2024 MEETING OF THE SOUTHERN SECTION OF THE AMERICAN SOCIETY OF PLANT BIOLOGISTS

March 22-24th
Dauphin Island, Alabama
at the
DAUPHIN ISLAND SEA LAB

Meeting Sponsors

Meeting Code of Conduct

2024 Southern Section American Society Plant Biology Annual Meeting
PROGRAM-Schedule

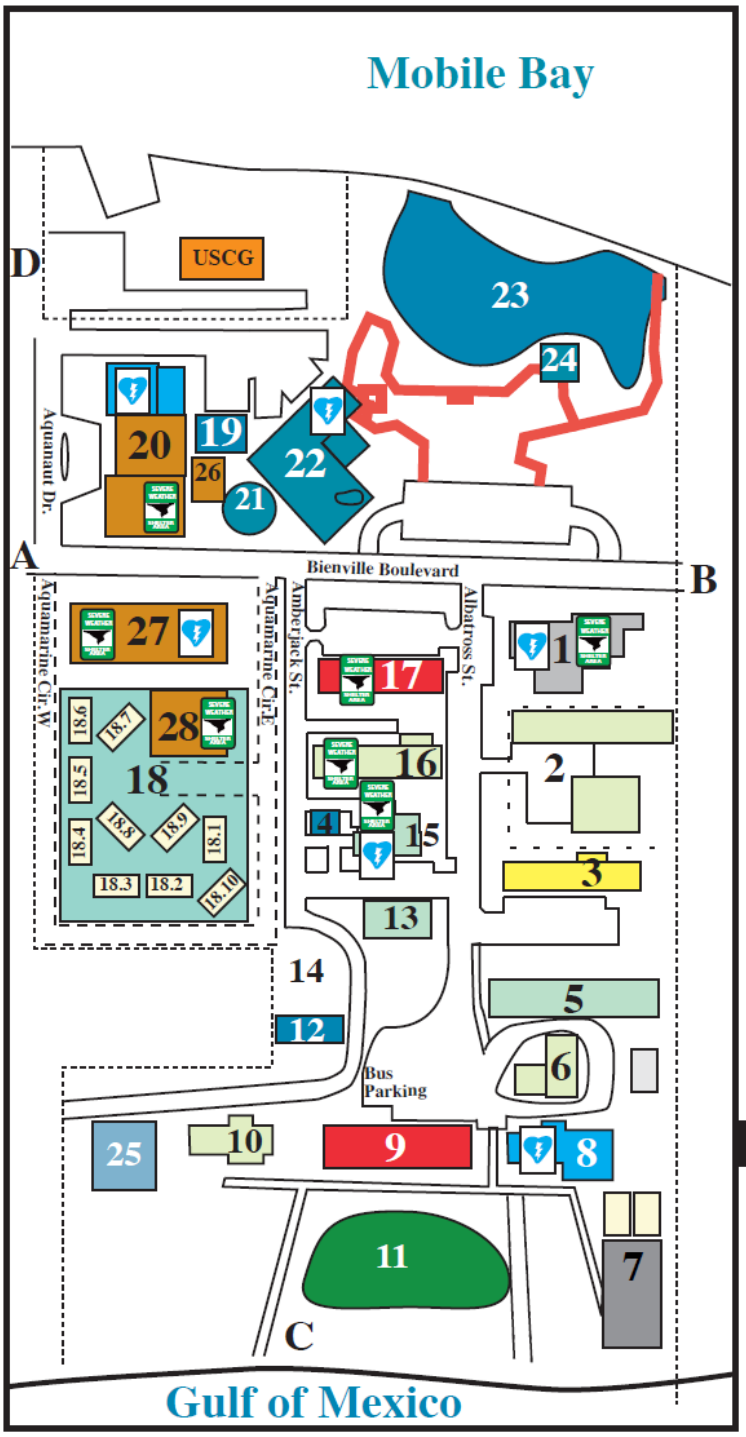
Friday, Mar 22nd		
		Room
3:00 – 7:00 pm	**Meeting Registration**	Atrium
4:00 – 8:00 pm	**Poster Setup**	Atrium
5:15 – 5:30 pm	**Welcome Session – Opening Remarks** *Chair: Colleen Doherty* *Vice-Chair: Aaron Rashotte* *Sea Lab representative: TBD* *ASPB Ambassadors*	Main
5:30 – 6:20 pm	**General Session 1 - Moderator: Aaron Rashotte**	Main
5:30 – 5:55 pm	**"***Selective Breeding and Domestication of Lemnaceae For Use in Sustainable Agriculture", Ryan Sartor, NC State University*	
5:55 – 6:25 pm	*"Growing beyond Earth: Telomere tales of Arabidopsis thaliana in lunar regolith simulant and on the International Space Station" Borja Barbero, Texas A&M University*	
6:30 – 7:20 pm	**Kriton Hatzios invited speaker:** *Katayoon Dehesh, UC Riverside "Decoding Cellular Aging: Insights from Golgi Apparatus Dynamics"*	Main
7:30 – 9:00 pm	**Opening Night Mixer and Networking Event**	Atrium
Saturday, March 23rd		
7:30 – 8:45 am	**Breakfast**	Café
7:30 – 9:00 am	**Registration & Poster setup**	Atrium
8:45 – 9:00 am	**Welcome**	Main
9:00 – 10:30 am	**General Session 2 – Moderator: Colleen Doherty**	Main
9:00-9:25 am	*"Precision genome engineering with the mPing transposable element" C. Nathan Hancock, University of South Carolina Aiken*	
9:25-9:50 am	*"A cation/H+ antiporter enhances stomatal conductance and carbon assimilation in water-deficit plants" Amith Devrieddy, Oak Ridge National Lab*	

9:50-10:15 am	*"The Arabidopsis telomerase RNP: echoes of mammalian and ciliate enzyme complexes", Saundarya Mishra, Texas A&M University*	
10:15 – 10:30 am	**Break** (coffee and tea)	Atrium
10:30 – 12:00 pm	**CONCURRENT SESSIONS 1A & 1B**	Main & Classroom
10:30 – 12:00 pm	**Concurrent Session 1A – Moderator: Ryan Sartor**	Main
10:30-10:45	*"Advancing Soybean Seed Protein: Insights from QQS Gene Exploration" Ethan Brister, Mississippi State University*	
10:45-11:00	*"Development of Duckweed Synthetic Seed (SynSeed) for Efficient Germplasm Conservation" Doni Devi Thingujam, University of Alabama at Birmingham*	
11:00-11:15	*'Single nucleus multiomics reveals the drought-driven gene regulatory atlas in Arabidopsis" Jinbao Liu, University of Alabama at Birmingham*	
11:15-11:30	*"Taxonomically Restricted QQS Associated 1 (TRQA1): A Regulator of Plant Metabolism Controlling Protein and Starch Content" Rezwan Tanvir, Mississippi State University*	
11:30-11:45	*"Cis-Zeatin delays leaf senescence in Solanum lycopersicum under salt stress as shown by physiological and transcriptomic analysis" Malsha Thennakoon, Auburn University*	
10:30 – 12:00 pm	**Concurrent Session 1B – Moderator: Amanda Storm**	Classroom
10:30-10:45	*"Chemical insights into the Chlamydomonas quorum sensing signal molecule" Kirstin Cutshaw, Florida Institute of Technology*	
10:45-11:00	*"Dual Phase molecular regulation of leaf senescence by cytokinin isoforms" Omar Hasannin, Auburn University*	
11:00-11:15	*"The role of SnRK1 in Development and Stress Response: omics analysis of the rice snrk1 mutants" Maria Clara Faria Chaves, University of Arkansas*	
11:15-11:30	*"Galactolipids Regulate Systemic Immunity In Plants" Tatsushi Kurokawa, University of Kentucky*	
11:30-11:45	*"A Reverse Genetics Approach to Identifying JAGN1 function in Arabidopsis thaliana" Regina Bedgood, University of Alabama at Birmingham*	
11:45-12:00	*"Salt-Stress Accelerated Leaf Senescence is Delayed by N-conjugated trans-Zeatin type Cytokinin forms in Arabidopsis thaliana" Risheek Khanna, Auburn University*	

12:00-1:00 pm	**Lunch**	Café
1:00 – 2:00 pm	**Undergraduate Poster Session**	Atrium
1:30 – 2:30 pm	**Graduate, Faculty, and Professional Poster Session**	Atrium
2:30 – 3:45 pm	**CONCURRENT SESSIONS 2A & 2B**	Main & Classroom
	Concurrent Session 2A - Moderator: TBD	Main
2:30-2:45 pm	*"Sulfur Mutant Profiling of Arabidopsis thaliana on interaction with Pseudomonas syringae" Binoop Mohan, University of Alabama at Birmingham*	
2:45-3:00 pm	*"Effects of Simulated Microgravity On Plant Growth Promoting Efficiency of ISS Bacterial Isolates" David Handy, Florida Institute of Technology*	
3:00-3:15 pm	*"Investigating the effects of diffusible signals from plant growth-promoting bacterium, Azospirillum brasilense, on rice at developmental and molecular levels." Samuel Hoggard, University of Central Arkansas*	
3:15-3:30 pm	*"Investigating the concentrations and role of Chlamydomonas-derived riboflavin and its degradation products" Ryan Quick, Florida Institute of Technology*	
3:30 – 3:45 pm	*"12-oxophytodienoic Acid: A Mobile Signal of Induced Systemic Resistance" Simrandeep Kaur, Auburn University*	
	Concurrent Session 2B - Moderator: Nathan Hancock	Classroom
2:30-2:45	*"Conversion of Martian Regolith Simulants to Soil via the Legume-Rhizobia Symbiosis" Hayley Ernest, Florida Institute of Technology*	
2:45-3:00	*"Rhizobial evasion of terminal differentiation: Temporal/spatial dynamics of Sinorhizobium meliloti reproductive population growth in alfalfa nodules and implications for cheating by poor N2 fixers" Katherine Bilodeay, Florida State University*	
3:00-3:15	*"Dissecting the functions of Salmonella enterica effectors in plant infection" Jill Presel, University of South Alabama*	
3:15-3:30	*"Evaluating the Use of Aquatic Plants in Martian Agriculture" Sergio Solano, Florida Institute of Technology*	
3:30 – 3:45 pm	*"Analysis of Systemic Acquired Resistance by Monitoring Redox-Mediated Transcriptional Dynamics in Arabidopsis" Rezwana Rahman, Mississippi State University*	
3:45 – 4:15 pm	Coffee Break	

4:15 – 5:15 pm	**Kriton Hatzios invited speaker:** *Jose Dinneny, Stanford University "Diversity and adversity: exploring the mechanistic basis for variation in stress tolerance across the Brassicaceae"*	Main
5:15 – 6:15 pm	**General Session 3 - Moderator: Aaron Rashotte**	Main
5:15-5:45 pm	**"***Elimination of Pungency in Allotetraploid Brassica juncea Through Gene Editing of the Multicopy Myrosinase Gene Family", Loren Rivera Vega, Pairwise, Durham NC*	
5:45 – 6:15 pm	*TBD*	
6:30 – 8:00 pm	**Low Country Boil(!) and Awards Presentations – Henry Daniell Award, Oral presentations, undergraduate presentations, and travel awards.**	TBA
Sunday, March 24th		
7:30 – 9:00 am	**Breakfast and Poster Take-down**	Café & Atrium
9:00 – 9:15 am	**Morning remarks**	Main
9:15 – 10:05 am	**General session 4 - Moderator: Borja Barbero**	
9:15 – 9:40 am	*"Plant-based Recovery of Rare Earth Elements from Secondary Waste Sources" Colleen Doherty, North Carolina State University, Raleigh, NC.*	Main
9:40 – 10:05 am	*Investigating Thiamine Metabolite Damage and Repair: Role of the TenA Domain in Arabidopsis TH2 Protein, Ghulam Hasnain, University of North Georgia, Oakwood, GA.*	Main
10:05 – 10:30 am	**Break** (coffee and tea)	
10:30 – 11:30 am	**Kriton Hatzios invited speaker:** *Gloria Muday, Wake Forest University, "Flavonol antioxidants control the accumulation of reactive oxygen species to protect plants from environmental stress and to modulate development."*	
11:30– 12:00 pm	**General Meeting & Concluding Remarks**	

Dauphin Island Sea Lab Facilities Map



1. Administrative Offices, Registration, Classroom and Study Hall
2. Maintenance/Vehicle-Boat Yard
3. Albatross Hall (Closed)
4. Laundromat
5. DHP Computer Lab/Classroom 5
6. Endeavor Hall (Class Rooms)
7. Basketball, Volleyball Courts
8. Discovery Hall (Class Rooms/Offices)
9. Horizon Hall (Class Rooms/Offices)
10. Galathea Hall (Class/Meeting Room)
11. Sea Pines
12. Swimming Pool
13. Mesocosm Facility
14. Future Aquatic Center
15. Cafeteria
16. Challenger Hall (Dormitory)
17. Beagle Hall (Dormitory)
18. 1-10 Faculty Housing
19. Wet Lab Building
20. Wiese Marine Science Hall
21. Husbandry Building
22. Alabama Aquarium, Giftshop &
23. Living Marsh and Boardwalk
24. Ladner Pavilion
25. A. U. Shellfish Research
26. Wet Lab
27. Shelby Fisheries Center
28. Marine Mammal Research Center

A. To the Water Tower and Audubon Sanctuary
B. To Fort Gaines
C. To Gulf Of Mexico Beach
D. To DISL Research Vessels

* - OPEN TO THE PUBLIC

SEVERE WEATHER SHELTER AREA — Severe Weather Shelter

AED — Automated External Defibrilators

2022/10 RD

Dauphin Island Sea Lab
Alabama Center for
Marine Education and Research

Guest Computer Access:

SIGNON NAME: event **PASSWORD:** Welcome2DISL

Guest WiFi Access:

Only available in Shelby, MSH, Endeavor, Discovery Hall, Horizon, Cafe & Galathea

CURRENT SSID: MESC-Meet
PASSWORD: meetdisl!

