mildew (DM), caused by the Oomycete Hyaloperonospora parasitica, is the most economically important disease of kale in the region. Capable of infecting at any stage, it produces small dark spots on the abaxial part of the leaf during early stages. Affected areas may yellow, enlarge, and turn necrotic as lesions age. Sporulation and infection are favored by heavy dews and high humidity produced within the densely populated baby leaf canopies, particularly during periods of cool to moderate night-time temperatures (12 to 20 C). Since there is little tolerance for this disease in spring mix, growers must be preventative regarding management and they are most interested in cost-effective low-risk fungicides. The objective of this study was to evaluate various rates of dipotassium phosphorus acid (potassium phosphite) for management of kale DM and to compare it to an industry standard, mandipropamid (Revus SC). The experiment consisted of five treatments, an untreated check, K-phite at 2.0, 4.0, and 6.0 pts per acre, and Revus at 8.0 fl oz per acre, arranged in a randomized complete block design with four replications. Three foliar applications were initiated at the cotyledon stage and were sprayed at 6-day intervals. Compared to the check, all fungicide treatments reduced DM severity, with no significant differences among K-phite rates during the first assessment. However, only K-phite at the 6-pt rate performed as well as Revus during the later stages of the trial, providing

comparable residual control. In summary, potassium phosphite provided effective DM control on kale, and it could prove to be a low-cost, low-risk alternative to some of the more costly DM fungicides. Additionally, it could serve well in an effective rotation for management of pathogen resistance.

CS 7A-5. understanding the molecular mechanisms of drought in plants

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Plants might face an inherently harsh environment at any of their life stages. A series of adverse environmental factors, such as high or low temperature, deficient or excessive water, high or low salinity, have varying effects within plants, leading to molecular, cellular or phenotypic alternations. Among various abiotic stresses, drought is raising more concerns as its severity on agriculture is increasing as a result of climate changes, necessitating the elucidation of potential mechanisms underlying drought. Dedicated by evolution, plants have formed complete and sophisticated defense systems which sustain their survival under drought conditions. Typical adaptive alternations involve the synthesis of osmolytes, scavenging of reactive oxygen species, production of hormones, and strengthening of root systems. In the past decades, rudimentary studies on mRNA and small RNAs provided fragmented information on molecular events leading to the onset of drought in various crops, such as tomato, rice, and soybean. However, till now the omnibus regulatory networks remain unestablished, partly because drought affects plants' modulation by its intensity, rate, and duration, and partly because of the immaturity of previous technologies which limits the integrated study at various -omics levels. Here I set out to perform diverse -omics experiments at a set of drought time points in Arabidopsis and tomato, including rRNA reduction library, miRNA library, m6A-eCLIP library, and HiChiP library. With the help of Next-Generation Sequencing analysis, my data will present one integrative regulatory network for plant-drought interactions, including protein-coding genes, long non-coding RNAs, microRNAs, RNA methylation, transcription factors. Further, a variety of mutants corresponding to the crucial regulatory elements from this system will be constructed and wet-lab techniques will be used to validate the predictions deriving from data analysis. With the completion of this proposal, the experimental results will play a major role in stabilizing crop performance under drought and in the protection of natural vegetation.

CS 7A-6. Harnessing salinity tolerance from the wild: de novo domestication of Solanum cheesmaniae via CRISPR/CAS9 genome editing

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Agriculture is the human activity that utilizes most of the freshwater resources available globally. The increasing limitations of freshwater supply together with the substantial use of continuous irrigation systems are intensifying the progression of soil salinization over the arable lands worldwide. Soil salinization has a significant impact on crop development and productivity, which limits cultivation in marginal lands and the use of saline water for agriculture. A glycophyte crop, such as tomato (Solanum lycopersicum), requires high amounts of water while being highly sensitive to soil salinity. Introgression of salinity resistance into tomato cultivars via traditional breeding procedures remains challenging due to the polygenic nature of abiotic traits. We aimed to de novo domesticate tomato as a high-salinity resistant crop from its wild relative, S. cheesmaniae. Based on published data, the accession LA0421 originally from the seashores of the Galapagos Islands is characterized by less yield penalty when exposed to high saline conditions. Thus, we chose LA0421 as the genetic baseline for knocking out genes involved in domestication traits, including plant architecture, flowering, yield, fruit size, and nutritional value. We are using a multiplex CRISPR/Cas9 strategy to produce loss-of-function alleles for the domestication-related genes BIF, cycB, J2, EJ2, MULTI, SP, SP5G, and FW11.3 in order to create a novel, halophyte tomato harboring an inherent high-salinity resistance along with desirable cultivation traits. This proof-of-concept research aims to engineer crops using speedy reverse breeding that could potentially enable the use (at least partially) of seawater hydroponics or saline soils for food production.

CS 7A-7. The role of ribosomal gene in telomere length regulation of Arabidopsis thaliana

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Telomeres are important nucleoprotein structures at the ends of eukaryotic chromosomes. Telomeres are required for proper genome maintenance and regulation of cellular life span. Telomeres in plants consist of repetitive DNA tracts with hundreds of TTTAGGG repeats. Few genes involved in telomere length control are currently known. We previously analyzed telomere length in 19 natural populations and 480 recombinant inbred MAGIC lines of Arabidopsis thaliana and through QTL mapping identified OLI2/NOP2A gene as a master regulator of telomere length. Several other genes from the same genetic OLIGOCELLULA pathway are known, but their role in the regulation of cell proliferation and telomere biology is less understood. The goal of this research is to characterize the role of Arabidopsis OLI5/RPL5A gene in telomere length regulation. OLI5/RPL5A encodes ribosomal protein L5A that binds to 5S ribosomal RNA and is involved in rRNA export from the nucleus to the cytoplasm and ribosome assembly. We analyzed two mutant lines of OLI5/RPL5A gene: oli5-2 (SALK_089798) and oli5-3 (SALK_023075). Plants were genotyped for the presence of T-DNA insertions in the OLI5/RPL5A, and telomere length was measured by telomere restriction fragment (TRF) assay that utilizes DNA hybridization with digoxigenin (DIG) probe. Our results indicate that homozygous oli5-2 and oli5-3 mutants display 30% shorter telomeres as compared to wild type plants. We next analyzed genetic interaction of OLI5/RPL5A and OLI2/NOP2A genes by creating double mutants. Evaluation of telomere

length phenotype in the double mutants indicates that both genes belong to the same genetic pathway, as no additional telomere shortening is observed in the double mutants in comparison to either of the two single mutants. Overall, our work indicates that OLI5/RPL5A gene has an important role in the regulation of telomere length in Arabidopsis thaliana. This work was supported by the Russian Science Foundation (project number 21-14-00147 to E.V.S.)

Competitive Concurrent Session (CS) 7B

CS 7B-1. Functional characterization of PvWRKY1, a seashore paspalum WRKY gene potentially involved in plant development and stress response

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WRKY transcription factors (TFs) are named by the presence of the WRKY domain, a 60-residue DNA-binding domain containing a highly conserved WRKYGQK motif. They can be classified into three subfamilies based on WRKY domain numbers and zinc-finger structures. WRKY TF family is one of the biggest TF families and plays an important role in development and response to stress in plants. We have cloned PvWRKY1, a WRKY TF gene from seashore paspalum (Paspalum vaginatum), a halophytic warm season perennial grass that is tolerant of many environmental stresses, especially salt stress. PvWRKY1 is highly expressed in an extremely salt tolerant phenotype. To reveal its possible role in regulating plant development and stress response, we have introduced PvWRKY1 into both Arabidopsis and creeping bentgrass. Overexpression of PvWRKY1 in creeping bentgrass alters plant development by inhibiting tillering and promoting the growth at early stage. Upon salt stress, transgenic plants exhibit more severe damage, despite a higher proline accumulation and a higher chlorophyll content than wild type controls. Further characterization of transgenic plants will allow a better understanding of the molecular mechanism underlying PvWRKY1 involvement in plant development and stress response, providing information for the development of novel biotechnology approaches for crop genetic improvement.

CS 7B-2. Gene stacking for boosted plant growth and broad abiotic stress tolerance

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Abiotic stresses such as salinity, heat and drought have serious impact on plant growth and development, causing significant loss in yield and ornamental value. Biotech approach manipulating specific genes proves to be an effective strategy in crop trait modification. The Arabidopsis vacuolar pyrophosphatase gene AVP1, the rice SUMO E3 ligase gene OsSIZ1 and the cyanobacterium flavodoxin gene Fld have previously been implicated in regulating plant stress response and confer enhanced tolerance to different abiotic stresses when individually overexpressed in various plant species. We have explored the feasibility of combining multiple beneficial traits brought by each individual gene for superior plant

performance. To this end, we have simultaneously introduced AVP1, OsSIZ1 and Fld in creeping bentgrass. Transgenic plants overexpressing these three genes performed significantly better than wild type control and transgenics expressing each individual gene under both normal and various abiotic stress conditions, exhibiting significantly enhanced plant growth and plant tolerance to drought, salinity and heat stresses as well as phosphate nitrogen starvation, which were associated with a number of improved physiological and biochemical characteristics as well as altered expression of genes involved in plant stress responses. Our results suggest that AVP1, OsSIZ1 and Fld function synergistically to regulate plant development and plant stress response, leading to superior overall performance under both normal and adverse environments. The information obtained provides new insights in gene stacking as an effective approach for plant genetic engineering. Similar strategy can be extended to other crops species for trait modifications, enhancing agricultural production.

CS 7B-3. Characterization of genetic mechanisms influencing perenniality variation in Arabidopsis lyrata

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The key to understanding divergence in life history evolution of plants lies in the comprehension of how trade-offs between traits influence species fitness and environmental local adaptation. The goal of this project is to determine the molecular basis of adaptive variation involving perenniality in Arabidopsis lyrata. A. lyrata populations are known to grow in warm versus cold climates and are highly differentiated along a perenniality continuum. These aspects contribute to their local adaptation and makes A. lyrata a useful model system for studying how populations diverge and understanding how reproductive barriers arise. The underlying genetic mechanisms that influence physiological and morphological differences in A. lyrata populations are still unknown. However, several QTLs have been mapped in crosses between divergent populations, the largest of which is on Chromosome 2 (C2). The morphological differences between populations suggest genetic variation in the allocation of meristem turnover from vegetative to reproductive states is responsible. This research will involve using a multifaceted approach that combines genetics and reciprocal transplant experiments. Reciprocal hemizygosity tests will be conducted in both North Carolina (Mayodan) (MA) and Norway (Spiterstulen) (SP) A. lyrata populations using CRISPR to knockout protein function in genes of interest mapped in the C2 QTL region. The genes that were identified include: PIN1, PIN3, BRC2 and PILS2. These genes were chosen because of their influence on auxin signaling, homeostasis and lateral shoot development. CRISPR/CAS9 enabled manipulation and reciprocal-hemizyogosity tests will be used to discover whether the QTL allelic differences explain the perenniaity differences. Agrobacterium- transformation techniques will be used to insert perenniality genes of A. lyrata into knockout lines of A. thaliana to observe differences in phenotypic variation. If candidate genes of interest are the ones affecting perenniality, then A. thaliana will produce more vegetative shoots and push more of its meristem toward long-term vegetative growth.

