2026 JOINT MID-ATLANTIC SECTION ASPB & UMD PLANT SYMPOSIUM – PROGRAM

UNIVERSITY OF MARYLAND, COLLEGE PARK

MAY 27-28, 2026

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Joint MAS-ASPB & UMD Plant Symposium Schedule

Day 1, May 27th	
8:30 – 9:00 am	**Registration; light breakfast, set up presentations for Session I**
9:00 – 9:02 am	**Welcome Remarks:** Dr. Daniel Rodríguez-Leal
9:02 – 9:05 am	**Keynote speaker introduction:** Dr. Yiping Qi
9:05 – 9:55 am	**Keynote address – Dr. Sarah Assmann** (Pennsylvania State University) *Adventures in abiotic stress resistance*
9:55 – 10:10 am	**Coffee Break**
10:10 – 11:45 am	**Session I: Plant Genetics and Genomics** (Session chairs: Drs. Shunyuan Xiao and Parva Sharma)
10:10 – 10:35 am	Dr. Eric Lam: Rutgers University. *Metacaspases as an emerging family of plant mediators for biotic stress management*
10:35 – 11:00 am	Dr. Purushothaman Natarajan: University of Maryland, Eastern Shore. *3D Genome Architecture and Transcriptomic Regulation of Powdery Mildew Response in Susceptible, Tolerant, and Resistant Vitis Species*
11:00 – 11:25 am	Dr. Umesh Reddy: West Virginia State University. *From Genome to Function: Multi-Omics Insights into Capsaicin Biology and its Agricultural and Biomedical Implications*
11:25 – 11:35 am	Dr. Parva Sharma: University of Maryland, College Park. *Dissecting Stripe Rust Resistance in Wheat Using Multi-Strain Phenotyping and Reference-Free k-mer GWAS in Einkorn wheat*
11:35 – 11:45 am	Dr. Nayara Sabrina Freitas-Alves: University of Maryland, College Park. *Optimization of CRISPR sgRNA Length Enhances Base Editing Efficiency in Plants*
11:45 – 12:00 pm	**Meet our ASPB-MAS sponsors!** ANSA, AmpSeq, OPS, Genesee
12:00 – 12:40 pm	**Lunch for all registered attendees**
12:40 – 1:40 pm	**Poster Session: Odd numbers** (Set up presentations for Session II)
1:40 – 3:45 pm	**Session II: Biotic Stress** (Session chairs: Drs. Pierre Jacob and Pai Li)
1:40 – 2:05 pm	Dr. Kiran Mysore: Oklahoma State University. *Functional genomics approaches identify novel genes involved in plant innate immunity*

Day 1, May 27th	
2:05 – 2:30 pm	Dr. Allie Igwe: Virginia Tech University. *Distinct Taxa, Shared Functions: Microbial Community Stability Across Serpentine and Nonserpentine Soils*
2:30 – 2:55 pm	Dr. Harsh Bais: University of Delaware. *From Soil to Space: How Root Microbes Supercharge Plant Health*
2:55 – 3:05 pm	Dr. Pai Li: University of Maryland, College Park. *Questing the MLO mystery: why loss of host proteins results in complete resistance to powdery mildew*
3:05 – 3:15 pm	Dr. Prem Kumar: University of Maryland, College Park. *Wheat Pore-Forming Toxin-like gene provides broad spectrum resistance to fungal pathogens in transgenic tomato and strawberry plants*
3:15 – 3:25 pm	Ms. Leah Vrydagh: University of Maryland, Baltimore County. *Structural and Functional Analysis of the KH-Domain Protein FLK in Regulating Arabidopsis Defense and Development.*
3:25 – 3:40 pm	**Coffee Break, Set up presentations for Session III**
3:40 – 4:50 pm	**Session III: Abiotic Stress** (Session chairs: Dr. Xingyun Qi and Ms. Moonia Ammari)
3:40 – 4:05 pm	Dr. Aziz Eida: University of North Carolina, Chapel Hill. *Fighting drought from the roots: dissecting mechanisms of rhizobacteria-mediated drought rescue in Arabidopsis*
4:05 – 4:30 pm	Dr. Pierre Jacob: University of Maryland, College Park. *Genetic requirements for NLR signaling, implications for biotic and abiotic stress responses*
4:30 – 4:40 pm	Ms. Sanchari Kundu: Virginia Tech. *Studying Cell-Type Specific Transcriptional Responses To Salt Stress In Pennycress (Thlaspi arvense) Roots Using Single Nuclei Transcriptomics*
4:40 – 4:50 pm	Mr. Ajagbe Nathaniel: University of Maryland, College Park. *Deciphering the Molecular Mechanisms of Abscisic Acid-dependent Gene Regulation in Plants*
4:50 – 5:05 pm	**Presentation by ASPB Ambassadors and Immediate Past-President President Dr. Hong Ma.**
5:05 – 6:20 pm	**Grant Development Workshop** (conveners: Dr. Vijay Tiwari and Dr. Pierre Jacob) Panelists: POs from Federal Funding agencies
6:30 – 9:00 pm	**Reception dinner at Mulligan's Grill and Pub for all registered attendees** **3800 Golf Course Road, College Park, MD 20742**

Day 2, May 28th	
8:30 – 9:00 am	**Registration; light breakfast, set up presentations for Session IV**
9:00 – 9:05 am	**Keynote speaker introduction:** Dr. Shunyuan Xiao
9:05 – 9:55 am	**Keynote address – Dr. Roger Innes** (Indiana University Bloomington) *Leaf Surface RNA: How does it get there and what does it do?*
9:55 – 10:10 am	**Coffee Break**
10:10 – 11:45 am	**Session IV: Plant Development and Cell Biology** (Session chairs: Drs. Jose Feijo and Evan Littleton)
10:10 – 10:35 am	Dr. Wendy Ann Peer: University of Maryland, College Park. Title: *Cytosolic- and membrane-localized oxidized indole-3-acetic acid formation regulates developmental auxin transients*
10:35 – 11:00 am	Dr. Gwonjin Lee: West Virginia State University. *Adaptive Divergence of Meiotic Recombination Patterns in Maize Lineages from Distinct Climatic Origins*
11:00 – 11:25 am	Dr. Rakesh K Upadhyay: Bowie State University. *Polyamine Metabolic Plasticity in Plants: A Key Player in Growth, Development and Stress*
11:25 – 11:35 am	Ms. Anmol Kajla: University of Maryland, College Park. *Molecular Characterization of the Wheat C-locus Controlling Spike Architecture*
11:35 – 11:45 am	Ms. Eeshita Ghosh: University of Maryland, College Park. *CorNIchon Homologue proteins regulate calcium signaling and root development in Arabidopsis thaliana*
11:45 – 12:30 pm	**Lunch for all registered attendees**
12:30 – 1:50 pm	**Poster Session: Even numbers** (Set up presentations for Session V)
1:50 – 3:00 pm	**Career Development Workshop** (convener: Dr. Daniel Rodriguez-Leal) Panelists: Dr. Murli Manohar and Dr. Derick Jiwan.
3:00 – 5:20 pm	**Session V: Botany, Ecology and Evolution** (Session chairs: Dr. Caren Chang and Ms. Anmol Kajla)
3:00 – 3:25 pm	Dr. Daniel Buonaiuto: University of Maryland. *Sex-specific flowering responses to environmental cues: implications for plant fitness in a changing climate*
3:25 – 3:50 pm	Dr. Meghan Blumstein: University of Virginia. *Eastern Temperate Forests' Potential For Climate Adaptation*
3:50 – 4:15 pm	Dr. Eloisa Vendemiatti: West Virginia State University. *Dissecting the Genetic Basis of Trichome-Mediated Defense in Tomato (Solanum lycopersicum)*

Day 2, May 28th	
4:15 – 4:25 pm	Ms. Cindy Wang: University of Maryland, College Park. *Identifying the genetic mechanisms controlling stomatal CO2 responses in the strawberry plant Fragaria vesca*
4:25 – 4:35 pm	Dr. Megha Sampangiramaiah: Indiana University. *Extracellular RNA in and on Maize Leaves: A Hidden Layer Contributing to Microbiome Assembly*
4:35 – 4:50 pm	**Coffee Break**
4:50 – 5:30 pm	**Introduction of Ferdows Foundation by Dr. Shahla Butler** **Poster and Oral Presentation Awards (Ferdows Awards)**
5:30 PM	**Concluding Remarks; Poster take-down and Departure**

Abstracts from Talks
Wednesday, May 27th

KEYNOTE TALK

Adventures in abiotic stress resistance

Sarah M. (Sally) Assmann

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Optimal cultivation of rice (*Oryza sativa*) is highly water intensive, yet subsistence farmers often cannot afford to irrigate their rice paddies, resulting in yield loss. We are interested in elucidating physiological and molecular genetic mechanisms that underlie rice resistance to drought and heat stress, with potential applications to real-world crop improvement. In one project, we have identified natural variants in the heterotrimeric G protein α subunit, RGA1, as beneficial for rice drought resistance. In a second project, we are studying how transcriptome-wide variation in mRNA secondary structure regulates the expression of stress-related genes in rice and other organisms. These experiments rely on a method, Structure-seq, that we developed which combines chemical probing of RNA structure with high throughput sequencing to allow characterization of RNA secondary structure *in vivo*. and transcriptome-wide.