Meeting Abstracts

Oral Presentation Abstracts

Kriton Hatzios Invited Speakers

Decoding Cellular Aging: Insights from Golgi Apparatus

Dynamics Katayoon Dehesh University of California, Riverside

The intricate process of transcriptional reprogramming, initiated in response to stress, plays a pivotal role in orchestrating the adaptation of physiological and metabolic systems to environmental challenges. It impacts essential macromolecules such as membrane lipids, proteins, and DNA. A central element in this response is the rapid stress response element (RSRE), found in roughly 30% of stress-responsive genes. A highly effective tool for monitoring stress-induced changes in gene expression in response to various biotic and abiotic stimuli is a reporter system that utilizes luciferase (LUC) driven by four copies of RSRE (4xRSRE:LUC). To gain deeper insights into the underlying mechanisms driving these transduction events, we conducted a thorough examination using the 4xRSRE:LUC reporter line. This investigation led to the identification of a mutant characterized by an amino acid substitution in the 7th subunit of the hetero-octameric Conserved Oligomeric Golgi (COG) complex. Subsequent complementation studies confirmed the localization of cog7 in the Golgi, while stress tests revealed that this mutant displayed accelerated senescence and carbon deprivation when subjected to darkness, in comparison to wild-type plants. Further exploration, involving multiomics and biochemical analyses, uncovered compelling findings. These included the expedited induction of protein ubiquitination and autophagy, along with an unexpected increase in protein N-glycosylation during senescence in cog7 mutants in comparison to their wild-type counterparts. To investigate potential rescue mechanisms, we executed a revertant screen using the OvereXpressor (FOX)-hunting system. This approach demonstrated a partial but significant restoration of cog7 phenotypes through the overexpression of COG5. Conversely, it induced premature senescence in lines with reduced COG5 expression. In conclusion, these discoveries shed light on the critical role of COG-mediated Golgi functional integrity in ensuring cellular survival under energy-limiting conditions. This finding underscores the intricate interplay between Golgi-related processes and stress responses, highlighting the significance of these mechanisms in enabling cells to adapt and flourish in challenging environments.

Diversity and adversity: exploring the mechanistic basis for variation in stress tolerance across the Brassicaceae

Jose Dinneny, Stanford University, Department of Biology

Plants have diversified greatly in their ability to survive in vastly different environments, yet our understanding of the mechanistic basis for such differences is highly limited. We have established a phylogenetically informed collection of species in the Brassicaceae family amenable to molecular-genetic and genomic analysis to address this gap. Relative to other plant families, Brassicaceae species have the advantage that most plants are annuals, transformable using the rapid floral dip method, and most importantly, closely related to Arabidopsis, which is by far the best system for molecular studies of gene function. We have established efficient transformation vectors/protocols for most species and have successfully performed CRISPR/Cas9 editing, which allows us to learn how gene functions are diversified. I will present our work that establishes a comparative physiological and genomic understanding of these species and present evidence for the diversification of cell type functions and hormone signaling.

Flavonol antioxidants control the accumulation of reactive oxygen species to protect plants from environmental stress and to modulate development.

Gloria Muday, Wake Forest University, Department of Biology and Center for Molecular Signaling, Winston Salem, NC, USA, 27109, muday@wfu.edu

Flavonols are plant specialized metabolites with important functions in development and stress responses, which have been demonstrated using mutants with defects in genes encoding biosynthetic enzymes. In both Arabidopsis thaliana and Solanum lycopersicum, mutants with altered flavonol biosynthesis have changes in root development, guard cell closure in response to drought, and pollen thermotolerance. We are testing the hypothesis that the developmental and stress protection activities of flavonols are linked to their antioxidant capability by which they reduce accumulation of reactive oxygen species (ROS). ROS signals are produced by plants during hormone signaling and stress responses but must be balanced to positively regulate cell signaling without reaching levels that cause oxidative stress. Ethylene and abscisic acid increase ROS, as visualized with fluorescent dyes and biosensors, to drive root hair formation and guard cell closure, respectively. Environmental stress (drought and elevated temperature) increase ROS synthesis through activation of respiratory burse oxidase enzymes and mutants with impaired synthesis of flavonol antioxidants have greater responses to these stresses and elevated ROS. In tomato pollen, temperature stress increases ROS in wild-type and this response is more pronounced in the anthocyanin reduced (are) mutant, which has a defect in flavonol synthesis. The are mutant is hypersensitive to the negative effects of high temperature on pollen tube germination, elongation, and tube integrity. The increased ROS accumulation and pollen phenotypes in the are mutant are reversed by genetic and chemical complementation. RNA-Seq of are reveals genome-wide transcriptional responses to high temperature that are buffered in wild-type. Overexpression of a gene encoding an enzyme of flavonol metabolism can convey thermotolerance, suggesting a strategy to convey pollen thermotolerance to address the negative impacts of increasing global temperatures on plant reproductive success. These studies reveal important functions of flavonol antioxidants in productive ROS signaling. (Supported by NSF IOS 1939255 and USDA NIFA2020-67013-30907)

General Session 1

Selective Breeding and Domestication of Lemnaceae For Use in Sustainable Agriculture

Ryan C. Sartor, NC State University, Department of Molecular and Structural Biochemistry, Department of Biological and Agricultural Engineering Jay Cheng, NC State University, Department of Biological and Agricultural Engineering Michael Burchell, NC State University, Department of Biological and Agricultural Engineering François Birgand, NC State University, Department of Biological and Agricultural Engineering Praveen Kolar, NC State University, Department of Biological and Agricultural Engineering

The plant family Lemnaceae, commonly known as the duckweeds, are small, floating aquatic plants with several natural adaptations that make them well suited to help improve the sustainability of modern animal production systems. My lab primarily focuses on one species, *Lemna gibba* (aka *Lemna*). This plant has 3 natural properties that we are interested in exploiting: 1) High yields, with potential yields of over 25 tons/hectare/year. 2) The ability to thrive on existing animal wastewater and 3) The ability to produce high quality biomass with as much as 35% protein or 30% starch by dry mass in vegetative tissue. These properties make Lemna a prime candidate for use in nutrient recycling systems where animal waste is used as a fertilizer source to grow a high quality feed supplement. Sustainability increases come from decreasing GHG emissions associated with the production of synthetic fertilizers and decreasing the on-site emissions of Nitrous oxides and volatile ammonia. Additional benefits come from decreased use of pesticides and herbicides to grow feed crops and form decreasing nutrient release into the environment. Such a system can also have economic benefits by producing low-input, on-site animal feed. It is possible to utilize wild Lemna varieties for this purpose. However, as should be expected from any undomesticated species, challenges exist to mass cultivation resulting in yield instability. In order to be used as a reliable crop, Lemna must be domesticated and selectively bred in order to realize its full potential. This is a major focus of my group and we are working on several projects to develop improved varieties of Lemna for use in nutrient recycling and wastewater treatment. In this presentation I will discuss the progress we have made in terms of promoting sexual reproductive cycles in Lemna, cross-pollinating to generate new varieties and the design and construction of novel systems for high-throughput phenotyping in order to facilitate extremely fast improvement of this species with enormous potential.

Growing beyond Earth: Telomere tales of *Arabidopsis thaliana* in lunar regolith simulant and on the International Space Station

Borja Barbero Barcenilla*, Texas A&M University Ishan Kundel, Texas A&M University Emma Canaday, Ohio University Alexander Meyers, NASA Sarah Wyatt, Ohio University Dorothy E. Shippen, Texas A&M University

NASA envisions sustainable colonies on the moon and on Mars by 2050, and plants will play pivotal roles in these endeavors. Here we investigate how the telomeres and telomerase of *Arabidopsis thaliana* are impacted by space flight and growth on extraterrestrial soil simulants. We report that telomere length is steady in plants grown on the International Space Station (ISS), although telomerase enzyme activity is strongly induced, increasing by up to 150-fold in roots.

Ground-based studies affirmed telomerase activity is elevated in *Arabidopsis* by diverse environmental stressors, and this induction is independent of telomere length changes. There was a strong inverse correlation between genome oxidation and telomerase activity levels, suggesting plant telomerase may harbor a redox protective role that can help to facilitate survival in harsh environments. Recent studies show that *A. thaliana* can be successfully cultivated in lunar regolith, but arrests at a terminal vegetative state and activates multiple stress responses. We found that pre-washing the simulant with an antioxidant cocktail facilitated seed setting and viable second-generation plants, but plants grown in lunar regolith simulant displayed increased genome oxidation and reduced biomass compared to Earth soil cultivation. Moreover, growth in lunar regolith simulant resulted in progressive telomere shortening and reduced telomerase enzyme activity for a variety of different *A. thaliana* accessions and in a variety of different regolith simulants. These findings highlight both the promise and the challenges of ensuring genome integrity for successful plant growth in extraterrestrial environments.

General Session 2

Precision genome engineering with the mPing transposable element

C. Nathan Hancock[1]* Coauthors: Peng Liu[2], Zara Lacera[1], Madison Hamlin[1], and R. Keith Slotkin[2]; [1]University of South Carolina Aiken, Aiken, SC, USA; [2]Donald Danforth Plant Science Center, St. Louis, MO, USA

Identification and manipulation of the genes controlling agronomically important traits is crucial for crop improvement and food security. Utilizing the mPing transposable element from rice, we have developed mutagenesis resources capable of generating both knockdown and overexpression phenotypes. In collaboration with Keith Slotkin's Laboratory at the Donald Danforth Plant Science Center, this technology has been advanced by establishing a reliable method for sequence-specific targeting of mPing insertion in plant genomes. Linking Pong Transposase to CRISPR/Cas nucleases allows for the excised mPing elements to be inserted into the Cas targeted double stranded breaks. They have successfully used this technology to deliver enhancer elements, open reading frames, and gene expression cassettes into targeted locations in *Arabidopsis* and soybean genomes. To enhance mutagenesis efficiency, the Hancock laboratory is developing hyperactive versions of mPing and the Pong transposase proteins responsible for mobilizing these elements. The results demonstrate the potential of mPing-based mutagenesis for crop improvement and expanding crop genetic engineering capabilities.

A cation/H+ antiporter enhances stomatal conductance and carbon assimilation in water-deficit plants

Amith R. Devireddy*, Tao Yao, Kuntal De, Patrick Bewg, Jin Zhang, Biruk A. Feyissa, Raphael Ployet, Sara S. Jawdy, Nancy L. Engle, Miguel Rodriguez Jr, Madhavi Z. Martin, David J. Weston, Chung-Jui Tsai, Yuko Yoshinaga, Christopher Daum, Mengjun Shu, Timothy J. Tschaplinski, Kerrie Barry, Anna Lipzen, Jeremy Schmutz, Gerald A. Tuskan, Jin-Gui Chen, and Wellington Muchero

A major challenge in breeding or engineering water-deficit stress tolerance is the inherent tendency of most plant species to regulate stomatal closure, reducing water loss via transpiration. This regulation results in decreased CO2 assimilation, leading to reduced photosynthetic efficiency. In this study, genome-wide association studies and expression quantitative trait loci mapping in Populus trichocarpa identified a genetic locus associated with delayed leaf senescence in natural Populus variants exposed to water-deficit stress under field conditions. This locus encodes the Cation/H+ antiporter CHX20. Transgenic poplar lines overexpressing PtCHX20 displayed larger root biomass, slower xylem conductance, and higher relative leaf water content while maintaining stomatal openings, enabling transpiration and CO2 assimilation during water-deficit stress. Similarly, Arabidopsis thaliana transgenic lines overexpressing AtCHX20 showed increased stomatal conductance, transpiration, and osmolyte content under water-deficit conditions. Transcriptomic and metabolomic analyses in AtCHX20 transgenics revealed an over-representation of stress-tolerance-associated processes. These findings collectively suggest the crucial role of CHX20 in mediating water-deficit stress tolerance, preserving photosynthetic efficiency through a previously undescribed mechanism.

The *Arabidopsis* telomerase RNP: echoes of mammalian and ciliate enzyme complexes

Saundarya Mishra[1*#], Chinmay Phadke[1#], Jiarui Song[1], Claudia Castillo Gonzales[1], Edward Marcotte[2], Ophelia Papoulas[2] and Dorothy E. Shippen[1, 3]; [1]Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas, 77843-2128, USA; [2]Department of Molecular Biosciences, The University of Texas at Austin, Austin, Texas, 78712, USA [3]Author for correspondence: dorothy.shippen@ag.tamu.edu (#: Authors contributed equally)

Telomeres are repetitive DNA sequences that cap the ends of chromosomes. Telomeric DNA is synthesized by the telomerase reverse transcriptase, a large ribonucleoprotein (RNP) complex containing a catalytic subunit TERT and an intrinsic RNA template TR. Despite its highly conserved function, the protein composition and biogenesis of telomerase varies widely across different eukaryotic lineages. Previously, we characterized TERT and TR from the flowering plant Arabidopsis thaliana as well as two other associated proteins: dyskerin, a core component of human telomerase, and Protection of telomeres 1a (POT1a), a highly conserved telomere end-binding protein. Here we seek to identify and characterize additional telomerase accessory proteins that might be important for RNP maturation or enzyme activity. Quantitative mass spectrometry (qMS) carried out with plants expressing tagged AtTERT revealed AtLa1, a genuine La protein, and a member of a class of RNP maturation factors known to interact with the 3'UUU tail of pol III transcripts. Notably, the La-domain protein p65 is a core component of Tetrahymena telomerase enzyme. Unexpectedly, however, our qMS experiment failed to uncover dyskerin or AtPOT1a. To examine the in vivo role of AtLa1 in telomere biology, we obtained plants that overexpress AtLa1 (expression driven from a strong 35S CaMV promoter) or are deficient in AtLa1. Overexpression of AtLa1 did not significantly stimulate telomerase activity, indicating that AtLa1 is not limiting for telomerase enzyme activity. Because AtLa1 is essential for embryogenesis, we created inducible knockdown mutants using an estradiol inducible RNAi system. Experiments are currently underway to determine if AtLa1 is required for enzyme activity or AtTR stability. In parallel, we are conducting biochemical experiments to study AtLa1 interactions with telomerase. Electrophoretic Mobility Shift Assays (EMSA) revealed AtLa1, like

the ciliate La protein, binds AtTR. Binding does not require the evolutionary conserved pseudoknot (PK) and template region, but it does require the terminal 3' UUU and the 3-way junction. Intriguingly, dyskerin engages this same region of AtTR, suggesting that dyskerin and AtLa1 may compete for binding AtTR in vivo. We are now testing this hypothesis. Taken together, these experiments may yield new insight into the evolution and biogenesis of the plant telomerase enzyme.