CS 7B-4. Transient expression of the rabies glycoprotein in soybean

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This research aims to demonstrate the successful transient expression of the rabies glycoprotein antigen in soybean using agroinfiltration. Agroinfiltration utilizes bacteria to infect a plant cell and insert a desired gene into the plant's genome. When expressed on its own, the glycoprotein has been shown to form Virus-like Particles (VLPs), which structurally resemble the native virus without any chance of being infectious. These VLPs produced in plants have been shown to produce varying levels of immunity in animal studies. Soybean is a promising system as a common agriculture crop that offers protein rich seeds and extensive leaf material. The findings would present a proof of concept of a successful edible rabies vaccine for animals. While this research is primarily focused on working towards the goal of producing an edible vaccine for animals, this is a steppingstone toward a much greater goal. Edible vaccines for humans would overcome the limitations that hold traditional vaccines back from reaching many underdeveloped regions, where people are still dying from highly preventable diseases. We are currently using agroinfiltration with a plasmid containing two reporter genes, GFP and GUS, to transiently transform soybean plants in order to optimize our transformation protocols. Following this step, a reporter gene will be replaced with the glycoprotein gene and plants will be transiently transformed to express the glycoprotein VLP. This is working toward future research where the transformed soybean leaf tissue's effectiveness at producing protective immunity in mice when orally administered will be studied. Future work also includes the production of a transgenic line of soybean plants, which means the offspring of the plant will continue to express the glycoprotein VLP. Producing a transgenic soybean plant expressing the rabies glycoprotein that, when orally ingested, produces protective immunity in animals will help pave the way for future edible vaccines for humans.

CS 7B-5. characterization of oxidative stress impacts on lipid metabolism to enhance plant resilience

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Redox reactions, which involve a transfer of electrons between two molecular species, are ubiquitous within plant metabolism. For example, plants transform light energy into chemical energy using photosynthetic complexes embedded within thylakoid membranes. Specialized glycerolipids that make up these membranes in turn require redox reactions for the synthesis and modification of their fatty acyl chains. Previous research has shown that plants subjected to oxidative stress (e.g., high light, high temperature, drought, etc.) often exhibit conditions associated with an unbalanced flow of electrons (and redox reactions). Uncontrolled stress can then result in increased levels of reactive oxygen species (ROS) which are frequently damaging to overall plant health. It remains unclear how chloroplast membranes are impacted under oxidative stress conditions and subsequently repair their membranes to maintain photosynthesis and other metabolic pathways. Therefore, this project sets out to further understand the biochemical connections between chloroplast membrane lipids, redox-protection mechanisms, and oxidative stress using the model system Arabidopsis thaliana. To do this we are currently generating higher order mutants genetically crossing mutants in redox metabolism/protection (e.g., NADPH- dependent

thioredoxin c, catalase) and lipid metabolism pathways (e.g., fatty acid desaturases, lipid assembly enzymes). We are characterizing each of these novel mutants, in addition to their single mutant parents, through measurements of growth and photosynthetic parameters, lipid profiles, and lipid stress-repair assays. Ultimately, we predict that through an improved understanding of chloroplast membrane dynamics our results can inform engineering strategies that target enhanced plant resilience subjected to adverse environmental conditions.

CS 7B-6. Protection of telomeres 1b protects against telomere oxidation in Arabidopsis thaliana

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Theories of cellular senescence highlight the genetic role of telomeres and the metabolic role of Reactive Oxygen Species (ROS) in determining longevity. However, little is known about the interplay between them. Telomeres are the protective caps on chromosome ends, consisting of long stretches of G-rich repetitive DNA that must be maintained to ensure genome stability. Due to their sequence, telomeres are hotspots for DNA oxidation, especially 8-oxoguanine lesions. Oxidation of telomeric DNA can lead to changes in telomere length by dysregulating telomerase or DNA repair pathways. Mild oxidative stress is associated with incremental changes in telomere length, but more severe stress triggers substantial telomere shortening. PROTECTION OF TELOMERES 1 (POT1) is one of the most highly conserved components of the telomere complex and in humans it has been implicated in the response to oxidative stress. The flowering plant Arabidopsis thaliana encodes two highly divergent POT1 paralogs, POT1a and POT1b. POT1a is an essential telomeric factor, involved in telomerase regulation. Here we show that POT1b is a nuclear-associated protein that is not required for telomere maintenance under normal growth conditions. Plants lacking POT1b display delayed development and are hypersensitive to oxidative stress. Transcriptomics analysis of POT1b mutants reveals activation of multiple oxidative stress-related genes. Consistent with this observation, pot1b mutants exhibit elevated ROS as measured by DAB (3,3'diaminobenzidine) and DCF-DA staining. Strikingly, telomeres in plants lacking POT1b shorten in response to ROS. Using an ELISA-based assay to measure 8-oxoguanine, we found that plants deficient inPOT1b or the major antioxidant CATALASE 2 accumulate higher levels of 8-oxoguanine than wild type plants. We hypothesize accumulation of such lesions causes telomere shortening. We propose that POT1b functions to maintain redox homeostasis, and particularly to mitigate DNA oxidation at chromosome ends.

Competitive Concurrent Session (CS) 8A

CS 8A-1. The role of polynucleotide phosphorylase (pnpase) homeologs of Nicotiana benthamiana in plasmodesmata function

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Plasmodesmata (PD) link the cytoplasm of two adjacent cells, allowing intercellular trafficking of solutes, signaling molecules, protein, RNA, and ribonucleoprotein complexes. The regulation of plasmodesmata-mediated trafficking is essential for plant growth and development. A hypothesis called Organelle-nucleus-plasmodesmata-signaling (ONPS) was developed by our group to explain this regulation. This hypothesis states that specific changes in organelle (plastids or mitochondria) gene expression lead to signaling to the nucleus (chloroplast-to-nucleus retrograde signaling, CRS) that changes the expression of nucleus-encoded genes associated with plasmodesmal regulation. Following this hypothesis, silencing homeologs of a chloroplast RNA processing gene named PNPase (POLYNUCLEOTIDE PHOSPHORYLASE) revealed their conflicting roles in plasmodesmal regulation. PNPase is highly conserved in bacteria and organelles (plastids and mitochondria), where it functions in RNA degradation and maturation through its polyadenylation and exonucleolytic activities. Nicotiana benthamiana encodes two putative chloroplast-targeted PNPASE proteins (PNPase A and PNPase B) and two mitochondrial-targeted PNPASE proteins. To study their roles in plasmodesmal regulation, we targeted individual or both chloroplast-targeted homeologs (PNPase A-, PNPaseB- and PNPase A/B-) and mitochondrial homeologs (PNPaseC- and PNPaseD-) for virus-induced gene silencing (VIGS). GFP movement assays revealed that these homeologs have distinct effects on plasmodesmata-mediated intercellular trafficking. While PNPase A- and PNPase C- silenced plants showed increased trafficking, PNPase B-, PNPase D- and PNPase A/B- silenced plants showed decreased trafficking. We also found that the expression of PNPase homeologs varies in different tissues and at developmental stages (young, intermediate, and mature) of leaves in wild-type plants. To better understand the detailed mechanism of PNPase-initiated ONPS in plasmodesmal regulation, we will perform RNA-seq analysis in various PNPase- silenced plants.

CS 8A-2. Flower color evolution in Solanaceae and Convolvulaceae

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Color is one of the fascinating features displayed in life. Pigments produced by flowers absorb particular light based on the pigments' chemical structure and reflect the rest of light that is the color we perceive and attractive to pollinators. Besides a characteristic flower color, some flowers also grow multicolored flower patterns, such as spots and stripes. Flower color and color pattern evolution are thought to be driven by pollinators. Although the floral color is crucial to understand angiosperm evolution, investigating flower color evolution to include diverse species with a phylogenetic context is rare. In this study, we investigated flower color evolution in Solanaceae and Convolvulaceae, two Solanales sister clades that possess diverse flower colors and flower color patterns and many pollination syndromes. We analyzed flower images to determine the flower color and flower color patterns, the flower color diversity, and flower color evolution after the two families diverged from their

most recent common ancestor (MRCA) with a phylogenetic framework. The histogram of color distribution based on the hue value showed a bell-shaped curve near the yellow for both Solanaceae and Convolvulaceae; a bell-shaped curve near the purple range in Solanaceae, but a double peaks bell-shaped curve around blue and purple in Convolvulaceae. These results indicate that purple and yellow (besides white) are the most frequent flower colors observed in Solanaceae and Convolvulaceae. A significant number of Convolvulaceae species also evolved blue-colored flowers. Furthermore, there are more multicolored flowers observed in Convolvulaceae than Solanaceae, which contained more solid-colored flowers. The ancestral state reconstruction analyses suggest that the MRCA of Solanaceae is likely white; the other colors had evolved many times independently. Flower color evolution analyses using image data provide us critical insights into the flower color diversity and how flower colors have been shifted during evolution to adapt to the pollinators.

CS 8A-3. Structural modeling and in planta complementation studies link mutated residues of the Medicago truncatula nitrate transporter NPF1.7 to functionality in root nodules

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Symbiotic nitrogen fixation is a complex and regulated process that takes place in root nodules of legumes and allows legumes to grow in soils that lack nitrogen. Nitrogen is mostly acquired from the soil as nitrate and its level in the soil affects nodulation and nitrogen fixation. The mechanism(s) by which legumes modulate nitrate uptake to regulate nodule symbiosis remain unclear. In Medicago truncatula, the nitrate transporter MtNPF1.7 has been shown to control nodulation, symbiosis, and root architecture. MtNPF1.7 belongs to the nitrate/peptide transporter family (NRT1/PTR family, NPF) and is a symporter with nitrate transport driven by proton(s). In this study we combined in silico structural predictions with in planta complementation of the severely defective mtnip-1 mutant plants to understand the role of a series of distinct amino acids in the transporter's function. Our results support hypotheses about the functional importance of the ExxE(R/K) motif found on transmembrane helix 1 (TMH1) in proton(s) and possibly substrate transport. We also assessed residues contributing to a putative TMH4-TMH10 salt bridge in MtNPF1.7. Our findings add to the knowledge of the mechanism of alternative conformational changes as well as symport transport in NPFs and enhance our knowledge of the mechanisms for nitrate signaling. We are grateful to US National Science Foundation grant 1733470 for funding.