Session I: Plant Genetics and Genomics

Metacaspases as an emerging family of plant mediators for biotic stress management

Eric Lam

Department of Plant Biology Rutgers, the State University of New Jersey Rm. 216, Foran Hall 59 Dudley Road New Brunswick, NJ 08901

As sessile organisms, plants cannot run away from dangers and must constantly survey their surroundings and make appropriate responses by modifying their metabolism or development. Advances in the past two decades have revealed numerous pattern recognition receptors and kinases, as well as families of small signaling peptides that can act as phytocytokines, which play key roles in signal perception and propagation for a multitude of defense and developmental responses. However, the mechanisms coordinating these responses at the cellular and organismal levels remain poorly understood. Recently,

the highly conserved plant metacaspase family in plants emerged as a versatile switch that plays multiple roles from early signal perception to downstream signal propagation. In this talk, I will summarize the latest advances in metacaspase functions in general and focus on their roles in orchestrating the complex wounding response in particular.

3D Genome Architecture and Transcriptomic Regulation of Powdery Mildew Response in Susceptible, Tolerant, and Resistant Vitis Species

Purushothaman Natarajan*, Papaiah Sardaru, Sadanand Dhekney

Department of Agriculture, Food and Resource Sciences, University of Maryland Eastern Shore, Princess Anne, MD 21853.

Powdery mildew is a major fungal disease affecting grapevine production, with distinct variation in host response among Vitis species. To investigate chromatin-level mechanisms underlying disease resistance, Hi-C sequencing and RNA-Seq transcriptome analyses were performed on three Vitis species representing different stages of domestication and contrasting resistance levels: the domesticated Vitis vinifera (susceptible), the semi-domesticated Vitis labrusca (tolerant), and the wild Vitis rotundifolia (resistant). The Hi-C data are being used to assemble chromosome-scale 3D genome architectures to identify topologically associated domains (TADs) and long-range chromatin interactions linked to defense gene regulation. Integration of Hi-C and RNA-Seq datasets will reveal transcriptional networks and chromatin remodeling patterns that contribute to host response to the pathogen. Comparative analyses among the three species will decode how chromatin organization influences transcriptional reprogramming during pathogen infection. To our knowledge, this is one of the first integrative Hi-C and RNA-Seq comparisons of powdery mildew responses across Vitis species with contrasting susceptibility. Findings from this study will advance understanding of chromatin-mediated regulation in perennial crops and inform molecular breeding strategies for developing durable, disease-resistant grape cultivars.

From Genome to Function: Multi-Omics Insights into Capsaicin Biology and its Agricultural and Biomedical Implications

Umesh K. Reddy[1], Padma Nimmakayala[1], Carlos Lopez Ortiz[1], Subramanyam Chinreddy[1], Krishna Sai Karnatam[1], Juan Gerardo Flores[1], Mohankumar Armuganathan[1], Purushothaman Natarajan[2], and Vagner Benedito[2]

[1] Department of Biology, West Virginia State University, Institute, WV 25112
[2] School of Agriculture and Natural Sciences, University of Maryland Eastern Shore, Maryland, USA

Capsicum chinense is a globally important specialty crop valued for its diversity in pungency, color, flavor, and nutraceutical composition, yet the regulatory mechanisms underlying specialized metabolism remain incompletely understood. Here, we integrated genome-wide

association studies, transcriptome-wide association studies, spatial and single-cell transcriptomics, and CRISPR-Cas9 validation to dissect the genetic and cellular architecture underlying capsaicinoids, carotenoids, and volatile organic compounds across diverse germplasm. We identified major loci associated with metabolite accumulation, including Pun-1, Pun-2, transporter-associated variants, AP2 ERF and MYB transcription factors, phytoene synthase, and plastid-associated pentatricopeptide repeat proteins that coordinate specialized metabolic pathways. Spatial and single-cell transcriptomic analyses revealed tissue-specific organization of capsaicinoid and carotenoid biosynthesis within placental, vascular, epidermal, and pericarp cell populations. CRISPR-Cas9 editing of selected candidate genes confirmed their roles in regulating metabolite composition and transcriptional signaling. To further investigate capsaicinoid function beyond plants, complementary studies in Drosophila, glioblastoma U87 cells, and Caenorhabditis elegans demonstrated conserved yet context-dependent biological responses to capsaicin and capsiate. Single-nucleus transcriptomics of Drosophila brains identified sex-specific neural and glial responses involving Drosulfakinin signaling and circadian Clock regulation in response to dietary capsaicin exposure. In glioblastoma cells, TRPV1 loss shifted capsaicin responses toward mitochondrial apoptosis, while capsiate treatment in C. elegans extended lifespan, reduced oxidative stress and alpha-synuclein aggregation, and protected dopaminergic neurons. Together, these findings establish a multi-omics framework connecting plant genomics, cellular transcriptomics, genome editing, neuroscience, and translational biology to advance nutraceutical development and phytochemical-based therapeutic discovery in Capsicum.

Dissecting Stripe Rust Resistance in Wheat Using Multi-Strain Phenotyping and Reference-Free k-mer GWAS in Einkorn wheat

Parva Sharma[1], Paula Silva[2], Xianming Chen[3], Vijay Tiwari[1]

[1]Department of Plant Science and Landscape Architecture, University of Maryland, College Park
[2]INIA, Uruguay
[3]USDA-ARS, Pullman, WA (Wheat Health, Genetics & Quality Unit)

Bread and pasta wheat form a major foundation for global food security. One of the biggest threats for wheat production is a fungal disease known as stripe rust, caused by Puccinia striiformis f. sp. tritici. Recently a major resistance gene Yr15 that was deployed in global wheat breeding germplasm, broke down under evolving pathogen pressure. Quick discovery of new genes and alleles are a must to fight this unprecedented situation. To address this key concern, we utilized our simple and elegant diploid A-genome resources that include pangenomes, sequenced GWAS panel, sequenced RIL population and candidate gene validation resources such as TILLING and gene editing. We employed genomics enabled k-mer GWAS matrix to identify novel QTL against 8 globally distinct stripe rust isolates. Excellent resistance against these isolates was observed in the TmGWAS panel. We

identified major novel QTLs on chromosomes 5AL (100kb), 6AS (200kb), 6AL (100kb), 7AS (200kb) and 7AL (1.5Mb) in isolates PstS10-3404 (5AL and 6AS), PstS13-3860, PstV-14 and PstS10-3404 + PstS13-3457 resp. All these QTL regions have important genes involved in disease resistance like RGAs. Kinases, Ethylene responsive TF, F box LRR repeat containing and ABC transporter. Integration with a pan-genome framework enabled haplotype-based dissection of this region, revealing distinct resistance-associated haplotypes. Candidate gene analysis within the locus highlighted genes with typical disease-resistance signatures, including NLRs and kinase-related genes. These results provide a strong foundation for map-based cloning and functional validation of novel stripe rust resistance genes.These findings advance our understanding of stripe rust resistance and provide valuable targets for marker-assisted selection and gene discovery, supporting the development of wheat cultivars with improved and durable resistance.

Optimization of CRISPR sgRNA Length Enhances Base Editing Efficiency in Plants

Freitas-Alves, Nayara Sabrina[1], Chen, John[1], Cheng, Yanhao[1], Qi, Yiping[1,2]

[1]Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD, USA
[2]Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, MD, USA.

The Cas9-D10A nickase has been applied to engineer base editors (BEs) to induce base modifications using two main systems: adenine base editors (ABE) and cytosine base editors (CBE). The nCas9 is fused to a cytidine or adenine deaminase enzyme to catalyze the deamination of the target base (C-to-T and A-to-G conversions, respectively). However, several aspects might affect genome editing efficiency, such as the type of deaminase, the linkers used between the deaminase and the nCas9, and the chosen sgRNA. Here, we hypothesize that the length of the sgRNA can also affect genome editing outcomes in plants. Therefore, the aim of this study was to evaluate if base editing efficiency could be improved by altering the length of protospacers, using the CRISPR/nCas9-ABE8e and -CBE (A3A-130F) systems in mono- and dicotyledonous plants. Protospacer sequences of 16nt, 18nt, 20nt, 22nt, 26nt, 30nt, 34nt, 38nt, 42nt, and 48nt were designed to target two OsPDS target sites in rice protoplast. The most efficient protospacer sizes were used to revert a broken RUBY to a functional one in Nicotiana benthamiana leaves with co-agroinfiltration of a CRISPR/nCas9-ABE8e repair editor. Subsequently, rice stable transformation was performed with both CRISPR/Cas9n-ABE and -CBE editors, using optimized sgRNA lengths, targeting the genes OsDEP1, OsACC and OSALS. Next-generation sequencing (NGS) analysis of protoplast samples indicated that protospacers of 16nt, 18nt, 20nt, 22nt and 24nt induced the highest genome editing efficiencies. Overall, this study provides an important foundation for enhancing CRISPR sgRNA design and provides a basis for optimizing CRISPR base-editing strategies to advance plant genome engineering for agricultural applications.

Session II: Biotic Stress

Functional genomics approaches identify novel genes involved in plant innate immunity

Kiran Mysore

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World population is increasing at an alarming rate demanding more food with less land. Reducing crop losses from biotic and abiotic stresses is crucial to increase food supply. Plants employ various resistance mechanisms to combat potential pathogens. Nonhost resistance is one such mechanisms shown by a given plant species against all isolates of a specific pathogen that can cause disease in other plants. Using virus-induced gene silencing (VIGS)- and transposon (Tnt1) insertion mutant screening-based forward genetics approach, our lab identified novel genes that play a role in nonhost disease resistance in a hope to engineer durable disease resistance in crop plants. Functional characterization of couple of these genes (GCN4 and SUCROSE TRANSPORTER1) will be presented. Translational research has been initiated in a hope to develop durable disease resistance and multi-stress tolerant crops. One of the main bottlenecks for translational research is the recalcitrance of many crop species/varieties for Agrobacterium-mediated transformation due to plant defense responses and/or the inability of the plant to regenerate. Our lab has engineered Agrobacterium with a type III secretion system from *Pseudomonas syringae* to deliver proteins that suppress plant defense responses and improve plant transformation.