Concurrent Session 1A

Advancing Soybean Seed Protein: Insights from QQS Gene Exploration

Ethan Brister* and Ling Li; Department of Biological Sciences; Mississippi State University

Enhancing soybean seed protein holds profound significance, augmenting the nutritional quality of soybeans and addressing global concerns about food security and sustainable agriculture for both human and livestock consumption. Additionally, increased soybean seed protein is pivotal for diverse industrial applications, spanning high-protein animal feed to the production of plant-based protein products. The *Arabidopsis* orphan gene Qua-Quine Starch (QQS) has emerged as a key player in elevating protein levels in leaves and seeds across various plant species. Through a comparative analysis of RNA transcript levels in wild-type and QQS-expressing (QQS-E) soybean leaves, we successfully identified potential candidate genes associated with high-protein content. These genes exhibited higher transcript levels in QQS-E lines compared to the wild type, establishing a clear positive correlation with high protein content. To deepen our understanding of the interplay between these soybean genes and protein content, we conducted experiments involving the over-expression of these genes in wild-type *Arabidopsis* plants. Quantification of transcript levels and thorough analysis of leaf and seed composition in promising lines unveiled the potential of these candidate soybean genes to enhance leaf protein content in *Arabidopsis*. Armed with these compelling findings, we aim to illustrate the practical applications of these discoveries and their potential impact on augmenting soybean seed composition, thereby contributing to advancements in both agricultural and industrial sectors.

Development of Duckweed Synthetic Seed (SynSeed) for Efficient Germplasm Conservation

Doni Thingujam*, Karolina M. Pajerowska-Mukhtar, and M. Shahid Mukhtar; Department of Biology, University of Alabama at Birmingham

Duckweed has resurged as a valuable plant research model system for understanding various plant physiological processes as it offers advantages such as rapid growth, simple structure, and ease of manipulation. Moreover, it has also acquired significant interest as a sustainable food source due to its high nutritional composition. Therefore, the preservation of the genetic diversity of duckweed is crucial for plant research and potential food security. The traditional liquid and solid culture media have been used as methods for germplasm preservation and in vitro maintenance of duckweed plants in the laboratory. However, these methods for growing cultures require a large amount of space, are expensive, and are time-consuming to maintain a continuous culture. Considering these limitations, our study investigated the potential of synthetic seed

technology as a novel approach for both efficient clonal propagation and germplasm conservation of duckweed species. Our research primarily focused on establishing a protocol to produce synthetic seeds using varying concentrations of sodium alginate and calcium chloride. Furthermore, this study evaluated different culture media compositions to optimize seedling regeneration at various time intervals. The findings demonstrated the effectiveness of the proposed protocol for in vitro preservation through encapsulation of shoot tips and subsequent regeneration of whole duckweed plants. This method can extend germplasm storage, enabling a consistent supply of duckweed plants for sustainable food production and research.

Taxonomically Restricted QQS Associated 1 (TRQA1): A Regulator of Plant Metabolism Controlling Protein and Starch Content

Rezwan Tanvir* and Ling Li; Department of Biological Sciences; Mississippi State University

Arabidopsis thaliana orphan gene Qua-Quine Starch (QQS) and its interactor, Nuclear Factor Y subunit C4 (NF-YC4), are known to enhance leaf/seed protein levels, reduce starch content, and increase pest and pathogen resistance across various plant species while maintaining yield. Despite their great potential for crop improvement, their functional network is still elusive. Taxonomically Restricted QQS Associated 1 (TRQA1) is believed to be associated with QQS based on its expression profile. Analysis of RNA-Seq data revealed the suppression of QQS expression leads to a significant upregulation of TRQA1 expression. We have obtained Arabidopsis plants with overexpressed or suppressed (by RNA interference (RNAi)) TRQA1 expression and TRQA1 knockout to elucidate the impact of TRQA1 on plant metabolism. TRQA1 suppression can significantly increase plant protein content and decrease starch levels. Examination of TRQA1 promoter–GUS expression patterns under normal growth conditions reveals its ubiquity. Its expression was detected in virtually all plant organs throughout different developmental stages, consistent with publicly available RNA-Seq data. Specifically, TRQA1 exhibits exceptionally high expression along leaf veins and at root tips. TRQA expression was confined to the cytosol, and the TRQA promoter-coding sequence-GFP fusion protein was not detected in the nucleus nor plastids under normal growth conditions. Promoter motif analyses hint at TRQA1's potential involvement in diverse aspects of plant metabolism, stress resistance, and defense against pests and pathogens. These insights suggest TRQA1 may play a pivotal role in regulating carbon and nitrogen allocation, underscoring its applicability as a valuable tool for enhancing plant protein content. In summary, our research highlights the promising role of TRQA1 as a novel regulator of plant metabolism and fine-tuning protein and starch content while contributing to stress resistance and overall plant defense strategies.

Cis-Zeatin delays leaf senescence in *Solanum lycopersicum* under salt stress as shown by physiological and transcriptomic analysis

Malsha Thennakoon*, Risheek Rahul Khanna, Omar Hasannin, and Aaron M. Rashotte; Rouse Life Sciences, Department of Biological Sciences; Auburn University

Tomato (*Solanum lycopersicum)* is one of the highest globally producing vegetable crops that is often subjected to salt stress leading to leaf senescence and ultimately overall yield loss. The plant

hormone cytokinin (CK) plays a major role in responding to different abiotic stressors including salinity and is well known for delaying leaf senescence. However, CK has several different isoforms cis Zeatin (cZ), trans zeatin (tZ), Isopentyl adenine (iP) and Dihydrozeatin (DHZ), and it is unclear if they all function in the same way in CK-responsive actions like leaf senescence. While there are abundant studies on tZ, the studies conducted on cZ are limited. In part cZ is under investigated due to early difficulties in distinguishing between these isomers and several studies showing little to no active function towards these stressors. The objective of this study is to determine the physiological and transcriptional effects of cZ during dark induced and salt stress leaf senescence in tomatoes. This was done in mature (65d) tomato leaves treated with cZ (or a tZ positive CK control) +/- exposure to salt (150 mM NaCl). Early (2h) and late (72h) timepoints were examined to determine effects of CK and salt at different stages of senescence. Physiological analyses including Fv/Fm, and chlorophyll content were determined, and results suggested that both cZ and tZ play a role in delaying leaf senescence. Transcriptomic analysis indicates that four CK-related genes; SlHK4 (CK receptor), and three response regulators RR2, RR3 and RR9 were commonly upregulated at each CK+/- and NaCl +/- treatments at each time point indicating the cross talk between CK signaling and salt stress in delaying leaf senescence. Additional examination of differentially expressed genes (DEGs) by gene ontology analysis showed significantly enriched terms for chloroplasts (cellular localization) and processes of photosynthesis, stress response, and signal transduction etc. indicating the potential changes in the regulation of genes involved in these processes under salt and CK treatments. These results suggest that salt stress promotes leaf senescence and cZ and tZ are functioning as negative regulators in delaying senescence in tomatoes.

Concurrent Session 1B

Chemical insights into the *Chlamydomonas* quorum sensing signal molecule

Kirstin Cutshaw[1]*, Jake Labishak[2], and Andrew G. Palmer[1,3]; [1]Department of Ocean Engineering and Marine Sciences, [2]Department of Aerospace, Physics, and Space Sciences, [3]Department of Biomedical Engineering and Sciences; Florida Institute of Technology

Quorum sensing (QS) is an intercellular signaling process by which microorganisms couple phenotypic switching to population density by utilizing low-molecular weight signals, generically classified as autoinducers (AIs) or quorum sensing molecules (QSMs). While QS has been well-established across prokaryotes, more recently, there is evidence that unicellular eukaryotes such as yeast and algae may be capable of this phenomenon. We have previously demonstrated that the model photosynthetic eukaryote *Chlamydomonas reinhardtii* also exhibits a QS-like phenotype which regulates motility. Using a multi-pronged approach combining high-performance liquid chromatography (HPLC) and liquid chromatography mass spectrometry (LC-MS), we are working to identify the molecule(s) responsible for this phenomenon. Here I will discuss our current understanding of the nature of this molecule and the challenges associated with its detection. Identifying this molecule will give better insight into how these microorganisms perceive and respond to their environment and can contribute to devising better tools to identify QS in unicellular eukaryotes. Exploring QS in this broadly distributed genus can provide insights into microbial ecology, as well as agricultural and microbial biotechnology.

Dual Phase molecular regulation of leaf senescence by cytokinin isoforms

Omar Hasannin*, Risheek Khanna, and Aaron Rashotte; Department of Biological Sciences, Auburn University, Auburn, AL.

Cytokinins are key phytohormones in delaying leaf senescence, with various isoforms contributing to this process. This study focuses on understanding the physiology and the molecular pathways regulated by cytokinins during Dark Induced Senescence in *Arabidopsis* leaves, analyzed at four critical time intervals across this process: 2, 48, 96, and 144 hours. Through comprehensive omics analyses involving treatment with 11 naturally occurring cytokinin bases and conjugated N-glucoside isoforms, we uncover distinct temporal gene regulation patterns, marking a dual-phase cytokinin response in senescence regulation. In the initial phase, base cytokinin forms (iP, tZ, and DHZ) are shown to modulate chlorophyll catabolism, Senescence-Associated Genes, photosynthesis, and senescence-associated transcription factors, particularly at 48 and 96 hours. The late phase reveals that N-glucoside cytokinin forms significantly induced Photosystem II and Light Harvesting Complexes, while suppressing autophagy at 144 hours. These findings highlight previously unknown nuanced roles of different cytokinin isoforms in the senescence process, underlining the need for a detailed analysis to fully comprehend their regulatory mechanisms.

The role of SnRK1 in Development and Stress Response: omics analysis of the rice snrk1 mutants

Maria Clara Faria*, Department of Crop, Soil and Environmental Sciences; Vibha Srivastava, Cell and Molecular Biology Program and Department of Crop, Soil and Environmental Sciences; Rinalda Proko, Cell and Molecular Biology Program; Martin Egan, Cell and Molecular Biology Program and Department of Entomology and Plant Pathology, University of Arkansas, Fayetteville, Arkansas, USA.

SnRK1 (Sucrose Non-Fermenting Related Kinase 1) plays a crucial role in the regulation of cellular metabolism and energy balance in plants. The SnRK1 is the plant ortholog of the evolutionarily conserved SNF1/AMPK protein kinase family and it is activated by starvation and stressful conditions. This study aims to understand the role of SnRK1 in plant development and stress response using rice as a model organism. Rice contains three functional paralogs of SnRK1: SnRK1a (LOC_Os03g17980), SnRK1b (LOC_Os08g37800), and SnRK1c (LOC_Os05g45420). Of these SnRK1a and SnRK1b bear high sequence homology. Based on this, CRISPR/Cas9 mutagenesis was carried out to generate snrk1c single-mutant and snrk1a+b double-mutant lines. The phenotypic analysis of the 7-d-old seedlings showed that SnRK1a/b has a major role in the growth of rice during early vegetative stages. Transcriptomic analysis corroborated these findings, highlighting that SnRK1a/b plays a major role in controlling developmental and stress response during the early vegetative stage. In addition, both mutants showed developmental defects (short plant stature and lower grain yield) and higher susceptibility to *Magnaporthe oryzea*, which causes rice blast disease. Correlating with the phenotypic assessment, our proteomic analysis showed that snrk1a/b mutants exhibited downregulation of defense and catabolic pathways, but upregulation of anabolic processes (governed by Target of Rapamycin

(TOR) signaling). Further, our “omics” analysis reveals that SnRK1 plays a crucial role in stress response by regulating genes and proteins involved in hormone signaling, heat shock proteins, carbohydrate metabolism, glutathione metabolism, and MAPK pathway. This suggests that certain proteins within these pathways could be the potential targets of SnRK1. Overall, this research provides a comprehensive understanding of SnRK1's role in regulating energy homeostasis in the plant by modulating metabolic processes to ensure the plant’s survival and environment adaptation.

Galactolipids Regulate Systemic Immunity In Plants

Tatsushi Kurokawa[1]*, Ruiying Liu[2], Yu Keshun[1], Wendy Havens[1], Pradeep Kachroo[1], Aardra Kachroo[1]; [1]Department of Plant Pathology, University of Kentucky; [2]North Dakota State University

Systemic acquired resistance (SAR) is a unique form of long-lasting systemic immunity that provides protection against a broad-spectrum of pathogens in plants. SAR involves the generation of a mobile signal at the primary infected site, which upon translocation to the distal tissues, prepares the plant to better resist future infections. Multiple, chemically diverse SAR inducers that have been identified to-date. The phosphorylated sugar derivative, glycerol-3-phosphate (G3P) and C9 dicarboxylic acid, azelaic acid (AzA) are two such SAR inducers. AzA, which acts upstream of G3P and confers SAR by increasing G3P levels, is generated upon the hydrolysis of C18 unsaturated fatty acids present specifically on the galactolipids, mono- and di-galactosyl-diacylglycerol (MGDG and DGDG). MGDG and DGDG are major components of chloroplast membranes and therefore the most abundant lipids in plants. Previous studies have shown that mutants deficient in MGDG (mgd1) or DGDG (dgd1) synthesis, are impaired in SAR as well as photosynthetic activity. In addition, the dgd1 mutant is defective in gene expression responsive to another important SAR activator, salicylic acid (SA). Notably, transgenic expression of a bacterial glucosyltransferase (GT) in the dgd1 background, which introduces glucose instead of galactose into the membrane lipids partially restores photosynthetic functions, but not SAR functions. Using genetics, biochemical and molecular analyses, my work further elucidates the importance of the DGDG galactolipid in modulating various aspects of the plant’s systemic immune response including generation and systemic movement of multiple SAR inducers.