CS 8A-4. Altered branching in a weak BRI1 mutant

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BRASSINOSTEROID INSENSITIVE1 (BRI1) is one of the leucine-rich receptor kinases in plants which is involved in brassinosteroid (BR) signal transduction. BRI1 localized in plasma membrane can form a heteromeric complex with BRASSINOSTEROID INSENSITIVE

1-associated receptor kinase 1 (BAK1) as a co-receptor in BR signal transduction. BRI1 is composed of six different domains: signal peptide, extracellular domain, transmembrane domain, intracellular-juxtamembrane domain, kinase domain, and C-terminus. The kinase domain of BRI1 becomes activated via auto- and transphosphorylation when BR binds to the extracellular domain of BRI1. We have found that a mutant occurring spontaneously in Arabidopsis thaliana (L.) showed a mild bri1 mutant-like phenotype such as a compact plant architecture with thickened and dark-green leaves, short petioles, and less-elongated inflorescences. An investigation of branching in response to light signals (R:FR) showed that there was an increased number of rosette leaves and a decreased correlative inhibition index (indicating stronger branching) in bri1 mutants compared to wild type, regardless of high or low red light to far red light ratio (R:FR). Several phosphorylation residues involved in a BRI1 kinase function such as S1044 and T1045 in the kinase domain were previously identified. We hypothesized that there may be a mutation in the kinase domain resulting in the BR defective phenotype. Sequencing analysis of the BRI1 gene confirmed that there was a point mutation which substituted valine for alanine in the kinase domain of the mutant. More work will be conducted to elucidate the impacts of the mutation on BRI1 receptor kinase function, the downstream signaling pathway, and the role of BRI1 in branching.

CS 8A-5. Understanding the role of a cysteine protease in regulating STAY-GREEN in maize

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Stay-green is an important trait that can improve plant productivity by extending the period of photosynthetic assimilation. The genetic regulation of this quantitative trait is highly complex. Through a comprehensive systems genetic analysis, we have identified 64 candidate genes that have a role in the regulation of senescence in maize (Sekhon et al., 2019; The Plant Cell). One of the candidate genes, mir3, encodes for a cysteine protease putatively involved in proteolysis and, therefore, N remobilization and signaling. We also reported delayed onset of cysteine protease activity in a stay-green inbred (PHG35) compared to the non-stay-green inbred (B73). These results suggest that differential activity of mir3 can be contributing to the stay-green phenotype. To confirm the role of mir3 in the regulation of the stay-green trait, we are characterizing a diverse set of inbred lines with varying degrees of stay-green phenotype. Association of the distinct mir3 alleles with cysteine protease activity and C/N status will be presented. Characterization of the maize mir3 in Arabidopsis is underway using transient and stable transgenics. Together, these data will elucidate the role of mir3 in senescence and allow the manipulation of the stay-green trait in maize.

CS 8A-6. Analysis of stalk geometrical and metabolic phenotypes underlying maize stalk strength

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Stalk lodging drastically affects crop productivity in maize (Zea mays L.). Stalk strength is positively correlated to stalk lodging resistance and is one of the key factors affecting stalk lodging. Stalk strength is a complex trait and is influenced by genetic and environmental factors. Maize genetic improvement for stalk lodging resistance has been limited due to the lack of reliable and field-deployable phenotyping approaches. Identification of key intermediate traits contributing towards stalk strength, which could be more readily phenotyped unlike strength, could provide vital clues for understanding the biomechanical components of stalk strength. To identify the key geometric and metabolic determinants of stalk strength, we analyzed variation in a set of 16 hybrids derived from 8 diverse inbreds, each crossed with a stiff stalk (B73) and a non-stiff stalk (Mo17) inbred line. Measurement of stalk flexural stiffness (an estimator of stalk strength) with DARLING (Device for Assessing Resistance to Lodging IN Grains) revealed substantial phenotypic variation among hybrids. We are currently examining different mechanistic and biomechanical properties of these stalks, contributing towards the variation in stalk bending strength, including rind penetrometer resistance, stalk linear density, high-resolution near-infrared spectral data, etc., using both novel and existing phenotyping approaches. These data will be combined using advanced statistical/machine learning techniques to identify the most informative phenotypes underlying stalk strength. This would allow us to begin to generate a comprehensive picture of the genetic architecture of stalk lodging resistance.

CS 8A-7. Ionome profiling of Arabidopsis thaliana on interaction with Pseudomonas syringae

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Ionome can be described as a complete composition of mineral nutrients and trace elements of an organism and in general it represents the overall inorganic makeup. Sugars play a vital role in plant growth and development and thus sugars are crucial in various metabolic and signalling pathways in plants and it is quite obvious that sugars are inseparable part of the plant immune response. There are fewer evidences that support the role of micronutrients towards immune response in Arabidopsis and thus this study mainly focuses on in-depth fundamental research to incorporate various multi omics approaches to link gene regulatory networks with nutrient distribution. Arabidopsis thaliana species was used in the interaction study against a one of the deadliest phytopathogens plant pathogen Pseudomonas syringae. During an infection process it is clear that the available micronutrients are accessible for both the host and the pathogen but the unclear mechanism through which the pathogen and

the host responds to the available nutrients need to be clearly understood to shed light into the immune response of the host and the pathogen. DC3000 wild type strain and HrcC- strain with mutation in the Type three secretion system was used to infiltrate Arabidopsis thaliana. Pathogen infiltrated plant samples were collected from 0th to 72nd hours and analyzed using ICP-MS. Data mining and reverse genetic screening was used to extensively identify the key candidates that play crucial role in the Ionome response accordingly Transcription factors and their associated genes were identified as targets. Transcription factor and target interaction was identified using CHIP and EMSA technique and the sulfate transporters was found to interact with the target molecules. In light with understanding about the sugar transporters and their interaction with the effector molecules it can be estimated that effectors can interact with the plant sugar transporters in taking over the host machinery leading to immunogenicity.

Competitive Concurrent Session (CS) 8B

CS 8B-1. Understanding mir828-tas4-myb network-regulated anthocyanin biosynthesis in arabidopsis mitochondrial uncoupling protein (UCP1-3) mutants

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microRNAs (miRNAs) are small non-coding RNAs that play roles in fine-tuning plant growth, development, and responses to various nutrient and/or biotic and abiotic stresses. Polyphenolics such as anthocyanins and flavonoids have antioxidant properties and accumulation in response to various genetic (e.g., fusca phenotypes) environmental stimuli including accumulation of photosynthate end product sucrose, phosphate or nitrogen deficiencies, and most biotic and abiotic stresses. microRNA828 (miR828) negatively regulate anthocyanin biosynthesis via an autoregulatory feedback loop where miR828 targets Trans-Acting Small interfering RNA locus4 (TAS4), which itself produces an antisense siRNA [3'D4(-)] that targets the transcription factors MYB113 (also a target of miR828), PRODUCTION OF ANTHOCYANIN PIGMENT1 (PAP1)/MYB75, and PAP2/MYB90. In Arabidopsis thaliana, the mitochondrial Uncoupling Protein (UCP) gene family is composed of three members called AtUCP1-3. UCPs dissipate the proton electrochemical gradient established by the respiratory chain, thus affecting the yield of ATP synthesis and production of reactive oxygen species (ROS). Because polyphenolics, in particular anthocyanin, accumulation is associated with stress hormone abscisic acid (ABA), sugar signaling, drought responses, light-mediated and oxidative stresses, we are interested to characterize the crosstalk between UCP regulation of ROS and the miR828 stress-response regulon to better understand physiological and molecular adaptations in response to the environment. We report the preliminary results of ongoing experiments to characterize sugar signaling responses of ucp mutants by analyzing their small RNA and mRNA profiles by deep sequencing compared to untreated seedling libraries and in select sRNA biogenesis mutants and a transgenic PAP1 overexpression genotype.

CS 8B-2. Cold soil temperature reduces stress of winter flooding on the root system of Quercus phellos

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Green Tree Reservoirs (GTRs), bottomland hardwood forests intentionally flooded during winter to mimic the historical ecology, often experience a shift in species composition away from desired red oaks like willow oak (Quercus phellos). This study investigated whether cold soil temperatures induce reduced root activity during winter, and if the reduced activity increases root tolerance to winter soil flooding. Soil temperatures of potted seedlings were either held constant at 15°C, or transitioned to cold winter temperatures (10°C and 5°C treatments) which were held constant until spring. Root respiration, measured at treatment soil temperatures, was used as an indicator of overall root activity. Respiration was also measured at fixed temperatures of 15°C and 5°C to provide Q10 values as an indicator of acclimation to low soil temperatures in winter. Lower soil temperatures led to lower root respiration rates. However, Q10 values were unaffected, which does not support acclimation to cold temperatures, but rather indicated that temperature directly and reversibly affected root respiration rates. Additionally, half of the seedlings were subjected to soil waterlogging from December through early February. For a given soil temperature treatment, waterlogging did not appear to reduce root respiration as compared to roots from soil that was not waterlogged. Survival and spring growth were high even for seedlings kept at 15°C soil temperature during winter. However, there were morphological signs of stress response in the 15°C treatment that were less prevalent or absent in the 10°C and 5°C soil temperature treatments. Together these results support our hypothesis that flooding at cold soil temperature is less of a stressor than flooding at high soil temperature for willow oak roots. The implications of our results for flood stress that occurs during winter dormancy will be discussed.

CS 8B-3. RD29A and RD29B relay pgpr-mediated drought tolerance by differentially regulating CBF and NAC transcription factors

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In recent decades, drought has become a global problem for food security and agricultural production. A variety of strategies have been developed to enhance drought tolerance, but largely unsuccessful since most drought-responsive genes (DRGs) stimulate a stomata closure and in turn suppress plant growth and yield. To access if and/or how plants could enhance drought tolerance without trading off growth and development, we screened and isolated a plant growth-promoting rhizobacterium, Paenibacillus polymyxa CR1, capable of 1) priming drought tolerance and concurrently 2) increasing root growth in plants, e.g., Arabidopsis and soybean. In parallel, we uncovered that P. polymyxa CR1 3) induces the

expression of two DRGs, Response to Desiccation (RD)29A and RD29B, 4) of which pattern upregulations are controlled by a diurnal rhythm. Besides, RD29A and RD29B act as 5) 'memory' genes; their transcript levels are increased to a greater extent when plants encountered P. polymyxa CR1 for the second time compared to an initial exposure. In line with these findings, T-DNA insertion mutant Arabidopsis of RD29A or RD29B displayed enhanced susceptibility to drought, without any change in stomata behaviors or growth rates, than wild-type plants. Hence, we conclude that RD29A or RD29B are unique, efficacious generic materials that can potentially aid in upgrading the plants own survival capacity against drought without reducing yield potential.