Distinct Taxa, Shared Functions: Microbial Community Stability Across Serpentine and Nonserpentine Soils

Allie Igwe

Department of Biological Sciences, Virginia Tech University. Blacksburg VA

Serpentine soils represent chemically extreme environments characterized by low nutrient availability and elevated concentrations of magnesium and heavy metals. These conditions create strong environmental filters that can alter microbial community composition. In this study, we used shotgun metagenomics and functional annotations from 23 paired sites of serpentine and nonserpentine soils to compare microbial communities and their metabolic potential. While we observed substantial bacterial and fungal taxonomic differences between soil types, we found that many core functions were conserved across communities. Even so,

specialized functions such as siderophore biosynthesis, phosphonate metabolism, and stress-responsive regulatory pathways were identified and reflect local adaptation to serpentine geochemistry. We also identify the specific taxa that contribute to these specialized functions and quantify how redundant these functions are within and across soil types. This pattern suggests that functional redundancy allows microbial ecosystems to maintain key metabolic capabilities despite shifts in community composition while environmental conditions still select for specialized functions that facilitate local adaptation. These findings advance our understanding of how soil microbiomes maintain functional capacity under extreme environmental conditions and identify functions that may be particularly important, or particularly at risk, in serpentine ecosystems. This work has broad relevance for microbial ecology, environmental genomics, conservation biology, and efforts to predict microbiome responses to environmental change.

From Soil to Space: How Root Microbes Supercharge Plant Health

Harsh Bais*

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Our knowledge of plant beneficial bacteria in the rhizosphere is rapidly expanding due to the intense interest in utilizing these types of microbes in agriculture. The lab to the field. A component associated with understanding the functional role of the root-associated microbiome is slowly progressing. We know more about the synthetic community of microbes that help plants to abate various stress responses. In addition, the microbe-derived products in the rhizosphere also have a fundamental significance in how plants associate with specific microbes and how plants respond to various abiotic and biotic stressors. This presentation will highlight the roles of selected root-microbiome isolates in plant responses to pathogens (biotic stress), drought (abiotic stress), and the suppression of human pathogens related to food safety, in both terrestrial and space-like environments. A deeper understanding of how plant growth-promoting rhizo-microbiome interact with plants will support the development of more effective and reliable biological inoculum applications for field use.

Questing the MLO mystery: why loss of host proteins results in complete resistance to powdery mildew

Pai Li[1], David Bloodgood[1], Qiong Zhang[1], Ying Wu[1], Christina Zhou[1], Cheng-I Wei[2], Yiping Qi[1,3], and Shunyuan Xiao[1,3]

[1] Institute for Bioscience and Biotechnology Research, University of Maryland.

[2] Department of Nutrition and Food Science, University of Maryland College Park.
[3] Department of Plant Sciences and Landscape Architecture, University of Maryland College Park.

Loss of one or more Mildew Locus O (MLO) genes in host plants confers complete resistance to powdery mildew (PM), a disease caused by obligate biotrophic fungi of the order Erysiphales that depend on living host tissue to survive and thrive. Whether loss of MLOs results in activation of an unknown host immunity mechanism that blocks fungal penetration or impairment of a host process that is essential for pathogenesis remains to be determined. To quest this mystery, we knocked out the MLO2, MLO6 and MLO12 genes in Arabidopsis mutants in which key defense signaling pathways or programs are impaired and tested if mlo-mediated immunity still holds. Disruption of EDS1, PAD4 and SID2, three essential genes required for immune signaling channeled down from both PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI), leads to super-susceptibility to adapted powdery mildew. However, the eds1/pad4/side+mlo2/mlo6/mlo12 (designated eps3m) sextuple mutant is completely immune to the pathogen. We then employed CRISPR to generate mutations in PEN1, PEN2 and PEN3, three genes serving critical roles in cell wall-based resistance against fungal penetration, in eps3m. The eds1/pad4/sidemlo2/mlo6/mlo12+pen1/pen2/pen3 (designated eps3p3m) nonuple mutant remains completely immune to all tested powdery mildew fungi, despite that the eps3p sextuple mutant becomes susceptible to even non-adapted powdery mildew species that infect other dicot plants. Importantly, developmental, cytological and molecular analyses showed no sign of activation of canonical defenses such as early leaf senescence, ROS production, and PR gene induction in eps3m and eps3p3m, suggesting that mlo-mediated immunity can be uncoupled from host defense activation. Next, we examined if fungal penetration induced callose deposition is changed in eps3p3m compared to eps3p. Excitingly, we found that callose deposition occurs at 6 hrs post-inoculation (hpi) in eps3p3m, a few hrs earlier than that in eps3p, implying that earlier callose deposition due to loss of MLOs may underlie mlo-conferred immunity. To further test this hypothesis, we are employing multiplexed CRISPR mutagenesis to knock out four callose synthase genes (CalS) in the eps3p3m background. T2 transgenic lines carrying disruptive mutations in these CalS genes will enable a direct assessment of this hypothesis. Together with our studies of MLO2's focal accumulation at fungal penetration sites, these experiments will help elucidate the mechanisms underlying mlo-mediated immunity to powdery mildew fungi.

Wheat Pore-Forming Toxin-like gene provides broad spectrum resistance to fungal pathogens in transgenic tomato and strawberry plants

Prem Kumar Ganesan[1], Eman Elagamey[2], Juan Debernardi[3], Shunyuan Xiao[1,4], Alex Broadway[1], Nidhi Rawat[1*]

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[4] Institute for Bioscience and Biotechnology Research, Rockville, MD 20850, U.S.A.

Wheat pore-forming toxin-like (PFT) gene was reported by Rawat et al. (2016) previously to provide Fhb1 mediated resistance to Fusarium graminearum infection in resistant wheat cultivar Sumai 3. To investigate the effect of PFT in another plant system, we ectopically expressed it in dicot plant model plant Arabidopsis thaliana, which does not have any PFT ortholog or homolog and observed a broad-spectrum resistance to several necrotrophic and hemi-biotrophic fungal pathogens. In this follow up study, the wheat PFT gene was transferred to a diploid tomato cv. Moneymaker and an octaploid strawberry cv. Camerosa as both of these varieties are susceptible to several pathogens. The PFT transgenic tomato plants were challenged with Fusarium oxysporum f. sp. lycopersici, Verticillium dahliae, Alternaria linariae, and Botrytis cinerea (T1 and T2) whereas the PFT transgenic strawberry plants (T0) were challenged with Botrytis cinerea and Colletotrichum fioriniae. In both the experiments, transgenic plants of PFT tomato and strawberry showed significantly less disease severity index and fungal biomass with significant disease resistance against the fungal pathogens tested. In an in vitro antifungal assay, the F. graminearum spores treated with the purified and Dylight594 labelled PFT protein retarded the spore germination during 24h incubation period when compared to the buffer control. Glycan binding kinetics assays of purified PFT with different carbohydrates exhibited strongest binding with chitin monomer N-Acetyl-D-Glucosamine (NADG). These results support a model in which PFT targets fungi via GlcNAc/chitin recognition through its lectin domains and suppresses infection through its pore-forming toxin-like domain. Collectively, this work establishes PFT as a transferable, broad-spectrum antifungal trait effective across diverse plant genetic backgrounds.

Structural and Functional Analysis of the KH-Domain Protein FLK in Regulating Arabidopsis Defense and Development

Leah Vrydagh, Faridah Folorunsho, Dennis Lee, Seth Glassner, Geneva Mon, and Hua Lu.

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Plant disease resistance is a complex process that is maintained in an intricate balance with development. Increasing evidence indicates the importance of post-transcriptional regulation of plant defense by RNA-binding proteins. The Lu lab discovered a novel role for a known flowering time regulator FLOWERING LOCUS KH DOMAIN (FLK) in pathogen defense. FLK encodes a putative RNA-binding protein with triple K homology (KH) repeats. Each KH domain spans approximately 70 amino acids and harbors a conserved GXXG motif. The XX amino acid residues are mostly positively charged and are presumably important for RNA interaction. We hypothesize that substituting this motif with negatively charged aspartic acids

will disrupt RNA binding without compromising overall protein stability. Site-directed mutagenesis was conducted to generate FLK KH mutant variants using FLK-GFP as a backbone. These variants include GDDG replacement of the GXXG motif in individual KH domains, KH double mutants (KH1/2, KH2/3, and KH1/3), and KH triple mutant (KH1/2/3). These constructs were introduced into the flk-1 mutant via Agrobacterium-mediated transformation. The resulting plants have been examined for flk-1-conferred phenotypes. This research will clarify how the KH domains influence FLK structure and fu0nction in plant defense and development.

Session III: Abiotic Stress

Fighting drought from the roots: dissecting mechanisms of rhizobacteria-mediated drought rescue in Arabidopsis

Dr. Aziz Eida
Department of Biology, University of North Carolina, Chapel Hill.

Abiotic stresses, such as drought, have severe impacts on plant growth and crop productivity. The Food and Agriculture Organization estimates global economic costs associated with droughts at $34 billion annually. Microbial biostimulants containing beneficial rhizobacteria are a promising and eco-friendly approach to mitigating water scarcity in agriculture. However, the molecular mechanisms by which rhizobacteria rescue plants from drought stress remain poorly understood. Using a combination of transcriptomic and genetic approaches, we are dissecting drought rescue mechanisms in the model plant *Arabidopsis thaliana* and the growth-promoting rhizobacterium *Pseudomonas simiae* WCS417. Time-resolved transcriptomic analysis of *Arabidopsis* during drought stress and bacterial inoculation revealed significant enrichment of differentially expressed genes related to iron starvation responses, iron uptake, transport, and homeostasis across multiple timepoints in both root and shoot tissues. Strikingly, we identified a set of iron homeostasis genes that are transcriptionally induced by drought but suppressed by WCS417, and vice versa, suggesting the bacterium actively counteracts the plant's drought-induced iron deficiency response. Elemental analysis of iron in plant tissues is consistent with these transcriptomic signatures. Ongoing genetic dissection using iron homeostasis mutants is providing mechanistic insight into how WCS417 interfaces with plant iron signalling during drought, opening a new avenue for understanding plant-microbe interactions for stress resilience.