A Reverse Genetics Approach to Identifying JAGN1 function in *Arabidopsis thaliana*

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

To prevent a collapse in the global food system, it is critical to understand the molecular mechanisms plants use to respond to biotic and abiotic stressors. The role of the unfolded protein response (UPR) elicited by endoplasmic reticulum (ER) stress is particularly important as the UPR plays the central role in plant stress responses to elevated temperatures, drought, or pathogens. UPR alters gene expression and post-transcriptional responses to counteract cellular damage incurred by stress-induced misfolded protein accumulation. Jagunal Homolog 1 (JAGN1) gene in humans is documented as an important player in ER stress and immunity. Mutations in this gene cause severe congenital neutropenia, a life-threatening immunodeficiency. However, the ortholog gene in plants has yet to be studied. To identify the function of this protein in *Arabidopsis*

thaliana, I have used the only available T-DNA insertion line within this gene to perform ER stress and pathogen infection assays. Interestingly, the plant line displays an over expression phenotype and has thus been named atjagn1-OX. Here I will present my work using atjagn1-OX to characterize the function of JAGN1 in *Arabidopsis* thaliana following stress and the subsequent production of an artificial microRNA line to silence the JAGN1 expression.

Salt-Stress Accelerated Leaf Senescence is Delayed by N-conjugated trans-Zeatin type Cytokinin forms in *Arabidopsis thaliana*

Risheek R. Khanna[1]*, Omar Hasannin[1], Malsha Thennakoon[1], Martin Černý[2], and Aaron M. Rashotte[1]; [1]Rouse Life Sciences, Department of Biological Sciences, Auburn University, Auburn, AL 36849 USA; [2]Department of Molecular Biology and Radiobiology, Faculty of AgriSciences, Mendel University in Brno, Zemedelska 1, CZ-61300, Brno, Czech Republic

Exposure to salt stress reduces photosynthesis performance resulting in accelerated leaf senescence. The plant hormone Cytokinin (CK) can function physiologically to retain photosynthetic performance and pigment content resulting in delayed natural leaf senescence. trans-Zeatin (tZ) is the most well-studied CK-type in *Arabidopsis thaliana*, yet the modified N-glucosylated (tZNGs: tZ7G, tZ9G) that endogenously exist at more than 50-fold the base tZ form remain to be studied for their functional roles in leaf senescence. Here we use modified dark-induced leaf senescence CK bioassay to examine salinity exposure senescence effects in relation to tZ, tZ7G, and tZ9G treatment for photosynthesis functionality and transcriptome and proteome changes. Application of all CK forms maintained PSII efficiency (Fv/Fm) and higher chlorophyll levels compared to salt only treatment. Differentially expressed genes (DEGs) treated by tZ, tZ7G, and tZ9G under salt-treated and developmental leaf senescence were identified using RNA-Seq analysis. Both tZNGs exhibited a delayed regulation of gene expression, with most DEGs occurring at 72h after treatment, in comparison to the tZ base that immediately affected DEGs. Gene Ontology (GO) enrichment analysis revealed DEGs from tZNGs treatments were involved in CK signaling and photosystem related biological processes. In contrast to the delayed numbers of DEGs in the transcriptome for tZNGs, proteomic analysis revealed no delay of changes between tZNGs and tZ for numbers of differentially abundant proteins (DAPs), which were identified as early-responsive at 2h in salt-treated and developmental leaf senescence. tZNG and tZ regulated DAPs showed significant enrichment of chloroplast- and senescence-related BPs in GO analysis. Overall, this study indicates functional activity of tZNGs in delaying salt-treated *Arabidopsis* leaf senescence and highlights unique transcript and proteome regulation patters of tZNGs compared to tZ.

Concurrent Session 2A

Sulfur Mutant Profiling of *Arabidopsis thaliana* on Interaction with *Pseudomonas syringae*

Binoop Mohan[1]*, and Shahid Mukhtar[1,2,3] , [1]Department of Biology, 464 Campbell Hall, 1300 University Boulevard, University of Alabama at Birmingham, Alabama 35294, USA; [2]Nutrition Obesity Research Center, 1675 University Boulevard, University of Alabama at Birmingham, Birmingham, AL 35294, USA; [3]Department of Surgery, 1808 7th Ave S, University of Alabama at Birmingham, Birmingham, AL 35294, USA

Sulfur can play a crucial role when it comes towards plant pathogen interaction. The role and mechanism of sulfur in plants and metabolic pathway involved are clearly understood but its role in plant pathogen interaction in unclear. This work initially started with analysis of transcriptome data providing role of sulfur related genes in pathogen interaction. The sulfur mutants will therefore provide broader understanding resistance and susceptibility of these mutants against pathogen. Transcriptome data provided clues towards the differentially expressed genes in wild type of *Arabidopsis* Col-0 at 24-hour interval till 72 hours and the selected genes were used to construct a mutant strain to see its activity when infiltrated with the pathogen. Our approach was to unravel the molecular and physiological aspects of the plant-pathogen interaction across the *Arabidopsis* mutants and compare it with the wild type of *Arabidopsis* Col-0. The work was designed to include the pathogen assay by infiltrating the plants with *Pseudomonas syringae* pv. tomato DC3000 and HrcC-. The ROS assay and DAB staining will be done to estimate the response of the mutants to pathogen infection and for DAB we use *Pseudomonas syringae* effector AvrRpm1 strain for the infection. For molecular analysis, we used the selected transcription factors and did a gene expression analysis from samples infected with *Pseudomonas syringae* pv. tomato DC3000 and HrcC- at 48 hours' time. In conclusion, Sulfur mutants play a strong and influential role in plant-microbe interaction and the supporting molecular and physiological data support the interaction kinetics.

Investigate the effects of diffusible signals from plant growth-promoting bacterium, *Azospirillum brasilense*, on rice at developmental and molecular levels.

Samuel Hoggard*, Matthew Calhoun, Mary Galloway, Hunter Price, Carli Wattigney, Arijit Mukherjee Department of Biology, University of Central Arkansas, Conway, AR 72035.

Plants form associations with beneficial microbes, including arbuscular mycorrhiza (AM), rhizobia, and plant growth-promoting bacteria (PGPB). In these associations, the host plants benefit from improved growth in exchange for carbohydrates for the microbe. Studies in legume-rhizobia symbiosis (LRS) and AM symbiosis have shown that a molecular dialogue between the symbiotic partners is required to initiate these interactions. Furthermore, genetic and biochemical studies identified the plant and microbial signals and the host genetic pathways involved in these symbioses. For instance, 'Nod factors' are secreted by rhizobia bacteria during LRS, and 'Myc factors' are secreted by AM fungi during mycorrhizal symbiosis. Interestingly, the direct application of these microbial signals on plants can promote their growth, and naturally, these are already commercialized. The same level of understanding doesn't exist for interactions

between plants and PGPB. One recent study showed that diffusible signals from *Azospirillum brasilense*, a PGPB, stimulated growth in *Arabidopsis thaliana*. We established an experimental system where diffusible signals from *A. brasilense* could promote rice growth. We recently performed an RNA sequencing experiment to identify the transcriptomic changes in rice plants in response to the diffusible signals from *A. brasilense* and identified 2,516 differentially expressed genes (DEGs). We identified plant genes encoding flavonoid synthesis genes, defense responses, receptor kinases, transcription factors, and hormone pathways differentially expressed. Our results suggest the involvement of these genes in the host genetic pathways regulated by the diffusible signals from *A. brasilense*. We also validated the RNA-seq results by confirming the expression pattern of eight DEGs using RT-PCR. In the future, we plan to identify the chemical nature of these microbial signals, which can have important implications for improving agriculture sustainably.

Effects of Simulated Microgravity On Plant Growth Promoting Efficiency of ISS Bacterial Isolates.

David Handy[1]* and Andrew G. Palmer[1,2]; [1]Department of Ocean Engineering and Marine Sciences, [2]Department of Biomedical Engineering and Sciences; Florida Institute of Technology

Traditional agriculture and wild plants rely heavily on microbes to help maintain plant health. These microbes are mostly found in the rhizosphere and are highly diverse. Plants rely on their microbiota for assisting in nutrient uptake, modulating disease resistance, and more. These benefits are made possible by various microbial activities including nutrient solubilization and phytohormone regulation. Such plant-microbial associations could prove beneficial to space agriculture as well, providing food security beyond Earth's atmosphere. The work presented here involves studying International Space Station-derived plant microbes and their efficiency in supporting plant growth in simulated microgravity. Previous studies of the plant microbiome on ISS has revealed slight differences in the species present between ISS and ground controls. We hypothesize that changes in PGP phenotypic expression may be the cause of these discrepancies as plants prioritize microbes performing useful phenotypes.

Investigating the concentrations and role of *Chlamydomonas*-derived riboflavin and its degradation products

Ryan Quick[1]*, Kirstin Cutshaw[2], Andrew G. Palmer[1,2]; [1]Department of Biomedical and Chemical Engineering and Sciences, [2]Department of Ocean Engineering and Marine Sciences; Florida Institute of Technology

Chlamydomonas reinhardtii is a common species within many soil systems and is a model organism for algae studies. Understanding how it communicates with other microorganisms is paramount to understanding how it can be utilized for control and generally understanding its niche within the environment. In plant-microbial associations, riboflavin and its degradation products have been implicated as a potential redox signaling molecule and/or antioxidant. Prior studies have suggested that riboflavin exuded by the *C. reinhardtii* may similarly impact the activity of microbial populations in soil through a variety of possible mechanisms. One of these

mechanisms includes quenching Gram-negative bacterial quorums which could influence mutualistic and pathogenic symbioses. Here I explore the possibility of *C. reinhardtii* to release sufficient riboflavin into the environment utilizing an HPLC technique to see if enough riboflavin is produced to counter the photolabile nature of the molecule.

12-oxophytodienoic Acid: A Mobile Signal of Induced Systemic Resistance

Simrandeep Kaur*, Ashna Adhikari, Sang-Wook Park, Affiliations: Department of Plant Pathology, Auburn University.

In plants, growth and defense trade-offs have been a major pitfall for the genetic engineering of disease resistance. To understand if and how plants co-ordinate growth and defense responses, we exploit the role and mode of plant growth-promoting rhizobacteria (PGPR)-mediated 'induced systemic resistance (ISR),' a phenomenon capable of priming broad-spectrum durable disease resistances without the usually accompanied growth penalty. During our recent studies on the effect of PGPR in plant drought responses, we identified several PGPR strains that cannot enhance drought tolerance but - instead - are able to develop ISR against various microbial pathogens. These ISR inducers demonstrated differential gene expressions compared to ISR non-inducers, drawing new insights that imply 12-oxo-phytodienoic acid (OPDA) as an ISR signal. The expression of a subset of marker genes in Arabidopsis and cotton plants during ISR activations indicate that: i) Local root tissues induce and activate OPDA and JA-Ile signaling, as well as trigger SA signaling but not SA productions. In the meantime, ii) Systemic leaf tissues activate OPDA (but not JA- Ile) and SA signaling. Nonetheless, leaf tissues do not de novo synthesize OPDA, JA-Ile, and SA. These results together hint that OPDA produced in root tissues must travel via phloem cells to leaf tissues, where they activate OPDA signaling (but not converted to JA/JA-Ile). iii) In support of this scenario, we observed that OPDA (but not JA) can upregulate SA signaling marker genes via NPR1- dependent pathway (i.e., via GRX transcriptional regulators and TGA transcriptional factors). Together, we propose that ISR requires the accumulation of OPDA in local tissues that moves to systemic tissues where it activates GRX-mediated OPDA and SA response defense gene expression pathways.

Concurrent Session 2B

Conversion of Martian Regolith Simulants to Soil via the Legume-Rhizobia Symbiosis

Hayley Ernest[1]* and Andrew G. Palmer[1,2]; [1]Department of Ocean Engineering and Marine Sciences, [2]Department of Biomedical Engineering and Sciences; Florida Institute of Technology

Bio-regenerative life support (BRLS), the use of plants and other photosynthetic organisms to supplement life support and provide food security, is a mission critical system for future off-world expeditions and settlements. In order for a BRLS to remain sustainable in an off-world environment it must leverage existing (*in situ*) resources on site, thereby reducing the dependance on resupply missions. For example, the use of lunar and/or Martian regolith as a substrate for agriculture has received considerable attention. However, Martian regolith lacks many of the macro- and micro- nutrients essential for plants. As a result, cultivating in these conditions limits healthy plant growth and development. Considering that the organic nitrogen and carbon necessary for plants are not available in sufficient quantities on either the Moon or Mars, we must find sustainable methods to introduce them into the regolith in a sustainable fashion. Members of the Fabaceae family form mutualistic symbioses with nitrogen-fixing bacteria of the paraphyletic group, known as Rhizobia. *Trifolium repens* (clover), is a well-established member of this family that requires minimal resources and has historically been used in agriculture to increase soil fertility due to this critical plant-microbial interaction. We hypothesize that growing clover inoculated with a compatible species of rhizobia and tilling it into Mars regolith simulant over multiple growth periods could sustainably alter regolith composition. This process would introduce nitrogen and carbon into this system. This BRLS addition could be a simple method for improving the conditions of Martian regolith as a substrate for plant growth. We observed the impact of clover tilling on efforts to grow an edible crop in Martian regolith simulant as preliminary support for this approach.

Rhizobial evasion of terminal differentiation: Temporal/spatial dynamics of *Sinorhizobium meliloti* reproductive population growth in alfalfa nodules and implications for cheating by poor N2 fixers

Katherine M. Bilodeau*, Darryl J. Trickey, Amanpreet K. Brar, Grace Wisdom, and Kathryn M. Jones; Department of Biological Science, Florida State University, Tallahassee, FL

Biologically-available nitrogen is a limiting factor in the biosphere. Many legume plants have overcome this limitation by hosting N2-fixing symbiotic bacteria within root nodules. Some legumes, such as *Medicago sativa* (alfalfa), form indeterminate nodules that induce a terminal differentiation program on the intracellular, N2-fixing portion of the *Sinorhizobium meliloti* symbiont population. Efficient N2-fixing strains often have poor persistence in the environment, limiting the benefit that efficient N2-fixing *S. meliloti* strains provide perennial alfalfa over multiple years. Our goals are to determine 1) How *S. meliloti* uses symbiosis with alfalfa to take advantage of host resources, amplify its population, and recolonize the soil, and 2) How poor N2-fixing *S. meliloti* strains (Fix-) may exploit this interaction at the expense of both the plant and

good N2-fixing strains. Our results show that as long as a Fix- strain can produce the correct signals for invasion of the nodule primordium, it can amplify its population in the host nodule to the same extent an an N2-fixing strain. Surprisingly, the amplified S. meliloti population can remain viable within both N2-fixing and Fix- nodules for >9 months. Furthermore, we have found that expression of the *S. meliloti* divJ gene, which serves as a reporter of rhizobial cell division, steadily increases over time throughout nodule tissue as older nodules senesce. These results suggest that good symbionts (N2-fixing strains) and poor symbionts (Fix- strains) have similar ability to exploit host plant resources as long as they are equally successful in colonization of host nodules.