CS 8B-4. The role of endogenous secreted peptide ligand-receptor in plant autoimmunity and cell death regulation

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Cell death is a ubiquitous process in all organisms and is often associated with autoimmunity. Precise control of cell death is crucial for plant survival. However, the mechanisms underlying autoimmunity and cell death remain poorly understood in plants. Cell surface-resident immune receptors are the frontlines of the plant immune system. A major group of plant immune receptors are plasma membrane-resident receptor-like kinases (RLKs), for example, RLK FLS2 perceiving bacterial flagellin in plant immunity. Some of which also regulate plant growth and development, such as BRI1 perceiving growth hormone brassinosteroids. The Arabidopsis thaliana genome encodes more than 600 RLKs and more than 1000 putative secreted peptides. So far, most of them do not have their cognate ligands assigned and their function characterized yet. Ligand perception by leucine-rich repeat RLKs (LRR-RLKs) often recruits coreceptor BAK1, also named somatic embryogenesis receptor kinase 3 (SERK3). SERKs function as coreceptors for multiple LRR-RLKs involved in plant immunity, growth, and development. Intriguingly, the depletion of BAK1 and its closest family member SERK4 triggers autoimmunity and spontaneous cell death with elusive mechanisms. By deploying a transient RNAi-based genetic screen for bak1/serk4 cell death suppressors towards Arabidopsis knockout collections, we have revealed that mutants of an LRR-RLK suppresses bak1/serk4 cell death. Interestingly, the mutants of this LRR-RLK resemble wild-type plants in morphology and response to bacterial and fungal infections, suggesting an uncoupled role in plant immunity and bak1/serk4 cell death regulation. Since this LRR-RLK localizes to the plasma membrane, we investigate the involvement of endogenous small secreted peptides in regulating bak1/serk4 cell death. Characterization of peptide ligands and receptors in bak1/serk4 cell death regulation offers an insight into the molecular and physiological mechanism underlying plant cell death and autoimmunity.

CS 8B-5. Indole-3-Butryic acid Response5 (IBR5) activity is regulated by calcium and calmodulin

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Being sessile organisms, plants should respond to environmental changes rapidly and accurately to tune their growth and development accordingly. Many environmental and hormonal signals are known to elicit changes in the cytosolic calcium levels and these changes are transduced through calcium binding proteins such as calmodulin (CaM). Upon binding with Ca2+, CaM undergoes conformational changes that allow it to interact and modulate the activity of other target proteins that contain a CaM binding domain (CaMBD). Indole-3-butryic acid response5 (IBR5), a dual specificity phosphatase, has been shown to be involved in plant auxin response. IBR5 contains a putative CaMBD, and both bacterially expressed and native IBR5 proteins interact with CaM in a Ca2+ dependent manner. Additionally, Ca2+/ CaM was found to modulate the phosphatase activity of IBR5, further indicating the connection between calcium and auxin signaling pathways.

CS 8B-6. Ion distribution and accumulation patterns in Arachis hypogaea seedling root

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Ions are important factors that decides the fate of growth and development in biological organisms. Understanding the mechanisms that regulates root growth and development is important for agriculture, environment and forestry. SEM/EDS and ICP-OES were used to investigate the native ionomic matrix and ion interactions in Arachis hypogaea seedling root. K and P were the most abundant endogenous macronutrients in Arachis seedling root as estimated by ICP-OES. A K-gradient with decreasing K concentration towards the mature region of root was identified. Near the root tip, SEM/EDS maps indicated uniform distribution of K and P which got compartmentalized with maturity in specific tissues giving rise to ionomic patterns. Pericycle cells surrounding the vascular tissues had high P content while mid-cortical cells accumulated high amount of Ca. Element compartmentation in seedling root tissues was observed in regions where the Casparian band was absent or not completely developed indicating tissue specific regulation of ion. Exogenous K and Ca ion accumulation in seedling roots was a function of cell type, developmental stage and growth media composition. K accumulated in developmentally younger region of root while Ca accumulated in mature root tissues. K had an antagonistic effect on Ca accumulation in root tissues. This is the first report on the distribution of endogenous elements and exogenous ion accumulation in Arachis radicle. It indicates a previously unknown tissue-specific ion distribution and accumulation patterns that reflects the strict regulation of ion at tissue and cellular level.

Posters – Competitive, Session 6

P1. Bioremediation of Mars Regolith simulants with cyanobacteria Anabaena cylindrica

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Sustainable food production on Mars is a mission critical concern that faces considerable challenges given the harsh environment of the Martian surface. Strategies exploiting existing resources onsite (In-situ Resource Utilization, or ISRU) will limit the transportation of resources from Earth to Mars, reducing costs, increasing the long-term sustainability of the colony, and offer improved food security. Mars regolith simulants (MRS) have been developed to guide mission planning for an eventual effort to colonize the surface of the Red Planet. These simulants have confirmed that plant growth in actual regolith is unlikely to be possible 'as-is', and it is therefore crucial to consider remediation of these substrates to support future colonization. On Earth, primary succession is the conversion of abiotic soil to a biotic one through the applications of pioneering species such as cyanobacteria. These photoautotrophs are capable of remediating inhospitable sediments through nitrogen fixation, altering mineral composition, and producing a variety of secondary metabolites. Here we have attempted to accelerate the process of primary succession in MRS using Anabaena cylindrica as an approach to improve plant growth in the simulant. Measurements of this cyanobacteria's viability within the simulant was determined by chlorophyll A content as well as its extracellular polymeric secretions (biofilm). Physical changes in the simulant were by its organic and inorganic changes . The resulting effects of this pre-treatment on plant growth was evaluated with the model angiosperm Arabidopsis thaliana. The beneficial impacts of these studies go beyond Mars colonization and may provide insight into growth in other inhospitable substrates, encouraging plant development and microbial habitation.

P2. Evaluating nutrient availability to support plant growth in Martian Regolith Simulants

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Sustainable food production is generally accepted as a requirement for long-term colonization of Mars. Previous work has established he viability of specific Martian Regolith Simulants (MRS) to support germination and growth of a variety of plants with additional nutrient supplementation. These studies focused on the use of 'complete' nutrient solutions, comparable to those required for hydroponic growth. However, maximizing the utilization of in situ resources depends on a refined understanding of which nutrients within the regolith can be utilized by plants (directly or indirectly) and which will need to

be brought from Earth (or elsewhere) to support food production on the Red Planet. This information will help to maximize payload efficiency and provide food security to the colony. In the present study, we examine if supplemental nitrogen is sufficient to support the growth of tomatoes (Solanum Lycopersicum) grown in one of these simulants (JSC-Mars-1A). We will also examine the effects of particle size on viability. Metrics used to determine the JSC-Mars-1A's ability to support plant growth were viability, plant height, leaf count, and leaf area. I will present on our early findings and their potential implications to sustainable food production on the Red Planet.

P3. Tissue culture treatment of wheat to induce mobilization of an mPing based activation tag

Angela Plemmons* and Nathan Hancock, University of South Carolina Aiken Biology and Geology Aiken SC 29801

Tissue culture treatment of wheat to induce mobilization of an mPing based activation tag. Angela Plemmons and C. Nathan Hancock University of South Carolina Aiken Transposable elements are DNA segments that can be mobilized around the genome to cause mutations. Activation tagging is a mutagenesis approach where random gain-of-function mutations are induced by an insertion of an enhancer sequence near genes. ;An activation tagging derivative of the mPing element from rice, mmPing20F , was inserted into the wheat genome along with the ORF1 and transposase genes required for mobilization. These transgenic wheat lines show mmPing20F transposition, but not at sufficient levels. However, studies on mPing tagging soybean lines suggest that higher transposition levels occur during tissue culture. We propose to pass the existing mmPing20F wheat line through tissue culture and measure if heritable transposition frequency increases. The methodology includes isolating immature embryos and transferring the tissue to fresh media every two weeks. Once we obtain plants, DNA purification and PCR analysis will be used to confirm if heritable transposition occurred. We will use qPCR to determine how many copies of mmPing20F are present as we expect to see an increase in mmPing20F copy number if significant transposition occurs.

P4. Analysis of phototropism and gravitropism in Arabidopsis thaliana: a ground control for spaceflight experiments

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The use of plants for space exploration will be important for bioregenerative life support systems. We seek to understand the basic biology of plants by exploring the mechanisms of tropic responses, like gravitropism and phototropism. This project provides a ground control for a spaceflight project performed on the International Space Station (ISS). We performed experiments with an analog that simulates (clinostat) microgravity of low Earth orbit by rotating plants in different planes. Our working hypothesis is that plants grown on the analog clinostat will exhibit similar results to those obtained in the spaceflight experiment on the ISS. We used three different genotypes to complete the experiment

including the WT Ler, PhyA and PhyB. The seeds were placed on petri dishes and received a cold stratification before being placed in a dark, and a red-light treatment was provided to stimulate germination. Upon germinating, the seeds are then placed under a white grow light for 96 hours. The petri dishes are then placed on a rotating clinostat for 48 hours with unidirectional photostimulation with either red or blue light. The roots and shoots were measured after the 48 hours of rotation. The measurements collected from this experiment will be compared to measurements from similar experiments conducted on ISS in order to analyze whether an analog clinostat can simulate similar tropic responses in plants as those produced in microgravity. ;

P5. Arabidopsis IRE1A-dependent in vitro bZIP60 mRNA splicing/ degredation assay

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Arabidopsis thaliana IRE1a is a unique enzyme consisting of an ER luminal domain and a cytoplasmic domain containing an auto-phosphorylating kinase domain and an endoribonuclease (RNase) domain. IRE1a is activated upon accumulation of unfolded protein in the ER and can oligomerize, trans-auto phosphorylate, and perform bZIP60 mRNA splicing or degradation of other mRNA targets to alleviate ER stress[1]. Phosphorylation of the kinase domains triggers IRE1a RNase activity.[2];Therefore, it is important to understand the mechanism of IRE1a phosphorylation and its effect on IRE1a target splicing and degradation. Previously, it has been shown that phosphomimetic mutations in the IRE1a Kinase domain can help understand its mRNA splicing RNase activity. We set out to design an assay to uncover insights on the IRE1a ribonuclease activity. Using recombinant protein purification of various AtIRE1a Kinase and Ribonuclease subdomain constructs generated by site-directed mutagenesis, combined with total leaf RNA, and qPCR-based quantification, we demonstrated that AtIRE1a can actively splice/ degrade AtbZIP60 mRNA; in vitro .;This assay will allow researchers to identify and monitor novel IRE1a dependent mRNA targets, providing a deeper insight into IRE1A mediated ER stress homeostasis.

P6. Common soil bacteria as possible antifungal biological control agents against Xylaria sp. and taproot decline

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In 2017 a new plant disease for soybeans was identified. Taproot decline (TRP) has quickly become a growing concern for its effectiveness at killing the soybean plants. Xylaria sp. has been identified as the fungus that causes this disease in soybeans. The common characteristics of TRP is the interveinal chlorosis on the leaves and a black stroma on a darkened taproot. TRP has been identified to exist within southern states including Alabama, Arkansas, Louisiana, Mississippi, and Missouri, and remains a great threat throughout the soybean growing season (Allen et al 2017). Xylaria, the fungus that causes TRP, is part of the ascomycetous fungi division, and often grows on decaying wood (Sharma et al 2018). A library of bacteria has been collected from samples of soil. These bacterial strains belong to the class Alphaproteobacterim, Bacillus, Proteobacterium, and Pseudomonas. Bacterial

strains are assigned then tested through an in vitro phase of testing using in vitro assays. The bacteria strains that show the most fungal inhibition are then moved to an in vivo phase of testing using biocontrol assays. Bacterial strains that show promising results in the in vivo phase will be identified to be potential biocontrol agents (BCAs) against TRP in soybean plants. This is an ongoing research project. There are still several strains to be tested in vitro as well as in vivo.