Genetic requirements for NLR signaling, implications for biotic and abiotic stress responses

Pierre Jacob

Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD 20742

NLRs are innate immune receptors conserved throughout the tree of life. In plants, NLRs are intracellular receptors that monitor the integrity of the immune system. As pathogens deploy virulence effectors to disrupt the immune system, NLRs initiate a spatially coordinated immune response that restricts pathogen propagation and triggers localized programmed cell death. Mechanistically, NLRs oligomerize into resistosomes that directly or indirectly regulate cytoplasmic calcium levels, thereby modulating growth, resistance and cell death. Although some resistosomes can form calcium-permeable channels, enzymatic activity from TIR proteins appears to be required for prolonged cytoplasmic calcium influx and cell death. While the function of these receptors have been examined in detail during the plant immune response, the impact of NLR signaling on resistance and cell death during abiotic stress responses is unclear. We employ genetic approaches to identify major regulators of this TIR/NLR signaling module and examine their role in abiotic stress responses.

Studying Cell-Type Specific Transcriptional Responses To Salt Stress In Pennycress (Thlaspi arvense) Roots Using Single Nuclei Transcriptomics

Sanchari Kundu and Song Li,

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Soil salinization threatens over 800 million hectares of agricultural land globally, rendering vast areas unsuitable for traditional crops. Winter cover crops like pennycress (Thlaspi arvense) offer a promising solution for these marginal saline lands, providing oilseed production on otherwise unproductive acreage. However, the cellular mechanisms underlying pennycress salt tolerance remain poorly understood, limiting efforts to optimize this emerging crop for saline environments. To investigate cellular responses to salt stress in pennycress, we examined 6-day-old seedlings treated with 75 mM NaCl. Root tissues were harvested post-treatment and processed for both bulk RNA-seq and single-nucleus RNA-seq. Bulk RNA-seq differential expression analysis identified 1,183 differentially expressed genes (padj < 0.05), revealing enrichment of photosynthesis-related processes and water deprivation responses among upregulated genes, while downregulated genes were enriched in oxidative stress response, defense responses, and immune system regulation. Single-nucleus RNA-seq yielded 28,022 high-quality nuclei across four samples (control and salt-stressed, two biological replicates each), detecting approximately 1,351 genes per nucleus in control samples and 1,666 genes per nucleus in salt-stressed samples, providing a comprehensive resource for dissecting cell-type-specific salt stress responses in pennycress roots. Cross-species marker gene identification revealed 10 major root cell populations. Comparative analysis revealed that salt stress dramatically altered cellular composition, with a 39% reduction in actively dividing meristematic cells, a 23% increase in vascular tissue populations (phloem and xylem pole pericycle), and a 40% reduction in root

endodermis cells. Cell-type-specific differential expression identified key regulators of these shifts, including upregulation of NRT1.5 (AT1G68570) in xylem pole pericycle and downregulation of auxin signaling genes ARF2 and AUX, linking reduced auxin transport to lateral root suppression. These shifts in cell-type proportions indicate that pennycress roots undergo defensive cellular remodeling under salt stress, prioritizing vascular expansion for ion exclusion and water management while suppressing growth-related processes. Furthermore, root growth measurements at 48 hours post-treatment revealed growth inhibition at 75 mM NaCl, directly correlating with the reduction in meristematic cells observed at 24 hours and demonstrating that early cellular compositional changes predict subsequent developmental phenotypes. These findings reveal the cellular mechanisms enabling pennycress salt tolerance and establish a cell-type-resolved transcriptomic atlas that can guide breeding efforts to optimize this winter cover crop for productive oilseed production on marginal saline agricultural lands.

Deciphering the Molecular Mechanisms of Abscisic Acid-dependent Gene Regulation in Plants

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The plant hormone abscisic acid (ABA) plays a central role in drought responses by inducing stomatal closure in guard cells and reprogramming gene expression across different plant cell types. Chromatin accessibility is a key determinant of gene activation. Previous studies indicate that ABA triggers extensive remodeling of chromatin structure, and its opening requires four related transcription factors (TFs), known as ABF1-4 proteins. Current understanding lacks the mechanisms by which ABF TFs coordinate with other proteins to initiate chromatin remodeling during ABA/drought responses. This project explores the molecular mechanisms of ABA-dependent gene regulation in Arabidopsis thaliana. We employed TurboID-based proximity labeling, followed by streptavidin affinity purification and mass spectrometry to identify proteins that interact with the ABF4 TF. Notably, histone acetyltransferase 1 (HAC1) was the second-most-abundant protein detected, after ABF4 itself. Histone acetylation is known to play a major role in transcriptional activation. Yeast two-hybrid experiments indicated that HAC1 directly interacts with ABF proteins. Using cell-type-specific epigenomic approaches, we have discovered that ABA triggers genome-wide changes in histone acetylation. Interestingly, ABA-induced acetylation coincided with genomic regions with ABA-stimulated chromatin accessibility and transcription. Next, we will determine if this acetylation depends on ABF TFs and investigate the role of HAC1 in ABA responses. This research will deepen our knowledge of plant genome regulation under drought stress, aiding efforts to improve drought tolerance in plants.

Abstracts from Talks
Thursday, May 28th

KEYNOTE TALK

Leaf Surface RNA: How does it get there and what does it do?

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Plants produce abundant extracellular RNAs (exRNAs) that can be isolated from the leaf apoplast (intercellular space) and from leaf surfaces. These RNAs are not packaged into extracellular vesicles (EVs), but are remarkably stable despite the presence of extracellular RNases. These exRNAs come from diverse sources, including microRNAs, but tRNAs and tRNA fragments are especially abundant, which suggests that secondary structure and post-transcriptional modifications may be important for exRNA stability. How and why these RNAs are secreted are central questions that we have been pursuing over the last two years. Our recent data indicates that exRNA is derived from vacuolar bulbs, which are vesicular structures inside vacuoles derived by invagination of the tonoplast membrane. These bulbs contain bulk RNA from the cytoplasm and can be released to the extracellular space by fusion of the tonoplast and plasma membranes in a localized and controlled manner. Once released, we posit that these bulbs are quickly ruptured, exposing the RNA to extracellular RNases, which degrade most single stranded RNA, but leaving double-stranded RNAs such as those found in tRNAs and rRNAs. We hypothesize that these stable RNAs play an important role in maintaining the homeostasis of plant microbiomes and in defending plants against pathogens. Our preliminary data indicate that leaf surface RNAs may also play a central role in host-induced gene silencing of fungal and bacterial genes, thus knowledge of how exRNAs are produced and stabilized may enable more effective use of exRNAs in crop protection.

Session IV: Plant Development and Cell Biology

Cytosolic- and membrane-localized oxidized indole-3-acetic acid formation regulates developmental auxin transients

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Catabolism of the auxin indole-3-acetic acid (IAA) to terminate cellular responses primarily occurs in three steps: 1) conjugation of IAA to Asp/Glu, 2) oxidation of the indole ring by DIOXYENASE FOR AUXIN OXIDATION (DAO), and 3) amidohydrolase cleavage of Asp/Glu. This study examines if IAA oxidation historically associated with membranes is mediated by DAO isoforms and if oxidized auxin product (oxIAA) retains nominal functionality. We show that Arabidopsis DAO1 does exhibit both soluble and auxin-dependent plasma membrane association and that oxIAA exhibits weak “anti-auxin” activity. Both soluble and membrane-associated DAO1 primarily oxidize IAAsp. DAO2 activity is enzymatically similar to DAO1 and occurs where IAA levels are high. DAO1 and DAO2 function synergistically in adventitious root formation and in temperature-dependent petal development. In vitro assays show that oxIAA acts as a molecular glue between repressor AUX/IAA (IAA7 and IAA17) and TIR1 auxin co-receptors, but is readily outcompeted by IAA. BioLayer Interferometry and yeast degradation assays indicate weak “anti-auxin” activity, as oxIAA enhances IAA7-TIR1 interactions while retarding IAA7 and IAA17 degradation. In a low auxin/quiescent interval that precedes auxin-triggered apical hook opening in etiolated seedlings, IAA7 gain- and loss-of-function mutants exhibited early apical hook opening similar to dao1, and application of oxIAA to etiolated dao1 apical hooks partially rescued the phenotype. The weak "anti-auxin" activity of oxIAA during a transitional growth is an important reminder of the evolutionary processes that forge adaptive plant growth responses.