Dissecting the functions of *Salmonella enterica* effectors in plant infection

Jill Presel*[1,2], Jonathon Audia[3], and Tuan Tran[2]; [1]Department of Biology, University of South Alabama, Mobile AL 36688; [2]Department of Leadership and Teacher Education, University of South Alabama, Mobile AL 36688; [3]Department of Microbiology and Immunology, University of South Alabama, Mobile AL 36688

Salmonella enterica serovar Typhimurium are primarily recognized as pathogens associated with human food-borne illness. *Salmonella* also can colonize plant tissue effectively. The pathogen relies on type III secretion systems (T3SS) to infect mammals and plants successfully and counteract the immune response. Understanding the molecular mechanisms underlying *Salmonella's* interactions with plants is essential for developing effective strategies to reduce its impact on human health and agriculture. Effector proteins, known for their role in modulating host-pathogen interactions, have been implicated in the virulence and pathogenicity of *Salmonella*. However, their specific functions and contributions to *Salmonella's* interactions with plants remain largely unknown. Here, we aim to investigate the function of *Salmonella's* effector proteins during its colonization of plants. By evaluating plant response to effector mutants and effector proteins heterologous expressed in plant tissue, we identified a number of effectors that either suppressed or triggered plant defense responses in leaf tissue. Gaining a better understanding of *Salmonella's* effector proteins and their interactions with plants will lead to essential strategies to aid in the prevention of food-borne illness through the consumption of contaminated produce.

Evaluating the Use of Aquatic Plants in Martian Agriculture

Sergio Solano[1] and Andrew G. Palmer[1,2]; [1]Department of Biomedical Engineering and Sciences, [2]Department of Ocean Engineering and Marine Sciences; Florida Institute of Technology

Human missions to Mars are expected to be costly due to the amount of supplies needed to support such missions. To offset some of these costs and provide steady production of supplies, in-situ resource utilization, is a necessity for humans on Mars. One application is in space agriculture, wherein Martian regolith can be used to provide nutrition to plants that can be harvested. *Lemna minor*, or duckweed, is of interest due to its high nutritional value, bioremediation ability, and feasible growth. This study aims to evaluate the use of duckweed for Martian agriculture by investigating its response to growing with different Martian regolith

simulants. These simulants are not always geologically uniform, where some constituents are insoluble while others dissolve and provide plants with nutrition in a eutrophication-like process. Growth of plants is tracked alongside metabolic responses and expression of genes involved with nutrient deficiency to determine the efficiency of duckweed as a Martian crop.

General Session 3

Elimination of Pungency in Allotetraploid *Brassica juncea* Through Gene Editing of the Multicopy Myrosinase Gene Family

Loren Rivera Vega; Pairwise, Durham, NC 27701

We identified a novel opportunity to enhance the availability of nutritious, fresh leafy greens for human consumption. Here, we demonstrated the efficacy of disarming the 'mustard bomb' reaction in reducing pungency upon the mastication of fresh tissue—a major source of unpleasant flavor and/or odor in leafy Brassica. Using gene-specific mutagenesis via CRISPR-Cas12a, we created knockouts of 17 functional copies of the type-I myrosinase multigene family in tetraploid Brassica juncea. Our greenhouse and field trials demonstrated, via sensory and biochemical analyses, a stable reduction in pungency in edited plants across multiple environments. Collectively, these efforts provided a compelling path toward boosting the human consumption of nutrient-dense, fresh, leafy green vegetables. Data from consumers who tried these salads was overwhelmingly positive. Pairwise introduced into food service this leafy green as a new, healthy option called Conscious™ Greens.

General Session 4

Plant-based Recovery of Rare Earth Elements from Secondary Waste Sources

Colleen J. Doherty*, Edmaritz Hernandez, Pagan Kanjana Laosuntisuk, Michael Kudenov, Allison Haynes, Alex Harris

The emerging significance of Rare Earth Elements (REEs) stems from their critical applications in modern technology and growing relevance in biological systems. Due to their widespread use in high-tech devices essential for decarbonizing the economy, including magnet and battery technologies, REEs are central to economic and strategic concerns. However, increasing awareness of REE functions in biological systems raises concerns about potential environmental impacts from their distribution. To address this, we're employing plant systems to investigate the biological effects of REEs and to develop plant-based solutions for REE recovery from waste streams like Acid Mine Drainage and Fly Ash. We have identified plants that thrive in these waste streams and accumulate REEs. We are using these plants to identify mechanisms of REE uptake. In addition, we are optimizing the growth and propagation of these plants to develop carbon-friendly systems for REE recovery.

Investigating Thiamine Metabolite Damage and Repair: Role of the TenA Domain in *Arabidopsis* TH2 Protein

Ghulam Hasnain, Associate Professor of Biology University of North Georgia

Metabolite damage and repair is a relatively new concept in metabolic research. Enzymes, often deemed highly specific, can make mistakes, and build toxic products. Genetic and genomic evidence across various organisms has highlighted a network of conserved enzymes involved in repairing damaged metabolites. Comparative genomics is a powerful tool for predicting genes involved in metabolite repair. An illustrative example is the identification of a Nudix enzyme responsible for detoxifying degraded thiamin diphosphate, a vital cofactor for key enzymes. Thiamin diphospho kinase (TDPK) can mistakenly phosphorylate damaged thiamin forms like oxy- and oxothiamin, leading to enzyme inactivation. A Nudix enzyme was found to selectively remove diphosphates from these damaged thiamin forms, preventing enzyme inactivation. However, the mechanisms underlying how plants deal with damaged thiamin products remain unknown. The *Arabidopsis* TH2/At5g32470 protein is a fusion of the HAD phosphatase domain with a TenA domain associated with thiamin metabolism. We hypothesize that the TenA_C domain of At5g32470 may possess thiaminase activity against thiamin breakdown products, enabling the salvage of degraded thiamin forms like oxothiamin and oxythiamin.

Undergraduate Poster Presentation Abstracts

U01 - Evaluating Quorum sensing in light-sensitive *Chlamydomonas reinhardtii* isolates

Trevor Mello[1]* and Andrew G. Palmer[1,2,3]; [1]Department of Ocean Engineering and Marine Sciences, [2]Department of Biomedical Engineering and Sciences, [3]Department of Chemical Engineering and Sciences; Florida Institute of Technology

Chlamydomonas reinhardtii is one of the few unicellular eukaryotes observed to display cell density-dependent phenotypic switching, the phenomenon known as quorum sensing (QS). *Chlamydomonas reinhardtii* isolates have high degrees of genetic variance, which we hypothesize can be leveraged for the identification of molecular elements associated with QS in this model unicellular photosynthetic eukaryote. In the present study, I am investigating the QS responses of two strains of *C. reinhardtii*: 4886 (2137 mt+) and 4889 (21gr mt+). These strains are particularly novel as they are light-sensitive and have been maintained in the dark on acetate-supplemented media since the 1980s. In this experiment, I have observed the impact of photosensitivity and the associated metabolic adaptations on the ability of these isolates of *C. reinhardtii* to QS. These 'unusual' strains of *C. reinhardtii* could provide insight into the relationship, if any, between primary production (photosynthesis) and QS. *Chlamydomonas* QS could ultimately provide useful insight into the search for other QS phenotypes among other eukaryotes.

U02 - Investigating transposition activity: Functional analysis of the *Zea mays* Mariner 20 element

Keith Nelson* and C. Nathan Hancock; Department of Biological, Environmental, and Earth Sciences; University of South Carolina Aiken

Plant transposons are DNA sequences capable of moving to a new location within the genome. Their mobility contributes to genetic diversity by altering gene function or regulation and they have been harnessed as tools for plant genome modification. The goal of this investigation was to study the transposition mechanisms of a Mariner-like element from *Zea mays* called ZmMar20, that showed evidence of recent transposition. Our hypothesis was that ZmMar20's transposition behavior would be similar to the related OsMar5 element from Oryza sativa, which exhibits up to 8 bp footprints and overexpression inhibition. Utilizing yeast transposition assays, we found that Zmar20 excision sites also showed footprints of up to 8 bp. However, increasing ZmMar20 Transposase expression using a high copy plasmid resulted in higher transposition, suggesting that overexpression inhibition was not occurring. To test if the Transposase was not efficiently entering the nucleus, we tested the effect of addition of a strong Nuclear Localization Signal (NLS) to N or C-terminal of the protein. Addition of the NLS to the C-terminal resulted in a significant increase in transposition, consistent with protein localization regulating transposase activity. The outcomes of this study advance our knowledge of Mariner element mobility and have implications for transposon based genetic engineering.

U03 - Shade avoidance response in *Arabidopsis* abscisic acid insensitive/absent mutants

Brett Harris* and Brian Kim ByungHoon; Department of Biology; University of North Alabama

Shade avoidance response is the developmental reprogramming in response to environmental light quality to avoid shade created by neighboring vegetation. This includes elongational growth, altered flowering time, and increased leaf elevation angle. Plant phytochromes have the ability to perceive nearby plants that compete for the sunlight by sensing the ratios of far-red (FR; λ = 730 nm) and red light (R; λ = 660 nm). In a dense vegetation, more FR light is present than R light due to consumption of R light by chlorophylls, reducing the ratio of R:FR light, which triggers the shade avoidance response. The phytohormone abscisic acid (ABA) regulates the growth and development as well as stress responses in plants. Interestingly, there have been some indications that ABA may also affect the shade avoidance response. However, it is not well-understood how shade avoidance response is affected under environmental stress conditions when the ABA content is high. By using a new computational approach, we were able to measure the leaf elevation angles of Arabidopsis plants in response to low R:FR light conditions. Previously, we found that a high concentration of ABA suppresses the leaf elevation angles under this light condition. Hear we report that the leaf elevation angles in the mutant plants that have impaired ABA production (aba1) or insensitive to ABA (abi4 and abi5) were also affected under a low R:FR light condition, suggesting that the stress hormone ABA plays an important role in leaf elevation.

U04 - Genetic variation in the model eukaryote Chlamydomonas reinhardtii provides potential tools for understanding quorum sensing.

McKenna Taylor[1]*, Lucy Turner[2], Josh Ahrens[2], and Andrew G. Palmer[2,3,4]; [1]Department of Aerospace, Physics, and Space Sciences, [2]Department of Ocean Engineering and Marine Sciences, [3]Department of Biomedical Engineering and Sciences, [4]Department of Chemical Engineering and Sciences; Florida Institute of Technology

Quorum Sensing (QS) is a phenomenon in which communication between cells causes a phenotypic switch in the population due to the cell density. In the unicellular photosynthetic model eukaryote *Chlamydomonas reinhardtii* (strain cc124), QS increases the swimming speed of the cells presumably to encourage migration prior to the onset of nutrient scarcity. *C. reinhardtii* is the first evidence for QS among eukaryotes outside of fungi and broadens the potential for this phenomenon in the microbial world. *C. reinhardtii* displays significant phenotypic variation among environmental isolates which may impact QS. Such variation could be useful in identifying signals and molecular pathways associated with this process. In the present study we are investigating the QS-associated behavior of the cc408 and cc3269 lines to determine if these *C. reinhardtii* isolates display quorum sensing behavior and how this compares to the common lab isolated strain.

U05 - Radiation Resistance and Recovery in *Anabaena cylindrica*

Indigo Boggs[1]* and Andrew G. Palmer[2,3]; [1]Department of Aerospace, Physics, and Space Sciences, [2]Department of Ocean Engineering and Marine Sciences, [3]Department of Biomedical Engineering and Sciences, [4]Department of Chemical Engineering and Sciences; Florida Institute of Technology

Radiation protection is a crucial consideration in space exploration and settlement. Among the various types is ultraviolet radiation (UVR) which can have significantly deleterious effects on biological organisms. Prior to the formation of the ozone layer, Earth was affected by these rays, along with the different bacterial species that were present significantly impacting the distribution and nature of life across Earth. Members of the phylum Cyanophyta (Cyanobacteria), among the oldest living organisms, evolved to grow under such heavily irradiated conditions. One common strategy among members of this phylum is the production of dense 'biomats' which can protect the bacteria from radiation while also providing a nutrient rich environment. Such biomats are crucial to the pioneering role members of this genus play in regenerating ecosystems and could be of significant benefit to space agriculture efforts. We hypothesize that the cyanobacteria *Anabaena cylindrica* has natural resistance and recovery systems to withstand UV radiation and still form biomats which could be used to support plant growth in regolith-based agriculture. While *A. cylindrica* resistance to UV-A and B radiation is well documented, less is known about the resistance to UV-C. We hypothesize that following exposure to UV-C, *A. cylindrica* retains its capacity to produce these biomats in the present study we provide preliminary data on UV-C resistance and recovery via. If *A. cylindrica* can withstand these UV-C rays, it is a promising species for future space colonies which could use it to fertilize regolith found on different planets.

U06 - Sustainable lunar agriculture: recycling treated regolith for enhanced plant growth on the Moon

Ishan Kundel*, Borja Barbero Barcenilla, and Dorothy E. Shippen; Department of Biochemistry and Biophysics; Texas A&M University

As humanity endeavors to explore beyond Earth, the cultivation of plants becomes indispensable. Despite progress, much remains uncertain regarding how plants would adapt to such environments. With NASA's initiative to establish a permanent lunar colony by 2050, the ability to cultivate crops on lunar regolith emerges as a vital imperative. We and others previously showed that Arabidopsis thaliana has the capacity to germinate on both authentic and simulated lunar regolith, yet further growth is stunted at the vegetative stage. To enhance plant growth, various soil remediation techniques have been explored, among which exogenous antioxidant treatment has shown promising results. Plants cultivated on this treated regolith simulant exhibited improved survival rates, stalk height, biomass, and germination compared to untreated regolith. In addition to physiological assessments, telomeres, the G-T rich sequences that cap the ends of chromosomes, serve as indicators of plant fitness and proliferation ability. We recently reported telomere shortening in plants grown on antioxidant-treated regolith simulant. To address the challenges posed by lunar regolith, recycling of regolith presents potential benefits including increased organic matter, reduced particle abrasiveness, enhanced water retention, and decreased heavy metal content. In this study, we investigate Arabidopsis thaliana growth on

recycled lunar regolith simulant. Our findings indicate that plants cultivated on recycled regolith simulant exhibit increased germination, stalk height, and biomass compared to controls. Furthermore, these plants demonstrated increased telomere length, approaching levels observed in Earth control plants. We propose that recycling antioxidant treated regolith simulant may offer a more sustainable approach to crop cultivation on lunar colonies, potentially mitigating challenges associated with lunar regolith cultivation.