P7. The effects of BAM9 and AMY3 on guard cell phenotypes in Arabidopsis thaliana

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The purpose of this research is to explore the implications that the interaction between the starch degradation proteins, BAM9 and AMY3, have on guard cell phenotypes in the plant; Arabidopsis thaliana. The β-amylase (BAM) family is recognized for its role in starch degradation and gene regulation among plants and in; Arabidopsis thaliana. The BAM family consists of 9 proteins (BAM1-9) that are diverse in structure and function although the specific functions of some are only mildly understood. Amongst these is BAM9 which is non-catalytic but is highly conserved amongst land plants, though, there is little to no research pertaining to this specific protein. In vitro assays have identified an interaction between BAM9 and starch degrading α-amylase, AMY3. It is hypothesized that the interactions between BAM9 and AMY3 may have some implications on the overall competence of the plant and its productivity and if taken away, can lead to consequences for stomatal function and plant growth. In order to evaluate this, wild type (WT) plants as well as; amy3, bam1 and bam9; knock out (KO) mutants of Arabidopsis were grown and guard cells from each were analyzed to understand the effect that the absence of the proteins have on guard cell phenotypes. In order to do this, epidermal peels were taken from WT plants as well as all KO mutants just before dawn. A propidium iodide starch staining method was optimized to measure starch accumulation; however, further optimization of confocal microscopy will be necessary. Stomatal apertures of WT plants and KO mutants are being analyzed using ImageJ software and compared to results of previous research. The data collected from this experiment will provide information that is fundamental in understanding the interaction of BAM9-AMY3 in the essential process of starch degradation.

P8. Bacterial influence on Xylaria sp. growth

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One of Mississippi's most valuable crops include soybean. Soybeans are a principal source of agricultural income in Mississippi with a farm gate value of $1.115 billion in 2017, which is 14.75% of the total 2017 farm gate value of all agricultural and forestry production in the state (Heatherly 2018). Consequently, soybean production in the state has been declining with the advance in years since 2018. This is predominantly due to Taproot decline (TRP), known to be caused by Xylaria sp. Infections occurring early in the growing season lead to diseases that are typically not observed until the advanced reproductive growth stages. These may include Phytopthera root rot, charcoal rot, red crown rot, stem canker, SDS, and

some nematode-related issues (Bennet 2016). Xylaria, TRP's casual agent, is a fungus belonging to the ascomycetous division, that often grows on dead wood. In soybean, the root system is affected throughout the crop's lifecycle resulting in physical disparities such as a darkened and black stroma on the taproot and interveinal chlorosis on soybean leaves. TRP has been troubling to much of the Mid-south and remains a risk factor for future soybean growing seasons. In this research, biocontrol methods are implemented to influence TRP in soybean. Tests involve a dual culture method where bacterial strains from class Alphaproteobacterim, Bacillus, Proteobacterium, and Pseudomonas are evaluated for probable inhibition activity against Xylaria fungus. The bacterial strains that exhibit inhibition of Xylaria growth are selected for second-phase in vivo biocontrol assays to observe and investigate the potential biological control agents (BCAs) present. Thus far, five of the twelve bacterial strains utilized in experimentation have presented significant hindrance of Xylaria growth. However, this is an ongoing research and bacteria from all classes are still being tested via in vitro biocontrol assays.

P9. Experimental system to study de novo root regeneration under biotic stress

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De novo root regeneration (DNRR) refers to the generation of adventitious roots from plant explants. DNRR is a vital process in agriculture as it makes crop propagation possible. Most studies on DNRR are performed in sterile conditions. However, the effect of a microbial presence on DNRR has yet to be explored in the systems of Arabidopsis thaliana , mung beans, N. benthamiana , and tomato. Technically, it is challenging to perform DNRR with microbes, because bacteria and fungi quickly overgrow and kill explants. This study aims to discover the effect a microbial presence has on root regeneration in these systems. In order to do this, Arabidopsis and the three other plant systems were grown in sterile and non-sterile conditions. The plant seeds were sterilized and grown in plates with autoclaved sand supplemented with ½ MS medium. To create a condition with microbes, we added a mix of soil microbes in the non-sterile plates. Leaves 1 and 2 of Arabidopsis were cut at 12 days after planting, and adventitious roots were counted on day 8, 10, and 12 after cutting. We found that the leaf explants incubated in a sterile condition developed a higher number of adventitious roots than those in the non-sterile conditions. We also applied this system to mung beans, N. benthamiana , and tomato by cutting the hypocotyls at 12 days after planting. A microbe presence negatively affected root regeneration in these plant systems as well. Future work is needed to explore the molecular mechanisms of how responses to abiotic stress interfere with signaling of root regeneration.

P10. Egg-specific expression of ORF1 and transposase in Arabidopsis

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The overall goal of this project is to develop a method for inducing tissue specific transposition of the mPing transposable element in Arabidopsis thaliana . Transposable elements are DNA fragments that can jump to different places in the genome, creating mutations. The transposable element mPing is mobilized by ORF1 and Transposase proteins that bind to the element and catalyze movement through a cut and paste mechanism. We hypothesized that inducing transposition in the egg cell would be an efficient method for inducing germinal mutations, without inducing somatic mutations. We made a pHEE 401E plasmid construct designed to induce egg cell-specific expression of ORF1 and TPase proteins. We predicted that this would mobilize mPing only during reproduction. The egg-specific plasmid was transformed into Arabidopsis plants that already contained an mPing: GFP reporter, which fluoresces when mPing is excessed. Using fluorescence microscopy, 7% of the first generation of plants with egg-specific expression showed GFP, while 92% of plants expressing ORF1 and TPase from a constitutive 35S promoter showed GFP. This was expected as the egg-specific promoter should not be expressed in most tissues, while the control construct is expressed in all tissues. In the second-generation progeny, we observed no whole plant GFP expression from plants with the egg-specific construct, while 66% of our constitutive control plants produced whole plant GFP. This indicates that heritable transposition is not occurring in the egg cell despite using an egg-specific promoter. Together this suggests there is delay between transcription and mobilization mPing . This is consistent with transport of the Transposase protein into the nucleus being delayed, separating the time between translation and transposition.

P11. Identification and analysis of candidate genes controlling telomere length in Arabidopsis thaliana

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In humans, the abnormal shortening or elongation of telomeres on chromosomes has been found to have a significant correlation to cellular disease and aging. Those with shorter than average telomeres are likely to age prematurely due to lack of protection, while improper telomere elongation may lead to a much higher probability of being afflicted with cellular ailments such as cancer. As the general telomere structure and composition is conserved across eukaryotic organisms, we utilize a powerful eukaryotic model systems Arabidopsis thaliana to discover factors controlling telomere length. Telomere length differs in Arabidopsis ecotypes (natural populations of this species), and this natural variation can be studied in the genomic context to identify important genes regulating population-specific telomere length. We measure telomere length with Southern blotting and utilize genome-wide association studies to pinpoint significant loci in the Arabidopsis genome. We create a database of candidate genes for further analysis and investigation. We then obtain T-DNA mutants in candidate genes and genotype them via PCR to ultimately identify homozygous or heterozygous T-DNA mutants. Further analysis of telomere length in these T-DNA mutants will uncover their potential role in telomere biology.

P12. Use of loop-mediated isothermal amplification (LAMP) for the detection of tomato mosaic virus

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Tomato Mosaic Virus (ToMV) is a rapidly spreading single-strand RNA virus that infects several agriculturally important crops. The virus's single-stranded genome acts like messenger RNA allowing for direct translation into viral proteins by host cells, thus quickly infecting and destroying;entire crop yields. As there is no cure for ToMV, early detection is key; the infected plants must be identified, isolated, and destroyed before the infection spreads.Our aim was to develop;a testing strategy that;could not only detect ToMV with specificity but also be implemented in the field and quickly determine infection.Prior to this study, the most common form of detection was by use of polymerase chain reaction. While accurate, this testing method is costly and takes time, leading to greater crop loss. Our method used a novel RNA extraction buffer in conjunction with Loop Mediated Isothermal Amplification (LAMP) and specifically designed primers to target the coding region of the viral RNA responsible for the production of coat proteins. Using this process, we were able to identify with specificity the infected plants in as little as five minutes and with limited equipment.

P13. Do temperature effects on gas exchange contribute to blueberry (Vaccinium spp.) adaptation to warm environments?

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Blueberry (Vaccinium spp.) genotypes adapted to northern climates are known to not tolerate warm environments, but little is known about how high temperatures impact these plants. We hypothesized that carbon deficit in warm environments due to low photosynthetic rates during the day and high respiration rates at night contributes to this response. We tested this hypothesis using southern highbush (V. corymbosum interspecific hybrids cv. Emerald and Biloxi), northern highbush (V. corymbosum cv. Elliot), rabbiteye (V. virgatum cv. Premier and Woodard), and wild blueberry species (V. caesariense genotype CVAC-793). We measured leaf net photosynthesis, dark respiration, and photosynthetic response curves to increasing light intensity using an infrared gas analyzer with a 16 mm leaf cuvette and a built-in LED light source. Plants were acclimated to 23°C temperature for 7 days before measurement. Then, plants were acclimated to 33°C temperature for 7 days and these measurements were repeated. High temperatures increased dark respiration rates in Elliot, Woodard, and Emerald, but other genotypes were not affected. The same genotypes exhibited higher photosynthetic rates at 400 μmol m -2 s -1 of light, except Woodard, at higher temperatures. The results suggest that carbon deficit might be the cause for northern highbush blueberry plant stress in high temperatures.

P14. Homology of a maturase to the heart of the nuclear spliceosome

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Structural and biochemical evidence links group II introns as evolutionary precursors of nuclear introns. The spliceosome binds and excises nuclear introns. Maturases are prokaryotic enzymes that aid group II intron excision. The evolutionary ties between group II introns and nuclear introns suggest a possible link between maturases and the nuclear spliceosome. Structural analysis of Prp8, a core enzyme of the nuclear spliceosome has demonstrated this link and revealed similarity to prokaryotic maturases. Maturase K (MatK) is a chloroplast group II intron maturase. Unlike prokaryotic maturases, MatK binds to multiple intron substrates suggesting possible evolution akin to the nuclear spliceosome machinery. In the chloroplasts, MatK is responsible for the splicing of 7 plastid-encoded introns. We investigated protein interactions of the MatK maturase using co-immunoprecipitation. We determined similarities to proteins that interact with nuclear Prp8 ribonucleoprotein complex. This data supports MatK as a model of early evolution of the nuclear spliceosome.