Adaptive Divergence of Meiotic Recombination Patterns in Maize Lineages from Distinct Climatic Origins

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Meiotic recombination is a fundamental biological process that generates crossovers (COs) during meiosis, contributing to genetic diversity in offspring. While several mechanisms underlying CO formation in plants have been characterized, a comprehensive understanding of CO patterns and their regulation, particularly across sexes and lineages, remains to be elucidated. In this study, we investigated variation and regulatory factors influencing meiotic recombination dynamics using male and female maize lines derived from distinct climate zones. Through analyses of CO landscapes based on multiple genome sequencing datasets

of hybrid crosses, we found that recombination patterns differ significantly between tropical and temperate lines in males, while remaining relatively stable in females. Transcriptomic profiling revealed that synapsis-related genes and heat shock proteins exhibit differential expression between temperate and tropical maize lines during male meiosis, suggesting pivotal roles for CO interference and stress-response pathways in shaping meiotic recombination dynamics. Additionally, through epigenetic analysis we observed that standing variation in DNA methylation, particularly CHH methylation may influence recombination variability in female meiocytes across lineages. These findings, together with continued investigations into genetic and epigenetic factors, deepen our understanding of meiotic recombination as shaped by genetic background and cultivar origin, advancing strategies for crop breeding and genetic diversification.

Polyamine Metabolic Plasticity in Plants: A Key Player in Growth, Development and Stress

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Polyamines are important growth regulators that are ubiquitously present in plants and play fundamental roles in cellular homeostasis, growth, development, and environmental adaptation. Emerging evidence highlights the remarkable plasticity of polyamine metabolism and its integration with multiple signaling and metabolic networks that coordinate plant responses to developmental and environmental cues. Genetic components involved in the biosynthesis and catabolism of these amines (Putrescine, Spermidine and Spermine) have been uncovered in various plant species. This presentation will explore the dynamic molecular-genetic regulation of polyamine metabolic flexibility that enables plants to optimize growth while maintaining resilience under stress, and holds the key to traits including root growth, disease resistance, drought tolerance and fruit development and ripening. Further, insights from molecular, genetic, biochemical, and omics-based studies suggest that polyamine metabolic networks function as central regulators of plant adaptive plasticity. Understanding these regulatory mechanisms offer promising opportunities for engineering stress-resilient crops and uncover hidden potential of these growth molecules in regulation of unknown biological processes in unexplored plant species.

Molecular Characterization of the Wheat C-locus Controlling Spike Architecture

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Innovative genetic improvements in food crops are essential for global food security. Spikelet number per spike and grain number per spike are critical determinants of yield in cereals. Increasing fertile spikelets per spike without increasing plant height or biomass offers an opportunity to boost productivity and improve breeding efficiency. The C-locus, also known as the compactum locus, is one of three major domestication loci influencing wheat spike evolution, along with the Q locus (responsible for free-threshing bread wheat) and the S-locus (controlling subspecies diversification in T. sphaerococcum). However, the molecular basis of the C-locus, controlling T. compactum diversification, has remained uncharacterized, largely due to its proximity to a low-recombination region, hindering targeted breeding efforts. We employed recombination-independent mapping tools, including the MutMap pipeline, which was assisted by a platinum-quality telomere-to-telomere genome assembly of the C-locus donor line ‘Corrigin’, to positionally clone the gene to chromosome 2D (261.0–273.0 Mb). Comparative genome analysis revealed a previously hidden centromeric inversion in club wheat as compared to the Chinese Spring genome, explaining the historical difficulty in mapping the C-locus and exposing previously concealed genetic variants. To identify the specific gene, we performed TILLING mutagenesis in 'Corrigin' and generated seven independent mutant lines exhibiting lax spike phenotypes. RNA sequencing of these mutants identified a single candidate gene harboring key SNP variations. This gene regulates internodal length throughout the plant, with enhanced expression specifically in the spike. Combining C-locus lines with genotypes carrying enhanced spike length creates a powerful platform to increase grain yield, which was evident by F2 lines displaying a range of spike lengths with proportionally increased spikelet numbers, demonstrating the independent modulation of both yield components. These results provide a molecular foundation for developing gene-editing strategies targeting meristem regulators to precisely modify yield components, supporting breeding efforts to increase wheat productivity, and ensuring food security.

CorNIchon Homologue proteins regulate calcium signaling and root development in Arabidopsis thaliana

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One of the COPII cargo-adaptors essential for proper protein targeting to their subcellular membranes are CORNICHON-HOMOLOGUE (CNIH) proteins. There are five CNIHs in Arabidopsis thaliana and have been shown to target ion channels like GLutamate Receptor-like channels (GLRs) to their correct location and modulate their activity. GLRs and other ion channels affect cytosolic Ca2+ levels in response to external stimuli. To understand the role of CNIHs in plant development through Ca2+-signaling, we took a family-wide reverse genetics approach. Phenotypic analyses of single and higher order AtCNIH mutants reveal that AtCNIHs are important for plant development, importantly for root development. Higher-order AtCNIH mutants show significantly impaired root growth, with 40-60% reduction in primary root length and altered root hair development. Our data suggest an epistatic and a possible agonistic and antagonistic function of different CNIHs, indicating a complex regulatory network to modulate the localization and functionality of ion channels. Ca2+ imaging in roots of AtCNIH mutants showed reduced Ca2+ response to GLR agonist L-Glutamate, and inhibition of Ca2+ responses by the GLR inhibitor AP5, suggestive of a GLR involvement. These findings support the hypothesis that AtCNIHs are essential for root development and may function as components of Ca2+ signaling regulatory mechanism.

Session V: Botany, Ecology and Evolution

Sex-specific flowering responses to environmental cues: implications for plant fitness in a changing climate

Daniel Buonaiuto

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Plants are immobile, most are hermaphroditic, and many can self-pollinate—traits that can create substantial barriers to effective mating. One of the most widespread adaptations plants have evolved to overcome these challenges is dichogamy—the temporal separation of male and female flowering phases. By reducing self-interference and promoting outcrossing, dichogamy enhances the adaptive capacity of plant populations in a rapidly changing world. Climate change has already driven major shifts in flowering time (i.e., phenology) across a diversity of plant taxa, making phenological change one of the clearest biological indicators of climate change to date. Yet few studies have directly compared shifts in the timing of male versus female flowering phases, and the physiological mechanisms controlling these sequences remain poorly understood. This gap presents a major obstacle to predicting changes in plant reproduction and demography under novel environmental conditions. Here, I present a suite of case studies including both monoecious and dioecious taxa that suggest male flowering phases respond more strongly to temperature cues than female phases. I discuss the implications of these differences for dichogamy and, more broadly, for plant fitness as the climate continues to change

Eastern Temperate Forests' Potential For Climate Adaptation

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The phenological emergence of leaves from buds of temperate eastern North American trees has advanced 5 to 10 days over the past 50 years. This early onset of spring creates a feedback with the climate system, making our ability to predict phenological timing a key component of predicting climate change. To date, scientists have detailed the environmental drivers and the genetic controls of leaf-out variation in plants, finding both significant plasticity and heritability. However, researchers have yet to unite this knowledge in large-scale predictive frameworks. My talk covers my lab's recent efforts to examine both the genetic and environmental underpinnings of variation in northern red oak (Quercus rubra) phenology

and how this data can be leveraged in conjunction with the phenocam network to improve regional prediction of climate response.

Dissecting the Genetic Basis of Trichome-Mediated Defense in Tomato (Solanum lycopersicum)

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Insect herbivory remains a major constraint to tomato (Solanum lycopersicum) production, driving substantial yield losses and continued dependence on synthetic pesticides. These inputs impose economic and environmental costs and are increasingly undermined by the rapid evolution of pest resistance. This work explores an alternative strategy centered on strengthening endogenous plant defenses through the deployment of glandular trichomes and their associated specialized metabolites. Here, we focus on type-IV glandular trichomes, specialized epidermal structures capable of producing acylsugars with demonstrated insect-deterrent activity, commonly found in wild tomato relatives but largely absent or developmentally restricted in cultivated backgrounds. Our central premise is that trichome development and acylsugar biosynthesis can be genetically decoupled and strategically reassembled to enhance resistance in elite germplasm. To address this, we integrate genetic mapping, transcriptomic profiling, and functional genomics to identify regulatory loci controlling trichome initiation, persistence, and metabolic output. Candidate genes are prioritized using RNA-seq–guided approaches and validated through targeted gene editing (CRISPR/Cas9). In parallel, we characterize acylsugar chemotype diversity across introgression lines to define structure–function relationships underlying bioactivity against key insect pests and foliar pathogens. Building on these insights, favorable allelic combinations are pyramided into cultivated tomato using marker-assisted selection, generating pre-breeding lines with enhanced resistance and minimal agronomic penalties. This work advances a framework for converting a complex, quantitative defense trait into a tractable breeding strategy. More broadly, it provides a scalable path toward reducing pesticide reliance, improving crop resilience, and promoting environmentally sustainable production systems.

Identifying the genetic mechanisms controlling stomatal CO2 responses in the strawberry plant Fragaria vesca

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Dynamically sized pores called stomata control gas exchange between plants and their environment. Specialized cells known as guard cells regulate the size of the stomatal

aperture to balance carbon dioxide (CO2) uptake with water loss through transpiration. Changes in atmospheric [CO2] are known to trigger rapid stomatal movements in many plants species. Research in the model plant Arabidopsis thaliana has discovered key genes that control stomatal movements. For example, in Arabidopsis guard cells the protein HT1 acts a sensor for CO2, thereby coordinating stomatal aperture size with [CO2]. However, we do not know how stomatal opening/closing is regulated in plants that more closely resemble agricultural crops. To investigate this question, we sought to identify potential stomatal regulatory genes in Fragaria vesca, commonly known as wild strawberry. Using time resolved gas exchange analysis we characterized the stomatal responses to changes in atmospheric [CO2] and to abscisic acid (ABA) hormone treatment in F. vesca. Using homology alignment, we identified the HT1 and SLAC1 orthologs in the Fragaria vesca genome. To test the functional conservation of the identified genes we performed a cross-species rescue experiment. We found that transgenic expression of F.vesca HT1 could restore stomatal [CO2]-responses in Arabidopsis ht1 mutant plants. To study the natural function of these genes, we are currently generating loss-of-function mutations in HT1 and SLAC1 in Fragaria vesca using CRISPR-Cas9. By better understanding the regulation of stomatal CO2 responses in crop-like plants, we can provide insight on trait improvement in the face of the continuing rise in atmospheric [CO2].