U07 - Efficacy of Sterilization on lunar and Martian regolith simulants

Davonya Cheek[1]* and Andrew G. Palmer[2,3,4]; [1]Department of Aerospace, Physics, and Space Sciences, [2]Department of Ocean Engineering and Marine Sciences, [3]Department of Biomedical Engineering and Sciences, [4]Department of Chemical Engineering and Sciences; Florida Institute of Technology

Due to the unavailability of authentic Martian and lunar regolith, simulants are heavily relied on for space-related research projects. This includes space agriculture, which is mission-critical for food security and supplemental life support in future off-world settlements. However, terrestrially sourced simulants like these are not sterile, often containing 106 cells/ml or more. Sterilization of regolith simulants is therefore an important consideration in space agriculture research and is the goal of this research. A series of Martian and lunar regolith simulants were autoclaved to determine the efficacy of this process. Survival of microbial species following sterilization attempts was then observed. We attempted this with both moderate and high-volume samples to gauge the efficacy of the process. Additionally, the effects of sterilization and reduction of the microbial load on plants' growth potential after sterilization were evaluated. Understanding what microbes exist in the simulants and how sterilization methods can alter their composition is beneficial in developing Martian and lunar regolith-based agriculture.

U08 - Testing Bacterial Isolates from the ISS for their Plant Growth Promoting Abilities

Kara Smoak[1]*, Aiden Kelly[1]*, Nathan Kawalski[1], Kristina Kowalik[2], and Andrew G. Palmer[2,3,4], [1]Department of Aerospace, Physics, and Space Sciences, [2]Department of Ocean Engineering and Marine Sciences, [3]Department of Biomedical Engineering and Sciences, [4]Department of Chemical Engineering and Sciences; Florida Institute of Technology

In microgravity environments, plants are exposed to a variety of stressors that can impact growth, nutrient acquisition, phytohormones, and susceptibility to disease. To counteract these stressors, plant growth promoting bacteria (PGPB) can be introduced to plants in this environment to serve the same role they do on Earth. During missions beyond Earth, plants need to grow and thrive to serve as a food source for astronauts; PGPB can relieve the challenges that plants face and allow for on-site production of food in these challenging environments. Here we have mined the plant-associated microbiome that has emerged aboard the Intentional Space Station (ISS) to identify potential PGPB with spaceflight experience. The bacteria tested here are passed through a pipeline of four assays: ACC deaminase, indole production, phosphate solubilization, and siderophore production assays. These assays test the bacteria for different traits associated with plant growth promoting phenotypes, like increasing the uptake of

important plant nutrients for example. Positive results, like evident colony growth, large halo sizes, or elevated absorbance values, would indicate the presence of bacteria exhibiting PGPB traits. Once identified, the bacteria can be tested with plants on microgravity simulators to evaluate their efficacy in space-like conditions or in lunar or Martian regolith simulants. The discovery of PGPB has the potential to enhance the growth of agriculture in microgravity environments, like the moon or Mars, which will provide a sustainable food source for future space missions.

U09 - Assembling a Phased, Chromosome-scale Genome for Sabal palmetto

Madison L. Zimmerman[1]*, Huiting Zhang[2] [3], Stephen Ficklin[2], Leslie Goertzen[4], Alex Harkess[5], and C. Nathan Hancock[1]; [1]Department of Biological, Environmental, and Earth Sciences, University of South Carolina Aiken, Aiken, SC 29801, [2]Department of Horticulture, Washington State University, Pullman, WA, 99164, [3]USDA Agricultural Research Service, Wenatchee, WA, USA 98801, [4]Department of Biological Sciences, Auburn University, Auburn, AL, 36849 [5]HudsonAlpha Institute for Biotechnology, Huntsville, AL, 35806

Sabal palmetto, a notable species in the Arecaceae family, is a palm native to the southeastern United States that plays a vital role in the coastal ecosystem. Despite its ecological and cultural significance, little is known about the genetic basis of its unique adaptations and growth characteristics. This project represents the first comprehensive effort to provide a haplotype-phased, chromosomal level, genome of S. palmetto. To do this, a combination of advanced sequencing technologies was used: PacBio HiFi sequencing long reads, Dovetail Omni-C chromosome conformation capture, Illumina shotgun DNA and RNA sequencing. This approach will allow for a complete and high-quality genome. The annotation process is expected to identify genes potentially associated with salt tolerance, drought resistance, and disease resistance; shedding light on the evolutionary adaptations of S. palmetto to its environment. Our study aims to identify expanded gene families in S. palmetto compared to other palms, providing insights into its resilience and versatility. Comparative genomics analyses will also elucidate evolutionary relationships with other monocots, offering a new perspective on palm species diversification. By conducting whole-genome sequencing and annotation, we seek to contribute to phylogenetic studies and future palm research. This work could have applications in horticulture, conservation, and enhancing our understanding of climate change resilience in coastal ecosystems.

U10 - Fiber analysis of plant material and biofertilizers for potential space-based agricultural systems

Trenton Causey[1]*, Emily Soucy[1,2], and Andrew G. Palmer[2,3]; [1]Department of Aerospace, Physics, and Space Sciences, [2]Department of Biomedical Engineering and Sciences, [3]Department of Ocean Engineering and Marine Sciences; Florida Institute of Technology

Distinguishing fiber (cellulose, hemicellulose, lignin) content within plant samples under different experimental treatments can provide insight into how plant structure is impacted by various stressors. Stressors faced by plants in space growth conditions include altered atmospheric pressure, limits on available nutrients, and notable mineralogical differences than those

experienced on Earth. These differences would affect the structure, and thus fiber content, of relevantly stressed plants. In space agriculture applications, this is of particular importance for evaluating edible:inedible biomass ratios. We are currently attempting to optimize the Van Soest gravimetric method of fiber digestion to quantify the hemicellulose, cellulose, and lignin content in plant cells. Following treatment, electron microscopy can be used to confirm potential differences of structural characteristics and bolster confidence in the methodology. However, the Van Soest method is labor and time intensive while also generating a significant amount of hazardous waste chemicals. We propose that quantifying the differences within these fibers using Fourier Transform Infrared Spectroscopy (FTIR), rather than the Van Soest method, could provide similar data in a manner that is more efficient, cost effective, and less wasteful of reagents as well as materials. Taking each of these analysis methods, further examination of fiber content under simulated space-based conditions will further both our knowledge and our ability to successfully cultivate life in environments unlike our own.

U11 - One giant leap for plantkind: exploring the growth of *A. thaliana* in regolith simulants derived from different lunar regions

Emma Dawson*, Cole Schulte*, Max Evans, Ishan Kundel, Borja-Barbero Barcenilla, and Dorothy E. Shippen; Department of Biochemistry and Biophysics; Texas A&M University

Enabling survival beyond Earth's limits is crucial for future extraterrestrial colonization, with plants playing a vital role in generating food, oxygen, and improving human well-being. Recent developments in space cultivation have revealed the successful growth of the model organism, *Arabidopsis thaliana*, in lunar regolith simulant LMS-1. However, plants displayed decreasing size and biomass with continuous propagation. In addition to LMS-1, other lunar regolith simulants are commercially available that replicate specific lunar environments with high fidelity. However, *A. thaliana* growth on these simulants has not been reported. Natural variation offers a potent means to uncover significant traits in biological organisms. In this study, we examine how distinct A. thaliana accessions grow on different lunar regolith simulants. Specifically, we compared the growth of *A. thaliana* accessions Hov-10 (Sweden), Pro-0 (Spain), and Col-0 (US) on Earth soil, and a variety of untreated lunar regolith simulants (LMS-1, LSP-2, and LHS-1), and antioxidant-washed lunar regolith simulants. As expected, diminished germination was observed on all accessions grown on each untreated regolith simulant we tested. Overall enhanced germination was observed on treated LMS-1 regolith compared to LSP-2 and LHS-1. Interestingly, the cold-adapted Hov-10 accession displayed the highest germination percentage across all regolith simulants, with Pro-0 displaying the least growth. This suggests an adaptation by Hov-10 towards lunar growth and a specific metal/nutrient availability in LMS-1 to lead to plant germination. By determining the optimal plant accession and regolith simulant, further studies can focus on uncovering the most favorable conditions for plant growth on the Moon surface.

U12 - Evaluating biomining and biofertilization effects of microbe Anabaena cylindrica in extraterrestrial regolith simulants

Haley K. Murphy[1,2]* and Andrew G. Palmer[2,3,4]; [1]Department of Aerospace, Physics, and Space Sciences, [2]Department of Biomedical Engineering and Sciences, [3]Department of Ocean Engineering and Marine Sciences, [4]Department of Chemical Engineering and Sciences; Florida Institute of Technology

As interest in space exploration increases, plans to visit and inhabit nearby extraterrestrial bodies are in progress. New challenges emerge in supporting the crews present on these future missions. Establishing a sustainable, on-site food supply will foster independence of settlers from the expensive, potentially failed, or delayed shipping of materials. In-situ resource utilization (ISRU) leverages resources available on site to support inhabitants with crop production. Regolith will be found on any rocky extraterrestrial body and is a promising candidate for a plant growth substrate with modification. Martian and lunar regolith will require supplementation of organic material and increased bioavailability of present inorganics. On Earth, autotrophic pioneering species of cyanobacteria simultaneously fix carbon and nitrogen. In space applications, they have survived microgravity, desiccation, and UV radiation. We hypothesize that these activities and traits will be useful on Mars and the moon to achieve deposition of bioavailable organic nutrients. The filamentous cyanobacteria *Anabaena cylindrica* is capable of growth in both Martian and lunar regolith simulants. In the current study, the species is introduced to extraterrestrial regolith simulants to quantify microbial growth, inorganic interactions, and nitrogen fixation to determine its ability to improve plant growth. Our findings impact understanding of how terrestrial ecology may be employed to support off-world settlement.

U13 - Metabolization of Bacterial quorum sensing signals by the model eukaryote *Chlamydomonas reinhardtii*

Adam Bach[1,2]*, Alan Brown[3], and Andrew G. Palmer[1,2,3]; [1]Department of Biomedical Engineering and Sciences, [2]Department of Ocean Engineering and Sciences, [3]Department of Chemistry and Chemical Engineering; Florida Institute of Technology

Numerous species of prokaryotes regulate symbiotic and pathogenic relationships, exhibit phenotypic switching based on cell density, and other chemical processes (i.e., metabolism) using a method known as quorum sensing (QS). In Gram-negative bacteria, QS allows bacteria to coordinate specific behaviors that typically use N-acyl L-homoserine lactones (AHLs) as a signal. AHLs are made up of an L-homoserine head group, and a variable length acyl tail which is species dependent. Preliminary evidence suggests that the model unicellular eukaryote, *Chlamydomonas reinhardtii* may be able to metabolize and incorporate AHLs thus altering cell densities associated with QS in Gram-negative bacteria. Using a combination of High-performance liquid chromatography (HPLC), Nuclear Magnetic Resonance (NMR), as well as isotopically enriched AHLs we will attempt to observe metabolization and specific incorporation of these bacterial QS signals into the metabolome of *C. reinhardtii*. Our findings could significantly impact our understanding of the cell densities required for QS in both bulk soil as well as around the rhizosphere.

U14 - Assessment of quorum sensing behavior in Melbourne, Florida-isolated *Chlamydomonas reinhardtii* strain

Lucy Turner*[1], Josh Ahrens[1], Andrew G. Palmer[1,2,3]; [1]Department of Ocean Engineering and Marine Sciences, [2]Department of Biomedical Engineering and Sciences, [3]Department of Chemical Engineering and Sciences; Florida Institute of Technology

Density-dependent phenotypic alteration within a cell population is a phenomenon attributed to the cell-cell signaling process known as quorum sensing (QS). The ability of prokaryotic organisms to QS has been extensively studied, but little is known about QS behaviors in unicellular eukaryotes. Understanding the mechanism of QS in eukaryotes can lead to advancements in agriculture, cosmetics, food and beverage, and treatment of fungal and yeast infections. *Chlamydomonas reinhardtii* (strain cc124), a unicellular eukaryotic model organism isolated from Amherst, Massachusetts, displays QS by adjusting swimming speed contingent on cell density. In this experiment, we explored the possibility of QS behavior in *Chlamydomonas reinhardtii* (strain cc2343), isolated from Melbourne, Florida. *Chlamydomonas reinhardtii* has incredible genetic variability between environmental isolates; therefore, different genetic adaptations are expected between cc124 and cc2343. Comparison of the two strains may assist in identifying the QS mechanism in *Chlamydomonas reinhardtii,* which would help us understand more about QS in eukaryotic organisms. Here, I will discuss the behavior of cc2343 in response to cell density.

U15 - Phytoremediation by Carnivorous Plants in Regolith

Emily Soucy*[1,2] and Andrew G. Palmer[2,3]; [1]Department of Aerospace, Physics, and Space Sciences, [2]Department of Biomedical Engineering and Sciences, [3]Department of Ocean Engineering and Sciences; Florida Institute of Technology

Heavy metals found in Martian and lunar regolith present serious concerns for regolith-based agriculture as a means to ensure food security off-world. The likelihood of heavy metal accumulation in crops grown in regolith will require substrate pre-treatments prior to their use. Phytoremediation presents a solution which may eliminate potential toxins and condition the regolith, helping convert it into soil. Carnivorous plants, such *as Utricularia gibba,* offer a partial remedy. In addition to being well-equipped to oligotrophic and low nitrogen environments, *U. gibba* is capable of accumulating Pb^{2+}, Mn^{2+}, Cr^{3+}, Cu^{2+}, and Zn^{2+}. Of these elements, Cr^{3+} and Mn^{2+} are found in both lunar and Martian regoliths in significantly higher concentrations than on Earth, thus posing a notable concern to plant growth and human health. We hypothesize that *U. gibba* will be able to successfully capture heavy metals from lunar and Martian regolith, providing support for the growth of edible plants once the regolith has been properly processed. In the present study, we explore the growth of *U. gibba* in regolith simulants and their capacity for metal uptake has been investigated.