P15. PvAVP1, a vacuolar H+-pyrophosphatase from Paspalum vaginatum confers plant tolerance to salinity and drought stress

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Abiotic stresses, such as salt and drought, significantly affect plant development and are the major limiting factors for crop quality and productivity. Manipulation of genes involved in plant stress response facilitates plant mitigation of adverse environment. Vacuolar H+-pyrophosphatase, an electrogenic proton pump, catalyzes the hydrolysis of inorganic pyrophosphate (PPi) and active transport of proton across the tonoplast to acidify vacuoles in plant cells. Protons pumping out of the vacuole creates an electrochemical gradient for the uptake of ions and other metabolites. H+- pyrophosphatases have been implicated in regulating plant growth and nutrient use efficiency as well as plant response to salt and drought stress. H+-pyrophosphatasemediated toxic ions sequestration and the accumulation of ions and other metabolites in the vacuole facilitate plant adaptation to various environmental conditions. We have cloned and studied PvAVP1, a vacuolar H+-pyrophosphatase gene from Paspalum vaginatum, a highly salt-tolerant halophytic warm-season perennial grass. Transgenic Arabidopsis plants overexpressing PvAVP1 exhibited significantly enhanced tolerance to salt and drought stress associated with characteristic morphological and physiological indexes. The results obtained provide additional evidence demonstrating the important role vacuolar H+-pyrophosphatases play in plant response to environmental adversities and suggest PvAVP1 is a potential candidate for use in important crops for improved plant performance under drought and salt stress.

P16. The impact of light intensity on organ-specific metabolite profiles in Scutellaria

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Scutellaria is a genus of 470 widely distributed herbaceous plants in the mint family (Lamiaceae). Root extracts from the East Asian species, S. baicalensis, have been widely used in oriental medicine for more than 2,000 years. Several flavones found in S. baicalensis extracts possess human health-related properties such as anti-cancer, antioxidant, antiviral, and neuro-psychologic activities. Up to this point, most research has focused on characterizing flavone biosynthesis in S. baicalensis, meaning our knowledge of flavone biosynthesis in other Scutellaria species is limited. This study aims to expand our understanding of organ-specific chemodiversity in less well-characterized Scutellaria species. In particular, we intend to examine the impact of light intensity on organ-specific flavonoid accumulation in S. racemosa and S. wrightii. To achieve this, we exposed young plants of each species to either dark or high-light conditions and collected leaf, stem, and root tissue samples. Tissue samples were also collected from control plants grown in standard greenhouse conditions. We then quantified concentrations of 14 flavones in these tissue samples with High-Performance Liquid Chromatography. Our analysis revealed whole-plant shifts in metabolite accumulation in response to light intensity, suggesting that flavone biosynthesis is regulated by inter-organ signaling mechanisms. This work furthers our understanding of interactions between environmental conditions and flavone production in understudied Scutellaria species, paving the way for efficient biotechnology production. This can potentially enable large-scale commercial production while reducing overharvesting strain on native plant populations.

P17. Detection of tomato mosaic virus using a novel at home RT-PCR approach

Kaylee Petraccione*, Molly Tancini, and Emma Lehmann, Department of Biology at Coastal Carolina University, Conway S.C

Tomato mosaic virus (ToMV) belongs to the genus Tobamovirus which consists of positive-strand RNA viruses. ToMV has put a constraint on tomato production around the world, destroying crops as it spreads. Current testing methods require expensive molecular equipment for highly complex molecular diagnostics and are limited to a laboratory environment. In the present study, specific primers were made to detect ToMV genes within tomato leaves. A modified RNA extraction protocol along with a simplified RT-PCR protocol with SYBR green was used to amplify and detect ToMV in a manner that could be replicated in a kitchen environment. Novel ToMV primers targeted against ToMV gene 2, encoding for the movement protein, and gene 4, encoding for the coat protein, were checked by BLAST analysis in GenBank for non-specificity to other viruses. Primers targeting the atpF gene were incorporated as an internal control to confirm positive results and successful RNA extraction. Initial tests demonstrated the efficacy of the methodology. Using this combined RNA extraction and RT-PCR approach can help detect ToMV in settings outside of a typical

laboratory and could lead to detection methods for various viruses when materials and facilities are lacking.

P18. Gene expression analysis reveals an important role for the ABC transporter WBC19 in Cu transport

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Metals such as iron, zinc, and copper are micronutrients that are commonly found in plants and their uptake and transport is highly regulated. Previous results have shown that Arabidopsis thaliana plants exposed to antibiotics exhibit changes in their Fe uptake. In addition, in Arabidopsis wbc19 mutants, known to be very sensitive to the antibiotic kanamycin, which is produced by the bacteria Streptomyces kanamyceticus , Zn and Cu uptake are significantly lower than in control plants. A better interpretation of the physiology of the roots of A. thaliana exposed to kanamycin is needed to understand this shift in their metal uptake.RNA-seq analysis was performed to compare gene expression in the roots of control and wbc19 mutant plants. There were 762 genes that were significantly upregulated or downregulated at adjusted p value <0.01. Among the significant upregulated genes, many of them are associated with metal homeostasis. The results specifically indicate that wbc19 mutants exhibit copper deficiency as seen from the upregulation of ferric reduction oxidase 4 (FRO4), copper chaperone (CCH), and also copper transporter 2 (COPT2) being among the top 15 upregulated genes. There is also a shift from the copper/ zinc superoxide dismutase to Fe- superoxide dismutase. These findings suggest that the primary role of wbc19 is in Cu transport.

Posters – Non-Competitive, Session 9

P19. The impact of age on plant sensitivity to bacterial quorum sensing signals

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Through the phenomenon of quorum sensing (QS), many unicellular organisms, both prokaryotic and eukaryotic, are able to couple phenotypic switching with cell density. This process regulates specific phenotypes, such as swarming, as well as virulence factor and biofilm production. These processes are regulated through a variety of signals generically classified as autoinducers (AIs). In Gram-negative bacteria, the most abundant AIs are a class of compounds known as; N -acyl; L -homoserine lactones (AHLs). The notion that plants are sensitive to these signals is supported by previously observed growth effects that include altered primary root length, increased production of auxin and ethylene, and the stimulation of transpiration. Understanding how plants respond to these signals is essential to mapping out host-microbial interactions. Earlier studies have determined that plant-sensitivity to

AHLs is largely dependent on the activity of the enzyme fatty acid amide hydrolase (FAAH) which hydrolyzes the amide of AHLs. Indeed, the growth effects observed in plants were due to the amidolysis products of AHL cleavage by FAAH, specifically L -homoserine. As a result, AHL sensitivity in plants should be a function of FAAH expression, which can change as a function of age. The present study attempts to determine if mature A. thaliana plants, which express reduced levels of FAAH relative to seedlings, are less sensitive to AHLs.

P20. Preliminary study on suppressiveness of decomposing Brassica oleracea leaves on Rhizoctonia solani

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Every year, plant pathogens cause billions of dollars in crop losses around the world. These losses lead to increased food costs, exacerbating the global issue of undernourishment. One particularly notorious plant pathogen is the fungus Rhizoctonia solani , which infects the stems and roots of several important crops. Efforts to develop host crops with resistance to R. solani have been unfruitful because so much of the pathogen's mechanism of infection remains unknown. Soil-borne pathogens can be inhibited by application of organic amendments to the soil. These materials are a promising alternative to synthetic pesticides that often have adverse effects on crops and ecosystems. One key aspect of the application of organic matter is the dynamic of decomposition. As the material decays, it releases many chemicals that can either suppress or stimulate the growth of pathogens. Isothiocyanates, chemicals that are released during the decomposition of Brassica species, have been shown to suppress R. solani. We investigated the relationship between the diversity of the microbial community decomposing Brassica oleracea (cabbage) leaves and the suppression of R. solani . The suppressive effect of filter-sterilized extracts collected from decomposing B. oleracea leaves over a period of four weeks was tested on R. solani in vitro. The decayed extract significantly suppressed R. solani regardless of the biodiversity of the decomposing community for the majority of the time of decomposition (P < 0.05). This result is important because the fact that filter-sterilized extract inhibited R. solani shows that the suppression was not due to competitive microbes. Some inhibitory chemical must have been produced, but tests to determine whether this chemical was an isothiocyanate were inconclusive.

P21. Environmental Ecology Plant Physiology Section

The Environmental and Ecological Plant Physiology (EEPP) section was the first theme-based section within the American Society of Plant Biologists. This section represents anyone interested in the broad category that our name implies. This section exists to provide a place to integrate leaf and plant- level responses to biotic and abiotic stress under field and laboratory conditions, to set molecular physiology in an ecological context, and/or to provide a basis for scaling root and shoot level responses to canopy, ecosystem and region for crops or natural vegetation. The mission of the section is to advance and promote the science and practice of Environmental and Ecological Plant Physiology. This includes (but is

not limited to): integrate the plant environmental physiology community and research opportunities within and outside ASPB, support, train and liaise with young plant environmental physiologists, and work with other societies to promote the missions of Environmental and Ecological Plant Physiology. The leadership committee is composed of a chair, vice-chair, secretary/treasurer, and outreach officer. Committee members serve a 4-year rotation, starting as outreach officer and progressing to chair. The outreach officer is nominated and voted upon by the section membership yearly. Our events include the annual EEPP meeting, hosting research symposiums, social and networking events, and hosting webinars via Plantae. Please considering joining our section and getting involved! Follow us on Twitter @EnvEcoPlant and to find current officers and most updated information visit https://eepp.aspb.org/.

P22. Isolation of a quorum sensing regulator in Chlamydomonas reinhardtii

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Quorum sensing (QS) is a phenomenon which occurs in both prokaryotes and some unicellular eukaryotes, coupling phenotypic switching to cell density. In the model photosynthetic unicellular eukaryote, Chlamydomonas reinhardtii, QS regulates swimming speed. This density dependent increase in swimming speed appears dependent on an as yet unidentified signal, we have dubbed the Chlamydomonas Swim Speed Factor (CSSF). Efforts to identify this signal have been limited by a slow isolation process as well as limited knowledge about the stability of the CSSF. Here we evaluate the replacement of a traditional organic phase extraction for the isolation of the CSSF with a solid phase extraction (SPE) approach. SPE dramatically reduces the amount of organic solvent required for isolation as well as the time required to complete the extraction. SPE approaches are likely to be more reproducible as well. Here we confirm the existence of the CSSF in the extracts isolated by our SPE approach. We also report on the stability of this signal as a function of time, pH, and temperature. We discuss the significance of our findings within the context of our search for the identity of the CSSF signal as well as the in vivo regulation of this novel eukaryotic QS phenotype.

P23. Nuclear movement in growing root hairs in Arabidopsis thaliana depends on multiple mechanisms

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Nuclear movement is a prominent feature of root hair growth. Root hairs are single-cell outgrowths from the main root. In root hairs, the nucleus maintains a fixed distance from the root hair tip by moving forward at the same rate as the tip grows. Interestingly, when this distance between the nucleus and the root hair tip is disrupted, root hair growth ceases.