Extracellular RNA in and on Maize Leaves: A Hidden Layer Contributing to Microbiome Assembly

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The Innes Laboratory investigates molecular mechanisms underlying host-induced gene silencing in plants, focusing on how regulatory RNAs move from plants to their pathogens and pests. Earlier this year, we reported that Arabidopsis leaves are coated with diverse RNA species, with tRNAs being especially abundant. To explore whether this phenomenon extends to crop species, we examined maize (Zea mays) grown under field conditions. We assessed whether field-grown maize leaves are also coated with RNA and, if so, whether these RNAs influence the make-up of the leaf surface microbiome. We also assessed whether endophytes residing in the apoplastic space differed from those on leaf surface. Leaf surface RNA and apoplastic RNA was isolated from five-week-old plants grown in fields at the University of Kentucky. Denaturing gel analyses and Illumina RNAseq and sRNAseq

revealed that maize, like Arabidopsis, secretes a broad range of long and small RNAs. The majority of maize-derived reads corresponded to non-nuclear rRNAs, with uneven read distribution suggesting that specific rRNA subregions resist degradation. We also identified abundant tRNA fragments, consistent with previous findings in Arabidopsis. At the microbiome level, most RNA reads mapped to bacteria, followed by fungi and archaea. The bacterial genera Siphonobacter, Chryseobacterium, and Sphingomonas were the most abundant. We are now developing synthetic bacterial communities representing the maize leaf surface microbiome to test their sensitivity to leaf surface RNAs. Building on these findings, our bioinformatic analyses identified 39 small RNAs belonging to diverse biotypes, including rRNA fragments (rRFs), multiple classes of tRNA-derived RNAs, as well as miRNAs and other ncRNAs. To assess their biological roles, we developed a high-throughput screening platform—Ex-MAP (Extracellular RNA Assay Plate)—containing synthetic versions of these RNAs. Using Ex-MAP, we co-incubated Pseudomonas syringae strain DC3000 with individual synthetic RNAs and observed that seven of them significantly altered bacterial growth compared to controls. We next assess whether these RNAs are taken up by P. syringae. We synthesized one rRF and one tRF with a fluorescent Cy5 label and then incubated these with bacterial cells along with a quencher to differentiate between extracellular and intracellular signal. Both RNAs were taken up P. syringae strain DC3000, providing a proof of concept that extracellular RNA can be taken up by bacteria and inhibit their growth. We are now extending this approach to test the impact of extracellular RNAs on synthetic bacterial communities (SynComs) representing the maize leaf microbiome. This work will help elucidate whether specific plant-derived extracellular RNAs influence microbiome composition and selection at the leaf surface.

POSTER ABSTRACTS
ODD NUMBER POSTERS #257-537
Wednesday May 27th

257 **Understanding waterlogging stress response in diverse maize germplasm using UAV-based LiDAR and SpATS for spatial trend modeling**

Manoj Subedi[1], Jeonghwa Kim[1], Tecle Weldekidan[1], Grace Chropka[1], Joseph Romano[1], Shreejana KC[1], Yin Bao[12], Qi Mu[1] [1]Department of Plant and Soil Sciences, University of Delaware, Newark, DE, 19716 [2]Department of Mechanical Engineering, University of Delaware, Newark, DE, 19716

Screening for waterlogging stress tolerance is essential to address the increasing incidence of extreme rainfall events resulting from climate change. Spatial trend in the field is the most common and influential source of error in field experiments. While conducting a waterlogging field trial, the issue of uneven land exacerbates errors, resulting in biased estimates. To address this, we used LiDAR, a remote sensing technique, to estimate the level of submergence of each plot during waterlogging conditions. We used SpATS (Spatial Analysis of Field Trials with Splines), a model developed by MX Rodríguez-Álvarez et al. 2016, to unravel the inherent spatial trends in the field. Additionally, we fitted a linear mixed effect model to quantify the effect of submergence level on waterlogging response. This way, we screened 50 diverse maize accessions in a randomized complete block design. Waterlogging treatment was applied by building berms around the field at 25 DAP which continued for 9 days. Waterlogged plants, in general, flowered late, were stunted and produced less leaves, less shoots and ears and had smaller/deformed tassels compared to the control. The SpATS model revealed a strong spatial trend along the submergence gradient, while the fitted linear mixed-effects model confirmed that waterlogging response varied strongly with submergence level. We also identified lines with potential susceptibility, as they consistently showed lower performance across traits; however, several lines demonstrated tolerance, though their response varied across traits.

277 **Novel insights into the transcriptional regulation of iron homeostasis using a whole-genome heterodimeric yeast one-hybrid approach.**

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Iron (Fe) deficiency-induced anemia is the most pervasive nutritional disorder affecting 30% of the world's population as their plant-based diets lack this essential nutrient. Plants utilize diverse mechanisms to regulate the uptake, trafficking and storage of Fe. While there have been recent advancements at elucidating the transcriptional regulation of Fe uptake in plants, the upstream regulators of the OLIGO PEPTIDE TRANSPORTER 3 (OPT3) - a key player in long-distance shoot-to-root iron signaling – remain unknown. To investigate combinatorial transcriptional regulation of OPT3, an HD-Y1H (heterodimeric yeast one-hybrid) screen was performed leading to the identification of TFs capable of binding to the promoter region of OPT3 (OPT3p) in the presence/absence of bHLH100, a key transcriptional regulator of Fe deficiency responsive genes. The binding of putative candidates was validated through electrophoretic mobility shift assays (EMSA) and further revealed cooperative effects of bHLH100 with additional transcription factors (TFs). To further assess the functional synergism between TFs, we performed transactivation assays in N. benthamiana leaves and measured the relative LUC/REN activity for different TFs co-expressed with bHLH100 and OPT3p driving the luciferase reporter gene. We are currently exploring the genome wide binding profiles of selective hetero-dimeric complexes using DNA-Affinity Purification Sequencing. We expect that this data will pave way to mechanistic insights into recognition, binding, and activation of Fe responsive genes driven by multiprotein TF complexes.

337 **Changes in root architecture and drought stress responses associated with DEEPER ROOTING 1 in apple rootstocks**

Brett Shelley and Lisa Tang Appalachian Fruit ResearchStation, USDA ARS

Apple (Malus x domestica) production in the U.S. depends on rootstocks for scion precocity and size control as well as ability to withstand abiotic stress such as drought, which severely limits fruit quality and yield. Understanding rootstock-conferred tolerance at the whole-tree level is critical for adapting trees to dry growing seasons and reducing irrigation costs. Overexpression of DEEPER ROOTING 1 (DRO1) in many crops was associated with narrower root angles and deeper rooting depths, indicating drought escaping mechanism upon soil water deficit. We evaluated the effects of overexpressing peach (Prunus persica) DRO1 in ‘Malling 26 (M.26)’ apple rootstock background on root growth and architecture, horticultural

traits, water potential, and other physiological parameters. Results suggested that DRO1 overexpression provides delayed onset of stress symptoms during a one-week drought period compared to the non-transgenic control. Time-course leaf transcriptomes during dehydration are being analyzed in both DRO1 lines and wild-type 'M.26' control to uncover the mechanism behind DRO1-associated drought tolerance with additional efforts being made to understand the interactions between DRO1 and the other signaling pathways in apple. Comparisons are also being made to determine if the effects of DEEPER ROOTING 1 drought tolerance can be passed onto different scions and among commercially utilized rootstocks.

395 **Disrupting Upstream ORF Translation for Enhanced Protein Production in Plants**

Authors: Jade Lyons, Chengsong Zhao, Jose Alonso, Anna Stepanova Institution: NC State University

Understanding how gene expression is regulated is essential for developing new biotechnological approaches to address agricultural challenges. Translation is a key regulatory layer of gene expression that shapes protein production. Advances in ribosome profiling and other high-throughput approaches have revealed that upstream open reading frames (uORFs) are pervasive regulatory elements within 5' untranslated regions (UTRs) that modulate translation of downstream main open reading frames. While uORFs are known to regulate translation, further studies are needed to understand how these cis-elements influence translational outputs. In this work, I combine reporter-based assays and CRISPR-mediated genome editing to investigate translational regulation via uORFs. Using a dual-luciferase reporter system in Nicotiana benthamiana, I assess the effects of 5' UTR variants with and without uORFs on luciferase activity. In parallel, I apply genome editing to modify endogenous 5' UTRs, such as that of EIN2, a central regulator of ethylene signaling, to explore how uORF architecture may influence translation in native contexts in Arabidopsis. Together, this work is providing insights into how uORFs contribute to translational control, paving the way to targeted gene expression regulation for smart crop design.