U16 - Optimization of the Pong Transposase Protein

Zara Lacera*, Madison Zimmerman, and C. Nathan Hancock
Department of Biological, Environmental, and Earth Sciences, University of South Carolina Aiken, Aiken, SC

Transposable elements (TEs) are segments of DNA that "jump" within an organism's genome. They cause mutations that result in genetic diversity and facilitate evolution. TEs are also being developed into genome editing tools because they can be inserted into targeted locations. We study the transposition behavior of mPing, an active TE from rice that is being developed for targeted insertion in plants. This element is mobilized by the ORF1 and Transposase (TPase) proteins from the related Pong element. We previously developed a hyperactive version of Pong TPase, named 1C-C12. In addition to mutations that disrupt a nuclear localization signal, this version of Pong TPase has two mutations V422E and A456V that both increase mPing mobilization in yeast. Our goal was to make a fused 1C-C12 TPase and ORF1 construct that could be expressed from a single promoter, to improve the efficiency of mPing-based genome manipulation in plants. We cloned a T2A peptide between our hyperactive TPase and the hyperactive ORF1 SC1 ONE version of ORF1 using bridge fusion PCR to link the ORF1 and TPase genes. This fragment was Gateway cloned into the pAG425 GAL plasmid to allow for testing of the construct with the yeast transposition assay. If this T2A construct shows improvement in yeast, we will test it using our *Arabidopsis* transposition assay.

U17 - **Unraveling the mysteries of plant defense hormone: Methyl Jasmonate signaling.**

Morgan E Wynn*, Daniel Rincon Diaz, Ansul Lokdarshi; Department of Biology, Valdosta State University, GA 31698

Understanding how plants defend themselves against various stressors, such as pathogens and herbivores, is critical to a sustainable food supply. Despite lacking a defined immune system, plants have developed a remarkable array of structural and biochemical defenses, including one of the most versatile plant hormones, Methyl Jasmonate (MeJA), which is actively engaged in defense against different types of abiotic and biotic stresses. While studies on MeJA-mediated defense responses have predominantly focused on transcriptional management (DNA to messenger RNA), the details of much faster regulation, translational control (messenger RNA to protein), remain underresearched. My work investigates how a cytosolic serine/threonine protein kinase, General Control Nonderepressible 2 (GCN2), phosphorylates its target, eukaryotic initiation factor 2 (eIF2) alpha in response to MeJA in the plant model, *Arabidopsis thaliana*. The GCN2-eIF2alpha module is a highly conserved eukaryotic stress response mode for regulating translation in all eukaryotes, generally referred as the integrated stress response. Utilizing immunoblotting, we show that MeJA requires light (therefore, chloroplast function) to activate the cytosolic GCN2-eIF2alpha module in a dose-dependent manner. Furthermore, a homozygous knock-out mutant for the GCN2 gene displays reduced growth under prolonged MeJA stress, suggesting GCN2-eIF2alpha as an essential component of the MeJA signaling pathway. Ongoing investigations are focused on understanding the biochemical activation mechanisms of the GCN2 protein and the effect of MeJA stress on global translation using polysome profiling and next-generation sequencing techniques such as ribosome footprinting. These advances will provide

deeper understanding of MeJA signaling in plants and aid in the future development of plants with better stress resilience/adaptation.

U18 - Does the NHP6A protein affect *mPing* transposition in yeast?

Kaili Renken[1] and C. Nathan Hancock[1]; [1]Department of Biological, Environmental, and Earth Sciences, University of South Carolina Aiken, Aiken, SC

DNA transposable elements are found in virtually all eukaryotic organisms and can jump around the genome and insert/excise themselves from the DNA. *mPing* is a *Tourist*-like miniature inverted repeat transposable element derived from the autonomous element, *Ping*. Currently, *mPing* is being developed into a targeting insertion tool for genome editing in plants. It has been shown that *mPing*, mobilized by transposase proteins, can be inserted into Cas9-cleaved sites. Our goal is to identify proteins that promote transposition and thus increase the efficiency of *mPing*-based gene discovery and genome editing. A study found that HMGB1 protein increased the mobility of a *Mariner*-type transposon, *Sleeping Beauty*, in mammalian cells by bending the transposase binding site to facilitate transposase binding. This project is testing if NHP6A, the yeast homolog of HMGB1, affects *mPing* transposition. An overexpression NHP6A construct was made amplifying NHP6A and Gateway cloning it into the pAG426 GPD plasmid. NHP6A null mutant yeast will also be transformed with a *hp_mPing:URA3* construct, designed to integrate into the genomic copy of *ADE2*. Using an *mPing* transposition assay that measures excision from *ADE2*, we will be able to determine the *hp_mPing* transposition frequencies with various levels of NHP6A. We anticipate increased transposition when NHP6A is overexpressed and decreased transposition in the NHP6A knockout. Moving forward, our next steps would be to test if expression of a NHP6A homolog in *Arabidopsis* will also increase transposition in plants.

U19 - STRESS-OUT: Stress Transcriptomics of Regolith Exposed Seedlings for Off World Utilization Techniques

Steven Elsaid*, Grace Brokaw, Annalyn Connor, Dr. Dhaka Sapkota, Dr. Robert Fitak, Dr. Julie Brisset Affiliations: Florida Space Institute, Exolith Lab, University of Central Florida

Within the next 2 decades, humanity will likely establish the first off-world settlement on the lunar surface. Plants have been identified as an important resource for improving mission success and crew morale in this challenging environment. However, lunar agriculture is not without challenges. Lunar regolith is an alkaline (pH>9), nutrient-poor, substrate prone to compaction and with high concentrations of phytotoxic heavy metals; notably Aluminum (Al), the 3rd most abundant element in the lunar highlands (≈10% of the total composition). Bioremediation, or the use of biological agents to degrade or sequester both inorganic and organic contaminants, has gained significant attention over the last decade as a potential alternative that is cost effective and sustainable. Compost worms display the ability to bioremediate heavy metals and compacted soils, including those with elevated Al concentrations, by consuming organic matter along with metal rich substrates. In this study, we used the compost worm *Eisenia fetida* to bioremediate lunar regolith simulant (LHS-1) for 20 weeks. Preliminary results have shown

dramatic increases in plant height, dry biomass, and seed germination for *Capsicum annum* and *Tagetes patula* when grown in remediated LHS-1. Current efforts are seeking to assess the genomic response of *Arabidopsis thaliana* to LHS-1, custom apollo simulants, nutrient amended LHS-1, and bioremediated LHS-1. This work will validate simulant based plant research by allowing a direct comparison of LHS-1 and apollo simulants to the work done at UF with genuine lunar samples. It will also showcase the efficacy of compost worm bioremediation at reducing regolith-induced stress on plants.

U20 - Identifying the causative mutations underlying a dwarf soybean phenotype

Samantha Marie Burns* and C. Nathan Hancock Affiliations: Department of Biological, Environmental, and Earth Sciences, University of South Carolina Aiken, Aiken, SC

Abstract: Glycine max, commonly known as soybean, is an important crop that make up 90% of all United States oilseed production. The U. S. is currently #1 in the world for soybean production and #2 in the world for soybean export. Identifying the genes that control soybean growth is critical to the genetic improvement of this crop. The Hancock laboratory identified a G. max plant line with decreased height, smaller leaves, and significantly lower yields in a mutant population. To identify the mutations present in the dwarf mutant, we Illumina sequenced the entire genome and assembled it to the Williams 82 reference soybean genome using Burrows-Wheeler Aligner. Picard was used to mark duplicates and convert it to a BAM file. Genome Analysis Toolkit (GATK) was then used to identify the variants present in the genome. Candidate mutations that disrupt genes will be identified using SNPEff. Once candidate genes are identified, the team will use PCR analysis of an F2 segregating population to determine which mutation is linked to the phenotype. Once the Hancock laboratory team can identify what gene is causing the mutation, further research into the function of the gene can be conducted.

U21 - Identifying the causative mutations underlying a dwarf soybean phenotype

Sam Burns* and C. Nathan Hancock Affiliations: Department of Biological, Environmental, and Earth Sciences, University of South Carolina Aiken, Aiken, SC

Glycine max, commonly known as soybean, is an important crop that make up 90% of all United States oilseed production. The U. S. is currently #1 in the world for soybean production and #2 in the world for soybean export. Identifying the genes that control soybean growth is critical to the genetic improvement of this crop. The Hancock laboratory identified a G. max plant line with decreased height, smaller leaves, and significantly lower yields in a mutant population. To identify the mutations present in the dwarf mutant, we Illumina sequenced the entire genome and assembled it to the Williams 82 reference soybean genome using Burrows-Wheeler Aligner. Picard was used to mark duplicates and convert it to a BAM file. Genome Analysis Toolkit (GATK) was then used to identify the variants present in the genome. Candidate mutations that disrupt genes will be identified using SNPEff. Once candidate genes are identified, the team will use PCR analysis of an F2 segregating population to determine which mutation is linked

to the phenotype. Once the Hancock laboratory team can identify what gene is causing the mutation, further research into the function of the gene can be conducted.

U22 - Analysis of Cas9 targeted *mPing* insertion in yeast

Megan Collins[1], Peng Liu[2], R. Keith Slotkin[2], and C. Nathan Hancock[1], [1] Department of Biological, Environmental, and Earth Science, University of South Carolina Aiken, Aiken, SC, [2] Donald Danforth Plant Science Center, St. Louis, MO

Transposable elements (TEs) are segments of DNA that naturally alter plant genomes by excising and reinserting when mobilized by specific transposase proteins. Our research focuses on the highly active *mPing* miniature TE from rice which relies on ORF1 and TPase protein provided by the *Ping* or *Pong* element for mobilization. Insertion of *mPing*-based activation tags was previously shown to upregulate nearby genes and recent studies have shown that fusing *Pong* TPase to Cas9 allows for targeted *mPing* insertion in plants. To improve the efficiency of this system, we are developing a yeast assay for Cas9-mediated targeted *mPing* insertion. We previously showed that the TPase:Cas9 fusion proteins can induce *mPing* transposition in yeast, while maintaining Cas9 function. We are developing a *mPing*-based activation tag and yeast reporter strain that will indicate when targeted insertion occurs. The hyperactive *mPing* carrying the GPD promoter was inserted into *MET15* gene to allow for selection of excision events. The target sequence we are using is an *ADE2* gene controlled by the *GAL2* promoter, paired with a gRNA specific to the *GAL2* promoter. We will measure the targeted insertion rate by screening for ADE2 expression in the *Met15* revertant colonies in the absence of galactose. We anticipate that TPase:Cas9 fusion constructs will allow for targeted insertion of *mPing*:*GDP* into the *GAL2* promoter, thus producing adenine on plates lacking galactose, resulting in white colonies. The development of this assay will provide an efficient method for studying the mechanism of targeted insertion so that we may further develop this system for plant genome engineering.

U23 - Characterization of the TH2/At5g32470 Fusion Protein and its Role in Thiamin Metabolism in Plants

Anthony Saca*, Ethan Soto*, Estrella Hernandez*, and Ghulam Hasnain, University of North Georgia, Oakwood, GA

Thiamine (vitamin B1) is essential for the proper functioning of many central metabolic enzymes. While plants and most bacteria can synthesize thiamine, animals must acquire it from their diet. Thiamine biosynthesis is well understood in both bacteria and plants. In bacteria, thiamine is phosphorylated by thiamin kinase (ThiK) and monophosphate kinase (ThiL). In contrast, in plants, thiamine monophosphate is first dephosphorylated (TH2) and then pyrophosphorylated by a pyrophosphate kinase (ThDPK). Notably, the enzyme responsible for thiamine pyrophosphorylation, ThDP, can also phosphorylate damaged forms of thiamine, such as oxothiamine and oxythiamin. In plants, a preemptive Nudix gene can selectively dephosphorylate the damaged forms of thiamine, but a salvage enzyme to repair the damaged forms is missing.

Comparative genomics evidence suggested that the TenA domain of the TH2 gene could salvage oxothiamine and oxythiamine. The TenA domain belongs to the TenA_C subfamily, other members of which have amino-HMP aminohydrolase activity. To test our prediction, we have mutated the putative catalytically important residues of TenA domain of TenA-HAD and tested the ThMP activity in the bacterial complementation assay. Currently, we are testing the oxothiamin/oxythiamin toxicity tolerance of a bacterial strain complemented with *Arabidopsis* TenA-HAD. To test the invitro TenA activity we are expressing the Arabidopsis His-tagged TenA-HAD gene in *E. coli* and will attempt to purify the protein. An attempt will be made to complement the *Arabidopsis* TH2 mutant with truncated TenA-HAD protein in which the TenA domain is truncated or mutated, hoping that the substrate of the TenA domain may build up.

Graduate, Faculty, and Professional Poster Presentation Abstracts

P1 - Role of *Arabidopsis thaliana* protein kinase General Control Nonderepressible 2 (GCN2) in Salicylic Acid Signaling

Ansul Lokdarshi* and Daniel Rincon; Diaz Department of Biology, Valdosta State University

Plants represent the ultimate source of nutrients for many organisms including bacteria, fungi, and animals. Therefore, understanding how plants defend themselves from pathogens and herbivores is critical to sustainable food supply. Even though lacking a defined immune system as animals, plants have developed a remarkable array of structural and biochemical defenses that are designed to detect invading pathogens and neutralize them before they are able to cause extensive damage. One of the key plant hormones that is actively engaged in plant defense towards different types of abiotic and biotic stresses is salicylic acid (SA). While the signaling events involved in SA mediated defense responses have been centered around transcriptional management (DNA to messenger RNA), translational (messenger RNA to protein) regulation remains underinvestigated. Our work provides new insights into the SA signaling at the level of translation, specifically by the protein kinase General Control Nonderepressible 2 (GCN2) and its target, eukaryotic initiation factor 2 (eIF2) alpha. The GCN2-eIF2alpha module is a highly conserved eukaryotic stress response node for regulating translation under different types of stresses in all plants. We show that eIF2alpha is phosphorylated in response to SA in a GCN2 dependent manner in the plant model, Arabidopsis thaliana. Interestingly, gcn2-1 seedlings (knock-out mutant for GCN2 gene) show wild-type like growth response under prolonged SA stress. Ongoing work is geared towards understanding the biochemical and molecular events leading to GCN2-eIF2alpha activation in response to SA, which will provide deeper understanding of SA signaling and general plant defense response.