Nuclear movement in Arabidopsis thaliana root hairs has been described to be dependent on actin filaments, but not microtubules. Movement along actin filaments is driven by myosin motor proteins and movement of the nucleus has been found to depend on myosin XI-I. Our project sought to test the role of myosin XI-I in nuclear movement during root hair growth. Using a myosin xi-i knockout, our experiments showed that the distance from the nucleus to the tip remains constant, suggesting there may be alternative mechanisms of nuclear movement during root hair growth. To determine whether these movements are actin-based or microtubule-based, we specifically disrupted either of these cytoskeletal networks using the actin-depolymerizing drug Latrunculin B (LatB) and the microtubule depolymerizing drug Oryzalin (Oz), respectively, in both wild type and myosin xi-i knockout (kaku1-3) seedlings. Not only did the nucleus move in the absence of myosin XI-I, but nuclear movement still occurred when the actin cytoskeleton was disrupted with LatB, suggesting that nuclear transport can take place along the microtubules. Conversely, myosin xi-i knockouts treated with oryzalin still demonstrated nuclear movement, suggesting that there may be actin-based processes that may involve different myosins, in addition to myosin XI-I. Together, these experiments demonstrate roles for both actin and microtubules cytoskeletons in the process of nuclear movement in growing root hairs. This suggests the presence of independent but redundant mechanisms to ensure proper nuclear positioning during root hair growth.

P24. Identification of selective rare earth element (REE) binding molecules and plant transcriptional responses for the development of a plant REE biosensor

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Rare Earth Elements (REE) are critical for modern electronics, but their mining and purification are expensive and environmentally damaging. Plants could provide a preliminary step in the purification process. This investigation aims to develop a plant-sensor system that could be rapidly tailored for different elements so that plants could facilitate extraction from the soil with a smaller footprint and lower costs than current methods. REE binding molecules (RBMs) were identified from previous publications and tested for REE specificity by expressing REE-binding candidates in Saccharomyces cerevisiae and performing spectrophotometric metal-binding assays with Pyrogallol red, a colorimetric reagent. Lanmodulin is a lanthanide-binding protein found in Methylobacterium extorquens that contains EF-hands very similar to Calmodulin but selects Ln 3+ s over Ca 2+ with a 10 8 -fold selectivity. In a second strategy, peptides will be extracted from plant lanthanide hyperaccumulators like Phytolacca americana and Lemna minor to select RBM. RBM will be modified by molecular engineering and directed evolution techniques to enhance REE selectivity and specificity in plants. As a third approach to identify RBMs, a molecular phylogenetic analysis of genomic sequences of Calmodulin and related proteins from prokaryotes and fungus will be constructed to study the sequence variance of Calmodulin and other EF-hands containing proteins. This analysis will be implemented to identify specific genome motifs to recognize non-characterized proteins with EF-hands in

metagenomes with potential REE binding affinities. Indirect transcriptional responses will also be used to build REE biosensors. P. americana and L. minor were grown, and RNA-Seq was performed to identify REE responsive transcripts. RNA-seq analysis will identify candidate transcripts for transcriptional reporters and prioritize putative REE-binding proteins. The biosensor will be constructed by developing a reporter system for RBMs and transcriptional reporters by fusing selected protein, peptides, and genomic regions to luciferase, or Green Fluorescent Protein (GFP), to incorporate into REE hyperaccumulating plants.

P25. Elucidating immune modulated subcellular movement of H+ and Ca2+ in Arabidopsis thaliana

Regina Bedgood*, Karolina Mukhtar Department of Biology; University of Alabama at Birmingham;Birmingham Alabama

Precise cellular pH homeostasis is fundamental for life and essential for the growth, development as well as the survival of all prokaryotic and eukaryotic organisms. To precisely execute all of these processes, plants maintain neutral pH within the cytoplasm and acidic extracellular spaces (apoplast). Accordingly, cytosolic acidification is associated with defects in growth and development and is a hallmark of many plant responses to biotic and abiotic stresses including pathogen infection. Likewise, plant immunity is also manifested by Ca 2+ signaling, and together with the cytoplasmic acidification represents an essential element of plant defenses. On contrary, virulent phytopathogens modulate diverse molecular and cellular processes including pH homeostasis and Ca 2+ fluxes to establish disease susceptibility. Here, we set out to elucidate the subcellular movement of H + and Ca 2+ in vivo by triggering immune stress in wild-type Arabidopsis thaliana transgenic lines carrying appropriate reporter constructs, followed by confocal microscopy. For spatiotemporal pH measurements, we use ratiometric, pH-sensitive fluorescent marker variants localized to the cytoplasm, the apoplastic cortex, or the cytoplasmic cortex. For subcellular Ca 2+ measurements, we employ Ca 2+ sensitive Cameleon reporter constructs localized to the ER and cytoplasm, imaged by FRET-based confocal microscopy. Different forms of plant immune responses will be induced by treating plant leaves and roots with flagellin, Pseudomonas syringae , and salicylic acid.

P26. WRI1 and DGAT1 overexpression in soybean embryo alters oil composition and starch metabolism

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Soybean commercial value is directly linked to its seed quality, especially its oil content. The numerous applications for its oil generates a big demand for cultivars with increased levels of seed lipids. Strategies for oil optimization include both breeding and genetic engineering. In this work, we evaluate the behavior of transgenic soybean lines that overexpressed two genes known to control oil accumulation in oilseeds. WRI1, a master regulator in

transcriptional control of oil biosynthesis, and DGAT1, considered a rate-limiting enzyme for triacylglycerol accumulation, were used to “push” and “pull” lipid production. Seed specific promoters were incorporated to restrict their expression only to embryo development. The biomass analysis of the transgenic mature seeds revealed that the total oil content was not increased but changes in the fatty acid composition and sucrose and starch content were observed. To have a better understanding of how the embryo metabolism was altered by the overexpression of these two genes, biomass and targeted metabolomics studies were performed at different points during seed development. The analysis showed that starch content and intermediates of starch biosynthesis were increased in the transgenic lines. These results form new questions regarding the role of the transient accumulation of starch during soybean development and how it is linked to the oil production. The new insights obtained in this work will be valuable for researchers when designing new strategies for oil improvement in soybean.

P27. CS 3B-6. Impact of fatty acid elongase-1 mutation on pennycress metabolism

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The aviation industry has expressed a growing interest in using alternative crops to produce renewable biofuel. Replacing traditional fossil fuels can help to reduce greenhouse gas emissions and combat global climate change. Thlaspi arvense, colloquially known as field pennycress, is a promising oilseed plant that can be grown in a double cropping system to produce renewable biofuel. Pennycress is widely undomesticated and there is abundant opportunity to improve its seed oil composition. Eliminating erucic acid in pennycress seed oil has been identified as a route to improve biofuel cold flow properties. Fatty Acid Elongase-1 (FAE-1) loss-of-function mutants were generated through CRISPR Cas-6 gene editing. The mutants fail to synthesize very long chain fatty acids including erucic acid. Understanding the impact of this mutation on plant metabolism is a key aspect of crop improvement. The overall hypothesis is that the elimination of FAE-1 alters the levels of metabolites involved in fatty acid elongation, specifically metabolites associated with the cytosolic Oxidative Pentose Phosphate Pathway (OPPP) and the Tricarboxylic Acid cycle (TCA). Indeed, the OPPP and the TCA cycle were previously shown to provide reductant and carbon for fatty acid elongation under the form of NADPH and citrate, respectively. To test this hypothesis, intracellular metabolites were extracted from developing pennycress embryos and leaf tissue. Then, extracted metabolites were quantified by liquid chromatography tandem mass spectrometry (LC-MS/MS). The results indicate that several intermediaries of central metabolism were significantly increased in mutant leaves and developing embryos. Pathway analysis revealed that seven pathways, including glycolysis/gluconeogenesis and the pentose phosphate pathway, were commonly impacted in both developing embryos and leaves. This study couples metabolomics with pathway analysis to describe fatty acid compositional changes in the promising oilseed crop pennycress.

P28. Unravelling the role of pennycress (Thlaspi arvense L.) proteins in the modulation of neutral lipid droplet abundance

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The finite nature of crude oil-derived fuels coupled with their adverse effects on the environment means the search for alternative, renewable sources of energy that are more environmentally friendly is paramount. Pennycress (Thlaspi arvense L.) has been identified as a promising alternative crop for aviation fuel production. It is an annual winter Brassicaceae which produces seeds with high oil content (26-39%). The average yield of pennycress seeds is 1,500 kg ha–1, corresponding to 600–1200 L ha–1 of oil, which is higher than that of soybean and camelina. While pennycress benefits from the fully sequenced genome and research tools of the closely related model plant Arabidopsis thaliana, there are still significant challenges associated with establishing gene function that would make pennycress much more valuable as a bioenergy oilseed crop. Transcriptional analysis of 22 pennycress accessions resulted in the identification of potential gene candidates whose expression levels were correlated with seed oil yield. Here, we show that protein products of six of these candidate genes- a lipid transfer protein homolog (LTP6), a lipid droplet associated protein homolog (LDAP3), an annotated lipase (α/β hydrolase), a long-chain acyl-coA synthase protein (LACS1), an endomembrane regulatory protein (RABA3), and a lipid storage and packaging protein (Oleosin)- mainly localize to lipid droplets when transiently expressed in Nicotiana benthamiana. The overexpression of coding sequences for these six proteins in N. benthamiana leaves resulted in a proliferation of cytoplasmic neutral lipids droplets. Analysis using GC-MS also indicated that the overexpression of these proteins increased the total neutral fatty acid content and somewhat altered the fatty acid composition of the infiltrated leaves. Our data point to possible roles of these six candidate proteins in the compartmentalization and/or stability of pennycress lipid droplets and represent interesting targets for genetic manipulation of pennycress seeds with increased oil content.

P29. Substrate supply and laccase specificity drive lignin composition during the switch from G- to C-lignin accumulation in Cleome hassleriana

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Catechyl lignin (C-lignin) is a linear homopolymer of caffeyl alcohol (C-monolignol) that possesses unique properties as both a biomaterial and substrate for biological funneling to small molecule precursors of chemicals. However, it appears to be limited to seed coats. Cleome hassleriana is emerging as a model system for studying C-lignin biosynthesis because the lignin synthesized in the seed coat switches from classical guaiacyl (G) lignin to C-lignin

at around 13 days after pollination (DAP). Here, we coupled targeted metabolite profiling and isotopic precursor labeling experiments to examine the provision and utilization of monolignols in Cleome seed coat. We show that, whereas lack of synthesis of C-monolignol limits C-lignin formation prior to around 12 DAP, coniferyl alcohol (G-monolignol) is still synthesized and accumulated after 14 DAP, even though the classical route to its synthesis has been suppressed. However, this G-monolignol is not incorporated into lignin, because C-monolignol is a strong competitive inhibitor of the oxidization of G-monolinol by seed coat laccases. Among potential C-lignin related laccases, recombinant ChLAC8 has significant level of activity with C-monolignol, but cannot oxidize G-monolignol. Glutamin289 of ChLAC8 appears to be critical for binding of C-monolignol. Suppression of ChLAC8 expression led to significantly reduced C-lignin content in the seed coats of transgenic plants. Feeding of C-monolignol to the Arabidopsis thaliana caffeic acid O-methyltransferase (comt) mutant expressing ChLAC8 led to appearance of C-lignin with over 5% of total lignin. ChLAC8 possesses the unusual property of oxidizing C-monolignol but not G-monolignol and plays a critical role in initiating C-lignin polymerization. We propose that, during the period of C-lignin biosynthesis, the seed coat possesses a mechanism to maintain levels of coniferyl alcohol while blocking its formation and polymerization through the classical monolignol pathway. This coupled with laccase specificity, determines the metabolic fate of G- and C-monolignols.