417 **Dissecting Stripe Rust Resistance in Wheat Using Multi-Strain Phenotyping and Reference-Free k-mer GWAS in Einkorn wheat**

Parva Sharma, University of Maryland, College Park, MD, USA Paula Silva, INIA, Uruguay Xianming Chen, USDA-ARS, Pullman, WA (Wheat Health, Genetics & Quality Unit) Vijay Tiwari, University of Maryland, College Park, MD, USA

Bread and pasta wheat form major foundation for global food security. One of the biggest threat for wheat production is a fungal disease known as stripe rust, caused by Puccinia striiformis f. sp. tritici. Recently a major resistance gene Yr15 that was deployed in global wheat breeding germplasm, broke down under evolving pathogen pressure. Quick discovery of new genes and alleles are a must to fight this unprecedented situation. To address this key concern, we utilized our simple and elegant diploid A-genome resources that include pangenomes, sequenced GWAS panel, sequenced RIL population and candidate gene validation resources such as TILLING and gene editing. We employed genomics enabled k-mer GWAS matrix to identify novel QTL against 8 globally distinct stripe rust isolates. Excellent resistance against these isolates was observed in the TmGWAS panel. We identified major novel QTLs on chromosomes 5AL (100kb), 6AS (200kb), 6AL (100kb), 7AS (200kb) and 7AL (1.5Mb) in isolates PstS10-3404 (5AL and 6AS), PstS13-3860, PstV-14 and PstS10-3404 + PstS13-3457 resp. All these QTL regions have important genes involved in disease resistance like RGAs. Kinases, Ethylene responsive TF, F box LRR repeat containing and ABC transporter. Integration with a pan-genome framework enabled haplotype-based dissection of this region, revealing distinct resistance-associated haplotypes. Candidate gene analysis within the locus highlighted genes with typical disease-resistance signatures, including NLRs and kinase-related genes. These results provide a strong foundation for map-based cloning and functional validation of novel stripe rust resistance genes.These findings advance our understanding of stripe rust resistance and provide valuable targets for marker-assisted selection and gene discovery, supporting the development of wheat cultivars with improved and durable resistance.

423 **A novel loss-of-function mutation provides an exciting route for einkorn domestication and the tracking of its evolutionary history**

Edward Haag, Anmol Kajla, Parva Kumar Sharma, HS Dhaliwal, Bikram Gill, Nidhi Rawat, Vijay Tiwari University of Maryland Department of Plant Sciences and Landscape Architecture

Crop domestication has played a key role in human evolution. Domestication syndromes: the pool of traits that enabled the transition from wild relatives to cultivated crops, were central to the expansion of early human populations. As one of the eight founder crops, einkorn wheat (Triticum monococcum) was the first wheat species domesticated, laying the foundation for modern civilization. Despite its historical significance, the wider utilization of einkorn is restricted by its non-free-threshing spike. While domesticated varieties are non-shattering, the kernels remain tightly enclosed in the glumes, hindering high-throughput mechanical harvesting. To address this bottleneck, we utilized a Sodium Azide mutant population to identify a novel free-threshing mutant. We then developed two independent MutMap populations derived from the free-thrashing mutant. We performed MutMap analysis by sequencing a bulked pool of the recessive mutants. SNP indexing of the bulked populations revealed an index of 1 (indicating 100% mutant allele frequency) across two significant peaks on chromosome 2A, located from 380 Mb and 410 Mb for both individuals.. Fine mapping using KASP markers enabled high-resolution narrowing of this interval, and candidate gene identification was achieved through systematic screening for genic mutations within the delimited interval. After analyzing the region we identified two novel genes with exonic SNPs. The primary gene at 405.3 Mb corresponds to a leucine-rich repeat (LRR) receptor serine/threonine protein kinase while the other gene at 406 Mb most likely relates to proline-rich extension-like receptor protein kinase (PERKs). Further, we will be validating between the candidate genes using TILLING approach using Cel-1 endonuclease (a mismatch-specific S1 nuclease derived from Apium graveolens) heteroduplexing to identify loss of function mutants. This research will demonstrate how we can use modern genomics to overcome historical domestication constraints, allowing einkorn wheat to be improved for practical cultivation while preserving its ancestral value.

425 **Evaluating DMR6 as a Susceptibility Factor in Durum Wheat – Fusarium graminearum Patho system**

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Fusarium head blight (FHB), caused by Fusarium graminearum, is a major challenge in wheat production due to its impact on yield and grain quality, particularly through the accumulation of mycotoxins such as deoxynivalenol (DON). While resistance breeding has advanced in hexaploid bread wheat (AABBDD), improving FHB resistance in tetraploid durum wheat (AABB) is often constrained by limited genetic diversity and fewer deployable resistance sources. An alternative and complementary strategy is to reduce host susceptibility by targeting plant genes that facilitate disease development, especially given that durum wheat is generally more susceptible than bread wheat to F. graminearum. Here, we tested whether the wheat ortholog of Downy Mildew Resistance 6 (DMR6), a known susceptibility gene in plants Arabidopsis thaliana and tomato, functions as a susceptibility factor for FHB in durum wheat. We identified that durum wheat has six copies of the DMR6 across chromosomes 2A, 2B, 5A and 4B. The closest orthologs were selected based on protein similarity on chromosomes 2A and 2B. Two truncation mutations on chromosome 2A, and two functional mis-sense mutation on 2B were obtained from UC Davis Kronos Tilling resource. Backcrosses were made of the individual mutants by crossing with wild type Kronos, as well as A and B genome mutants were crossed to obtain full-null mutant for the 2A and 2B homeologs. Homozygous individuals were selected for the various combinations and evaluated for FHB severity and DON accumulation. Disruption of DMR6 reduced both FHB severity and DON levels, supporting DMR6 as a susceptibility factor in durum wheat. Comparisons among single- and combined-homeolog disruptions indicated partial redundancy between the 2A and 2B copies and suggested that combined loss can provide stronger protection. Importantly, DMR6 disruptions did not impose detectable penalties on key agronomic traits, highlighting their potential utility for breeding. These results demonstrate that targeting susceptibility genes such as DMR6 can complement traditional resistance breeding and accelerate development of durum wheat with improved FHB resistance and grain safety.

439 **Transgene-free genome editing in citrus and poplar meristem tissues via biolistic ribonucleoprotein delivery of CRISPR-Cas9**

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The use of meristematic tissues offers a strategic approach to genome editing in woody species, especially those that are recalcitrant to conventional tissue culture, as these regions contain totipotent, highly regenerative cells capable of giving rise to whole plants. Here, we employed biolistic delivery of genome-editing reagents into the shoot apical meristem (SAM) of citrus and the axillary meristems (AXM) of poplar. The system was first validated using a GFP expression construct and subsequently applied for targeted genome editing. In citrus, edited plants were obtained at the CsNPR3 locus exclusively through the delivery of CRISPR/Cas9 ribonucleoproteins (RNPs), whereas plasmid-based vectors were unsuccessful. Similarly, genome editing in poplar was achieved using RNPs targeting the Pt4CL1 gene. Although chimeric events were detected, this approach provides a feasible and innovative framework for producing transgene-free edited perennial plants.

457 **The Role of SR45 in Regulating Photosynthesis Under Stress**

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As atmospheric CO2 levels rise and climate-related stresses intensify, understanding mechanisms that regulate photosynthesis under stress responses is increasingly important. The goal of this project is to investigate how the Serine/Arginine-rich 45 (SR45) regulates photosynthetic responses in Arabidopsis under stress. When comparing proteome between Col-0 and sr45-1 null mutant, common pathways were identified in both leaf and inflorescence tissues suggesting that the effect of SR45 on these pathways are independent of developmental stages. One such protein target that plays a role in photosynthesis is Ferredoxin-NADP+ Oxidoreductase 2 (FNR2), an enzyme that catalyzes electron transfers to produce NADPH, particularly under stressful conditions. Western blot confirmed the reduced FNR2 abundance in sr45-1 as identified in proteomics studies. Next, we assessed

the role of SR45 in abscisic acid (ABA) mediated stress responses associated with photosynthesis. Vegetative tissues from Col-0 and the sr45-1 mutant were compared to evaluate effects of ABA on stomatal conductance, stomatal aperture, stomatal reactive oxygen species (ROS) accumulation, and anthocyanin levels. Preliminary gas exchange measurements using a LI-COR 6000 indicate that Col-0 and sr45-1 respond similarly to changes in [CO2], however, Col-0 appears to be more sensitive to ABA than sr45-1. Stomatal aperture measurements show sr45-1 stomata are less open, and preliminary ROS measurements show more ROS accumulation in sr45-1 stomata. Anthocyanin content increased significantly in response to ABA with no genotype specific differences. Overall, these preliminary results suggest that SR45 may contribute to photosynthetic responses to ABA by influencing FNR2 abundance and regulating ABA-associated stomatal responses. More trials are needed to validate these preliminary findings in the future.

459 **Opposing Roles of Arabidopsis Apoplastic Amino Acids in Controlling Pseudomonas syringae Virulence**

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The leaf apoplast is the first internal plant compartment encountered by foliar bacterial pathogens after they breach the leaf surface. Long viewed primarily as a nutrient-rich extracellular space, the apoplast is now emerging as a dynamic immune-regulated metabolic environment in which plant-derived metabolites actively shape bacterial behavior. Recent work with Arabidopsis-Pseudomonas syringae pv. tomato DC3000 shows that free L-amino acids in the leaf apoplast do not simply support bacterial growth. Instead, their abundance, timing, and composition are remodeled during the onset of plant immunity, and these changes influence whether bacteria successfully activate virulence programs or not. Together, the evidence presented here supports a model in which pattern-triggered immunity modifies amino acid transport and accumulation in the leaf apoplast, leading to changes in glutamine, serine, valine, and other amino acids that delay or suppress bacterial virulence gene expression. These findings reposition plant-made apoplastic amino acids as immune-active metabolites that can either support bacterial proliferation or interfere with the onset of pathogenicity depending on concentration, timing, and immune context.