P2 - Computational exploration of uncharacterized cotton protein families through a CUPA-CURE

Amanda Storm*, Western Carolina University; Amanda Hulse-Kemp, USDA-ARS

High-quality genome reference sequences have been developed for *Gossypium* species. A striking similarity between the sequenced species is that they all share about ~15% of total annotated genes that are currently indicated as “protein of unknown function”. Sequence and structure analysis tools and databases have created the opportunity for students to perform initial characterization of functional features in these uncharacterized proteins. Additional insight can be gained by targeting multiple members within a protein family and comparing across whole families for family-specific features as well as unique diversification within subfamilies. As part of the Cotton Uncharacterized Protein Annotation Course-based Undergraduate Research Experience (CUPA-CURE), students each study a protein within different subfamilies of an uncharacterized protein family from the cotton genome based off of PhyloGenes PANTHER trees. Findings for each protein subfamily were compared across families. Sequence analyses are used to predict subfamily domain architectures and motifs, AlphaFold structure analyses compare surface and binding site features and transcriptomics data is mined for expression profiles. We find that, although protein families share sequence patterns, there are often distinct differences between subfamilies. Beyond diversified motifs, conserved surfaces and subcellular localization, additional domains are found in certain subfamilies, such as the reticulata-related (RER) family. Some differences between subfamilies suggest the need for further division of domain designations.

P3 - Xylem-specific SULTR3 gene knockout in *Populus tremula* x alba dysregulates photosynthesis and decreases TCA cycle intermediates

Samantha (Sammi) Surber[1]*, Antonella Aliste[1], Makenzie Drowns[2], William Patrick Bewg[2], Margot Chen[2], Scott Harding[2], and C.J. Tsai[1,2]; [1]Department of Plant Biology, The University of Georgia; [2]School of Forestry and Natural Resources,

The transport of sulfate throughout a plant is mediated by a family of genes known as sulfate transporters (SULTR). In the hybrid tree species Populus tremula x alba, the subgroup SULTR3 has variable expression across tissues. Whereas SULTR3;2a shows preferential expression to the woody tissue (xylem), SULTR3;4a has broader expression across the tissues, including the phloem and xylem of the stem and root. When these genes and genome duplicates are knocked out using CRISPR-Cas9 in poplar, assimilation of carbon and stomatal conductance is increased during the summer months. In contrast to increased assimilation, the TCA cycle intermediates are decreased in the xylem tissue of the trees. Sulfur’s known role during photosynthesis is the use of iron-sulfur clusters in the electron transport chain. However, these results suggest that vascular-expressed sulfate transporters affect the TCA cycle and photosynthesis in some manner. Ongoing experiments are investigating photosynthesis using LI-COR6800, chlorophyll content in leaves, and metabolic profiling to disentangle the genetic perturbation of SULTR3s and their influence on these essential processes in plants.

P4 - mRNA Splicing Variation in mop1-1 Mutant Maize

Kathryn M. Koirtyohann* and Karen M. McGinnis; Department of Biological Science, Florida State University

In plants, RNA-directed DNA methylation (RdDM) is an epigenetic pathway involved in the establishment and maintenance of heterochromatin. One mutant genotype of maize called mop1-1, deficient in a crucial component of the RdDM pathway, has been used extensively to study the effects of RdDM on different cellular and genetic processes. In other species, chromatin structure has been shown to have effects on mRNA splicing, and some variability in splicing has been observed previously in mop1-1 mutants. It is hypothesized that mop1-1 mutant maize has higher overall splicing variability than wild-type Mop1 maize. To examine variations in splicing across these genotypes, RNA sequencing data from wild-type and mop1-1 maize is being evaluated using multiple bioinformatic tools. The program EdgeR was utilized for differential expression analysis in the two genotypes, and a number of differentially expressed (DE) genes were identified. This included both upregulated and downregulated genes, consistent with a previous DE analysis for these genotypes. Further use of EdgeR for differential splicing analysis is being completed using the same RNA-seq data. Additionally, to examine splicing variation in mop1-1 plants more broadly, RNA-seq data was run through a program called VaSP, which quantifies intron usage during splicing events based on junction reads and gene-level reads. Variation between samples of each genotype was compared, revealing higher overall variation in mop1-1 plants than in wild type. This program is also being used to discover genotype-specific splicing events (GSS) unique to these mutants. Current results of the EdgeR and VaSP analyses will be presented.

P5 - Investigating the molecular mechanisms via which the plant growth-promoting bacterium, *Azospirillum brasilense*, improves growth in salt-stressed rice

Dylan Neuhaus*, Matthew Calhoun*, Samuel Hoggard, Hannah-Grace Fritz, Avery Gilkey, Arijit Mukherjee; Department of Biology, University of Central Arkansas, Conway, AR, USA

Major food crops, such as rice and maize, display severe yield losses (30-50%) under salt stress. Furthermore, problems associated with soil salinity are anticipated to worsen due to climate change. Therefore, it is necessary to implement sustainable agricultural strategies, such as exploiting beneficial plant-microbe associations, for increased crop yields. Plants can develop associations with beneficial microbes (e.g., mycorrhiza, plant growth-promoting bacteria (PGPB)). PGPB improve plant growth via multiple mechanisms, including protection against biotic and abiotic stresses. *Azospirillum brasilense*, one of the most studied PGPB, can mitigate salt stress in different crops. However, little is known about the molecular mechanisms by which *A. brasilense* mitigates salt stress. Previously, we established an experimental system in which *A. brasilense* inoculation improved plant mass in rice grown under high salt concentrations (100 mM and 200 mM NaCl), seven days post-inoculation (dpi). We hypothesized that *A. brasilense* inoculation would regulate the expression of rice genes involved in salt-stress response, nutrient and ion transport, and abscisic acid and jasmonic acid signaling, among others. Using RNA sequencing, we identified the transcriptomic changes in rice plants during *A. brasilense*-mediated salt stress tolerance at seven dpi. Our results identified key gene expression patterns in rice via

which *A. brasilense* help improve growth in rice. To identify the early plant transcriptomic changes in salt-stressed rice upon inoculation, we are currently performing a similar RNA-Seq study at one dpi. We have already submitted the RNA samples for sequencing and are waiting for the results. In this study, we expect to identify differentially expressed genes in salt-stressed rice involved in the initial perception and response to *A. brasilense*. Our findings will provide essential insights into salt stress mitigation in rice by *A. brasilense*.

P6 - Changes in sorbitol metabolism impact kernel sink strength and seed size

Nadia N. Mourad Silva[1]* (nmourad@ufl.edu), Katherine E. Gray[1] (kgray3@ufl.edu), Darcy A. Jordan[1] (darcy.jordan@ufl.edu), Alexander Hilo[2] (hilo@ipk-gatersleben.de), Hardy Rolletschek[2] (rollet@ipk-gatersleben.de), Ljudmilla Borisjuk[2] (borysyuk@ipk-gatersleben.de), Sylvia M. de Sousa Tinoco[3] (sylvia.sousa@embrapa.br), Jiahn-Chou Guan[1] (guanjc@ufl.edu), Karen E. Koch[1] (kekoch@ufl.edu); [1]University of Florida, Department of Horticultural Sciences, Fifield Hall 2550 Hull Rd., Gainesville, FL, 32611 USA; [2]Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, D-06466 Seeland-Gatersleben, Germany; [3]Embrapa Maize and Sorghum, Rod MG 424 Km 45, Sete Lagoas, MG, 35701-970 Brazil

Maize endosperm development is characterized by an oxygen-deficient microenvironment, requiring molecular and biochemical mechanisms for hypoxia acclimation and maintenance. Sorbitol dehydrogenase (SDH) catalyzes the conversion of (fructose + NADH)↔(sorbitol + NAD+) in endosperms during grain-fill. We hypothesize that SDH aids endosperm development in at least two ways, 1) by regenerating the NAD+ that maintains redox balance and glycolytic flux in the hypoxic endosperm, and 2) by enhancing sink strength through the metabolism of fructose and indirectly the import of its precursor sucrose. In support of our hypothesis, sorbitol accumulates in the endosperm region with lowest oxygen levels as determined by FTIR imaging in developing wild-type kernels. To evaluate the role of SDH, we characterized an Ac/Ds-induced sdh1 mutant which lacks detectable SDH activity and accumulates little to no sorbitol in kernels. We found that sdh1 mutant ears bear smaller kernels beginning at 15 DAP, with 13-17% less dry weight at maturity, suggesting an important role in kernel filling. Metabolic analysis of sdh1 mutant kernels shows elevated levels of fructose as well as sucrose (100% and 25% greater, respectively). Results indicate that conversion of fructose to sorbitol via SDH promotes sucrose metabolism and import. Work in progress focuses on characterization of Sdh1 over-expression lines, development of double-mutant kernels deficient in both sorbitol and starch biosynthesis, and an in-depth evaluation of metabolic impacts. Findings thus far highlight the role of SDH in kernel development with implications for metabolic engineering strategies to enhance sink strength and composition of the maize grain.

P7 - Pennycress powerhouse: Tailoring fatty acids for jet fuel production

Amira Rasoul*, Christopher Johnston PhD, Ana Paula Alonso PhD; University of North Texas (UNT)

The aviation industry has expressed a growing interest in using alternative crops to generate renewable jet-fuel. Replacing traditional fossil fuels with a renewable, domestic source can reduce carbon emissions, thus mitigating global climate change. *Thlaspi arvense* (pennycress) is an oilseed cover crop that can be grown during the offseason of corn and soybean to produce

renewable biofuel, increasing the economic viability of well-established farm operations. Pennycress is widely undomesticated and easily genetically modified, providing opportunities to enhance favorable traits. One such improvement is eliminating erucic acid in the seed oil to improve the biofuel's cold flow properties. To target erucic acid content, FATTY ACID ELONGATION-1 (FAE-1) loss-of-function mutants were generated using CRISPR/Cas9 gene editing. Mutants fail to synthesize very long chain fatty acids, including erucic acid. Utilizing an integrated multi-omics approach, we will investigate the impact of eliminating VLCFAs in developing plant embryos by examining key aspects such as metabolite quantities and gene expression profiles. This comprehensive analysis aims to elucidate the complexities of lipid metabolism, with the ultimate goal of providing valuable insight for future advancements in crop improvement.

P8 - Transcriptomic analysis of rice lines expressing DREB1a, the master regulator of abiotic stress response

Gerald Sormanti* and Vibha Srivastava; Department of Crop, Soil and Environmental Science, University of Arkansas, System Division of Agriculture

This study analyzed the transcriptome of transgenic rice plants expressing DREB1a at room temperature and cold stress conditions. Transgenic rice lines in cv. Taipei-309 expressing the stress (cold/drought/salinity)-inducible DREB1a gene (RD29a:DREB1a) were used in this study. 10-d-old seedlings on MS1/2 media were treated with cold-shock by placing them on ice pack for 20h, while the control seedlings were kept at the room temperature. Total RNA was extracted from the aerial portion of seedlings and submitted for RNA-seq. Bioinformatics analysis of the differentially expressed genes (DEGs) was done on iDEP platform (http://bioinformatics.sdstate.edu/idep93/). In a comparison of the DREB1a transgenic line with the wild-type, 3852 DEGs were found upon cold-shock treatment, whereas 611 DEGs were found at the room temperature (control). This data indicates that a subset of DREB1a regulon is active in the control conditions. Analysis of the DEGs showed that response to abiotic stress, biosynthesis of secondary metabolites, jasmonate pathway, and MAPK signaling were upregulated in the DREB1a transgenic plants. Notably, at the room temperature upregulation of response to abiotic stress occurs in DREB1a plants. Interestingly, photosynthesis and carbon fixation pathways were significantly upregulated at the room temperature. However, ribosome biogenesis was downregulated. This suggests that DREB1a plants are more efficient at energy generation, but they probably transfer that energy for secondary metabolism and stress response more than the growth processes. Previously, we showed that DREB1a transgenic rice is more tolerant to drought and salinity stress through upregulation of multiple stress response pathways including secondary metabolism. Here, we propose that the basal DREB1a expression in the non-stressed environment prepares the plants for a rapid stress response. However, whether this basal expression unfavorably affects plant growth and productivity needs to be investigated. In this ongoing study, we aim to determine the yield components of DREB1a plants and their stress response during reproductive growth. We will also conduct a transcriptomic analysis to understand genome-wide expression changes associated with the balance and tradeoff of stress and growth processes.

P9- Telomere analysis in Arabidopsis thaliana using Fluorescence In Situ Hybridization (FISH)

Pavel Ulianich*, Borja Barbero Barcenilla, Dorothy E. Shippen; Texas A&M University, Department of Biochemistry & Biophysics

Fluorescence In Situ Hybridization (FISH) is commonly used for localization of specific chromosomal sequences. The probe typically consists of a single-stranded sequence (DNA or RNA), complementary to the target, that is composed of regular nucleotides and nucleotides with labels that can be detected. The main challenges with this method include the complexity and the time-consuming nature of preparing labeled probes and their hybridization. Our goal was to develop a rapid, optimized FISH protocol for telomere localization with fluorescent labeled oligonucleotides. To optimize the length and sequence of the probe, we tested two different oligonucleotide sequences complementary to the telomeres of Arabidopsis thaliana, utilizing three fluorescent dyes: Texas Red, Cy3, Cy5 and Alexa 488. For the hybridization protocol, we experimented with various washing solutions and washing conditions. Additionally, we tested different concentrations of probes, the composition of the hybridization mix, and hybridization times. These steps are critical in optimizing FISH protocols. As a result, we developed an optimal protocol for the localization of telomeres in pistils using fluorescently labeled oligonucleotides. This optimized method was successfully applied to A. thaliana telomere-related mutants, where the formation of chromatin bridges was observed during anaphase. Current studies are focused on developing FISH methods for rapid and quantitative assessment of telomere length. Such a method would allow us to examine telomere tracts in single cells, opening a new avenue for exploring telomere dynamics in different environmental settings and in different mutant constitutions.