OTHER ABSTRACTS SUBMITTED

1. Understanding Salt Adaptation in a Wild Sand Bean, Strophostyles helvola.

Christy Zuelsdorf, **Farida Yasmin*,** Janice Kofsky, Yan Luo, Bao-Hua Song #: These authors contributed equally to this work. Department of Biological Sciences, University of North Carolina at Charlotte, NC, USA

Soil salinity is one of the major environmental factors causing crop yield loss worldwide. Many crop wild relatives can thrive in high-salt environments while most of the crops are salt sensitive. Here we aim to elucidate the salinity tolerance mechanisms in the sand bean, Strophostyles helvola, by phenotypic and transcriptomic comparisons between a beach-adapted genotype and an inland one. The sand bean is a diploid wild legume species closely related to soybean and black bean. Our results showed that beach and inland genotypes responded differently to salt treatment. The beach genotype, unsurprisingly, exhibited a higher seed germination rate and seedling growth rate under salt treatment than inland ones. Meanwhile, compared to the inland genotype, the beach genotype showed significantly more differentially expressed genes (DEGs) when treated with high levels of salt solution. Further analysis of the DEGs suggested that ABA pathway, indicative of regulation of stress-response, and transcription factors (MYB, WRKY, and NAC), might play important roles in salinity tolerance in beach genotypes. Meanwhile, the beach genotype showed significantly higher levels of constitutive expression (high gene expression at both control and salt-treated plants) of genes related to salt tolerance i.e. SOS-3 interacting protein 1, MAPKKK, and genes related to oxidation-reduction, indicating these genes are also important for salt

adaptation in the beach genotype. Interestingly, inland genotype showed increased levels of photosynthetic gene expression, which might be due to the attempt to counteract the detrimental effects of salt stress. These findings provided insight into a complex and dynamic response at molecular and cellular levels translating to whole-plant phenotype differences and conferring salinity adaptation. This study makes full use of wild genetic resources to provide a foundation to understand plant environmental adaptation, and further to develop salt-tolerant crops.

2. Metabolomic Response in Sweet Potato Wild Relatives (I. imperati and I. lacunosa)

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As climate change alters the agricultural landscape, crops will increasingly be raised on marginal soils, including those with high salt concentrations. With their greater genetic diversity, wild crop relatives of important agricultural products are being used more and more to elucidate the defenses that plants have evolved in order to handle these stresses. We used two relatives of the globally important crop sweet potatoes (Ipomoea batas) to understand the metabolomic response of plants undergoing salt stress. The salt tolerant beach morning glory (I. imperati) and it's glycophytic cousin the Whitestar morning glory (I. lacunosa) were exposed to 600 mM salt stress over a seven day period, and GC-MS analysis was performed to identify metabolites produced under both control and salt-stress conditions. Samples taken at 3, 24, and 168 hours indicate clear metabolomic differentiation between both species. The salt-sensitive species, showed induced differentiation between control and treatment conditions. The salt tolerant species produced more metabolomic overlap between control and treatments, indicating its constitutive metabolomic responses. Amino acids, sugars, and organic acids are among the metabolites produced by both species. I. imperati had increased concentrations of metabolites associated with the TCA cycle, indicating its ability to maintain metabolic conditions; I lacunosa showed up regulation of many metabolites, including amino acids such as asparagine, glutamine, and proline which are involved in stress responses in a wide variety of plants. Lowered production of metabolites related to the TCA cycle in I. lacunosa indicate a shift from growth to survival response. The metabolites produced by these plants were traced through possible metabolomic pathways in the production of secondary metabolites that may aid in survival during salt stress conditions that sessile organisms cannot escape. These diploid relatives of the sweet potatoes showcase the usefulness of wild crop relatives in modeling stress response in plants.

3. Improved plant regeneration in rice mediated by Baby boom and Wuschel transcription factors

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To achieve high rate of plant transformation, high rate of regeneration is important. This issue has been tackled, with various strategies but with limited success. Lately, an efficient tool based on the use of morphogenic transcription factors, Baby Boom (Bbm) and Wuschel2 (Wus2), has been described in maize and sorghum. This study tested the strategy on rice by comparing regeneration rates with and without these transcription factors, coupled with a desiccation inducible CRE/lox excision system in two varieties i.e., Nipponbare and Diamond. The vector PHP78891 (provided by Dupont) was used that contains CRE, driven by the drought inducible maize RAB17M promoter and loxP sites that bracket the CRE, Wus, and Bbm genes. A constitutive maize UBI promoter directs a GFP expression as reporter, which is placed outside the CRE/lox excision sites. The control vector had only the GFP expression cassette, without the aforementioned genes. In this study, instead of rice callus, mature excised rice embryos were transformed using the gene-gun with the two vector types. This first step, helped cut short, close to 6 weeks of the tissue-culture time. Transient GFP expression was scored with both the vectors. The percentage plant regeneration with the PHP78891 in Diamond was 66.6% compared to 46.6% in control and for Nipponbare, it was 83.3% compared to 58.5% in control. While the regeneration rates went up, the recovered plants did not contain GFP, requiring more work for this strategy to be adopted for rice transformation protocols. With the use of mature rice embryos, this study took 6 weeks to complete - from the day of DNA delivery to plant regeneration.

4. Plant immune system activation is necessary for efficient interaction with auxin secreting beneficial bacteria

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Plants continuously monitor the presence of microorganisms through their immune system to establish an adaptive response. Unlike immune recognition of pathogenic bacteria, how beneficial bacteria interact with the plant immune system is not well understood. Analysis of colonization of Arabidopsis thaliana by auxin producing beneficial bacteria revealed that activation of the plant immune system is necessary for efficient bacterial colonization and auxin secretion. A feedback loop is established in which bacterial colonization triggers an immune reaction and production of reactive oxygen species, which, in turn, stimulate auxin production by the bacteria. Auxin promotes bacterial survival and efficient root colonization, allowing the bacteria to inhibit fungal infection and promote plant health.

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KH1. Using every tool in the kit: a story of diversification enabling innovation
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Life in the Anthropocene has many challenges, among the most urgent is widespread global hunger and malnourishment. What if every community of small-hold farmers could improve

their own heritage crops to their own specifications? Realizing this DIY vision would require radical innovations in the way we conceptualize and execute crop improvement. Synthetic biology, sitting at one of the intersections of engineering and biology, offers theoretical and practical tools to guide such efforts. My group is interested in exploring the limits of rational re-tuning of developmental pathways to customize plant form for local agricultural conditions, while simultaneously striving to understand the molecular tuning knobs that control fundamental components of eukaryotic signaling (e.g., ubiquitin-mediated protein degradation, transcriptional repression/activation).

KH 2. The Evolution of G Signaling
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The most prevalent and best understood signal transduction pathway in Eukaryota is the G protein-couple pathway for the perception of a broad range of extracellular signals. In the canonical pathway worked out using ex vivo vertebrate cells and yeast, 7 transmembrane (7TM) plasma membrane receptors (GPCRs) are activated by ligand binding that then initiate the GPCR to catalyze the release of GDP bound to an associated, cytoplasmic G protein complex thus allowing the binding of GTP. This reaction is the rate-limiting step. This GTP bound form is the activated state that binds to different downstream targets to affect their activity. As the signal propagates, it is amplified. The inactive state occurs once the intrinsic GTPase activity of the G protein complex catalyzes the bound GTP. This intrinsic hydrolysis can be accelerated by cytoplasmic Regulator of G Signaling (RGS) proteins. Plants and protists have G proteins that are self-activating meaning they lack GPCRs. Instead, they have RGS proteins that share the topology of a GPCR and GTP hydrolysis is the rate-limiting step. Evidence suggests that this system architecture represents the ancestral state of G protein coupled signaling. Originally, it was thought that the primordial system used de-repression by receptor-RGS proteins and this evolved in metazoans to a threshold-based activation system but this understanding is itself evolving. Mathematical modeling shows that activation and de-repression systems have different behaviors and properties. Activation is faster but more sensitive to noise. In animals, GPCRs are numerous (~850 in humans) and receptor like kinases (RLKs) are minimal. The opposite is true for plants and it appears that RLKs play an important role in G signaling.

KH 3. Chemical signaling in plant defense
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Systemic acquired resistance (SAR) is a form of broad-spectrum resistance induced in response to local infections that protects uninfected parts against subsequent secondary infections by related or unrelated pathogens. Thus, SAR is more desirable than the "acquired immunity" of vertebrates, which only generates antigen-specific immunological memory. SAR signaling requires two parallel branches, one regulated by salicylic acid (SA), and the other by pipecolic acid (Pip), azelaic acid (AzA) and glycerol-3-phosphate (G3P). AzA and G3P function downstream of the free radicals nitric oxide (NO) and reactive oxygen species (ROS) and Pip functions by increasing NO/ROS levels. The plant galactolipids

monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) are also required for SAR and alpha-galactose-β-galactose sugars on DGDG are required for normal biosynthesis of NO. During SAR, SA, Pip, AzA, and G3P accumulate in the infected leaves, but only a small portion of these is transported to distal uninfected leaves. SA is preferentially transported via the apoplast, whereas phloem loading of AzA and G3P occurs via the symplast. Many of these chemical are associated with human disorders, although their underlying molecular mechanisms in such disorders are largely unknown. The evolutionary conservation of select signals and their precise role in SAR will be discussed.

KH4. Do plants feel pain? Stress signaling in Arabidopsis
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For animals, rapid, long-range signaling networks based on nerve conduction help integrate sensing and rapid response throughout their bodies. Neurotransmitters such as the amino acid glutamate operate through the activation of glutamate receptor channels to trigger nerve-to-nerve transfer of information. Plants also need to sense local signals, such as herbivore attack, and transmit this information throughout the plant body to rapidly activate defenses in undamaged parts. However, lacking nerves, they must employ a machinery that is different from the animals. Jasmonic acid-based responses are known to be triggered in both wounded and unwounded parts of the plant but precisely how, for example, a locally damaged leaf can rapidly communicate to undamaged leaves to prime their defenses remains poorly understood. We have found that glutamate acts as a wound signal in this system, triggering ion channels of the plant GLUTAMATE RECEPTOR LIKE family. Activation of this wound perception machinery then elicits a, likely electrical, signal that propagates throughout the plant. This systemic signaling system triggers an increase in intracellular Ca^{2+} as it moves that helps transmit wound information to distant tissues. This signaling system comprises two phases, firstly a rapid plant-wide movement through the vasculature. The signal then spreads throughout the undamaged tissues via cell-to-cell coupling through plasmodesmata, triggering defense responses through JA-dependent events. Thus, despite lacking a nervous system, plants employ a rapid, plant-wide signaling system that helps integrate responses across the plant body.