461 **Leaf conductance and hydraulics on photosynthetic adaptations in common beans from Andean and Mesoamerican gene pools**

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Enhanced, environmentally resilient photosynthetic gas exchange traits can support efficient plant growth and productivity under fluctuating environmental conditions. Such adaptations can reduce yield penalties under drought and heat stress, among other common environmental stresses. However, knowledge of existing photosynthetic gas-exchange adaptations in most economically important plants remains limited, especially in key C3 crops such as common beans, which are vital for global food security. We investigated the photosynthetic gas-exchange characteristics (including responses to light and CO2), leaf hydraulics, and the cellular characteristics of the photosynthetic gas-exchange sites of selected common bean genotypes from the Andean and Mesoamerican gene pools. Our findings indicated distinct differences in leaf anatomy and cellular features that influenced photosynthetic gas exchange in response to varying light and CO2 levels. These differences directly affect stomatal conductance to CO2, leaf mesophyll conductance, and water supply and loss from the leaves. Our analysis of the identified leaf hydraulic anatomies suggested possible adaptations to environmental temperature and moisture levels. Our results reveal unique combinations of photosynthetic traits in common beans, including leaf tissue and cellular characteristics, among common beans from the Andean and Mesoamerican gene pools that could be exploited for strategic manipulation of CO2 conductance, leaf hydraulics, and gas exchange characteristics, suitable for breeding efforts to improve photosynthetic efficiency and enhanced environmental stress resilience in common beans.

473 **Quantification of α-Tocopherol in Plant Extracts by HPLC–UV Reveals an Unusually High Vitamin E Source in Medicinal Plant**

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Vitamin E is a group of lipid-soluble antioxidants essential for protecting cellular membranes against oxidative damage, with α-tocopherol representing its most biologically active form. Although plant-derived foods contribute to dietary vitamin E intake, reported concentrations in fruits and vegetables are generally low, with commonly consumed sources such as spinach one of the highest vitamin E containing vegetables. In this study, high-performance liquid chromatography with UV detection (HPLC–UV) was employed to quantify α-tocopherol in selected plant extracts. Spinach was analyzed as a reference due to its well-documented vitamin E content. The method provided effective separation and reproducible detection of α-tocopherol across all samples. Quantitative analysis revealed substantial variation among the studied plants. Notably, several samples exhibited very higher α-tocopherol concentrations, with sample KY146 showing the highest concentration of 37.1 mg/100 g. Comparative analysis of spinach under identical conditions showed much lower α-tocopherol levels, with Baby (Smooth-leaf) spinach containing 2.4 mg/100 g and Asian spinach 1.7 mg/100 g. This research identifies a plant with unexpectedly high vitamin E levels, presenting a promising new natural source for this essential nutrient. Keywords: New source of α-Tocopherol; HPLC–UV; Nutritional analysis; Antioxidants; Natural product

477 **Deciphering the transcriptional regulation of the guard cell CO2 sensor HT1 (HIGH TEMPERATURE 1) in Arabidopsis thaliana**

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Pairs of guard cells form stomatal pores and are critical regulators of gas exchange, allowing CO2 uptake while facilitating water loss through transpiration. In C3 plants, this results in a low water-use efficiency of 1–3 g of carbon per kg of water lost. As of 2026, the atmospheric CO2 concentration has increased by over 50% from pre-industrial levels. With climate change, adverse and extreme weather conditions like drought are becoming more frequent. Both High [CO2] and the drought stress hormone abscisic acid (ABA) trigger stomatal closure to prevent water loss, impacting carbon assimilation and crop yield. In Arabidopsis thaliana, the guard cell

protein kinase HIGH LEAF TEMPERATURE 1 (HT1) is a key CO2 sensor. The ht1-2 null mutant has [CO2] insensitivity and constitutively closed stomata. Despite HT1's central role, the mechanisms regulating the transcription of HT1 in guard cells remain largely unknown. Our preliminary data show that ABA reduces chromatin accessibility in adjacent cis-regulatory regions of HT1, leading to transcriptional repression. The HT1 promoter contains motifs for ABA-regulated transcription factors, including MYB and AKS families. Further investigation revealed that myb30 T-DNA mutants exhibit impaired stomatal conductance at ambient CO2. Although quintuple aks mutants (aksx5) show no difference in stomatal responses, guard cell-specific overexpression of AKS1 increased stomatal conductance compared to wild-type plants. Using CRISPR-Cas9 mutagenesis, we are investigating the function of putative HT1 cis-regulatory regions in controlling HT1 transcription and stomatal responses. Additionally, we are generating higher-order MYB mutants to further elucidate molecular mechanisms governing stomatal control. Finally, we will identify interactors of the HT1 promoter using a CRISPR-based sequence proximity binding protein labeling (CSPL) assay.

483 **From Seed to Sequence: Genetic Profiling of Hemp Using Microsatellite Markers**

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Cannabis sativa L., or hemp, is a plant with prominent applications in industry, nutrition, medicine, and sustainable manufacturing, making it an increasingly important resource as global demand continues to grow. As this demand increases, maintaining genetic consistency within specific strains has become important, especially due to historically unregulated/undocumented breeding practices. This project aimed to establish a genetic profile for the Cherry Wine strain to serve as a reference for future comparisons across seed distributors. DNA was extracted from 20 Cherry Wine seeds. DNA concentration for each extraction was assessed using a Nanodrop spectrophotometer. Samples were then amplified using the polymerase chain reaction (PCR) targeting 19 microsatellite loci. The amplified fragments were separated using agarose gel electrophoresis, and alleles were scored based on molecular weight to construct the strain's genetic profile. This study produced a baseline genetic profile for Cherry Wine that can be used to evaluate consistency among distributors. This reference is important for supporting both growers and consumers of the hemp industry by reducing variability, improving strain reliability, and improving quality control.

485 **Deciphering Ethylene Biosynthetic Pathway Genetic Components In Duckweed Using Comparative Genomics and Transcript Profiling In Growth and Stress Conditions**

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Ethylene, a gaseous plant hormone, is known to play critical role in various growth, development and stress processes in land plants where its biosynthesis and signaling pathways are well characterized. Contrary to this, there is no well-established information in aquatic plants about ethylene biosynthesis and signaling components. Using comparative genomics, ethylene biosynthesis pathway encoding genes were identified in the genome of an aquatic plant duckweed (Spirodela polyrhiza). Findings reveal that duckweed genome possesses 2 ACS genes namely (SpACS1 and SpACS2) along with 3 ACO genes SpACO1, 2, 3, and three ACO-like genes- SpACS4-like, SpACO5-like, SpACO6-like. Evolutionary phylogenetic analysis reveals their closeness to the well-known other ACS and ACO proteins with presence of critical signature protein domains. Gene expression studies from Spirodela polyrhiza clone7498 indicate their functional presence with differential expressions in response to multiple stresses such as heat, cold, salt, copper and MeJA elicitor responses. Prominent gene expression patterns include-SpACS1 upregulation in heat, down regulation in salt; SpACS2 upregulation in copper and MeJA and down regulated in salt responses; SpACO1 upregulation in heat, copper, salt, and MeJA; SpACO2 upregulation in heat, copper, and MeJA; SpACO3 upregulation in copper, and MeJA; SpACO4-like upregulation in heat, copper, and MeJA and down regulation in salt; SpACO5-like upregulation in heat, copper, and MeJA and down regulation in salt; SpACO6-like upregulation in heat, copper, and MeJA and down regulation in salt. These expression patterns reveal a dynamic functional ethylene pathway in duckweeds. Overall, this study reveals the existence of a miniature size ethylene pathway suggests that aquatic plants also need this gaseous plant hormone and its precursor ACC.

487 **The Xenia Effect as a Potential Mechanism for Intra-Organismal Competition**

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Previous research demonstrated that different pollen donors can influence tomato maturation time, seed size, and trichome density via a phenomenon called the xenia effect. A fruit's pollen donor may also improve its competitive advantage by influencing sink strength and metabolite allocation. We hypothesized that greater genetic differences between the maternal plant and the pollen donor yield more pronounced xenia effects and will impact resource accumulation based on the pollen donor and that of the competing fruit. In this study, four heirloom tomatoes and wild-type S. galapagense were crossed with Tiny Tim tomatoes to produce competitive pairs of fruits on the same raceme. Flower pairs were isolated, emasculated, and then pollinated with combinations of self and foreign pollen before fruiting. Traits such as maturation time, sugar content, size, seed count, and exocarp thickness were recorded to identify potential xenia effects and competitive differences in sink strength. "Pair-type" and "role" groupings revealed significant differences that self-pollinated racemes were larger, less sweet, and had more seeds. Additionally, linear regression analysis between dependent variables demonstrated that internal mechanisms within the maternal plant, such as raceme dominance, seem to matter more in determining final fruit qualities than the pollen donor. Overall, significant xenia effects were catalogued and identified, but much more data needs to be collected. Understanding xenia is agriculturally relevant because targeted pollination can improve farmers' control over variables such as fruit sugar content and size. Furthermore, if xenia does influence sink strength, this could reveal a novel mechanism related to parental conflict theory and intraspecific competition.

491 **Deciphering Arsenic (As) Stress Tolerance in Cucurbitaceae: Insights from Physiological and Transcriptomic Analyses**

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Arsenic (As) contamination represents a major abiotic stress that undermines crop performance and food safety. To elucidate the molecular and physiological mechanisms underlying As tolerance, six cucurbit species, Lagenaria siceraria (Lagenaria), Cucurbita pepo (Pepo), C. maxima (Maxima), C. moschata (Moschata), Citrullus lanatus (Watermelon), and Cucumis sativus (Cucumber), were evaluated through integrated analyses of IC_{50} screening, physiological responses, As

accumulation, and orthogroup (OG)-level transcriptomics. IC_{50} values ranged from 8.94-17.28 mg kg^{-1}, reflecting broad variation in As sensitivity between species. However, physiological performance distinguished true tolerance, where Lagenaria maintained superior membrane integrity with minimal electrolyte leakage (2.2%) and stable photosynthetic performance (ΦPSII + 1.76%, ETR + 14.22%), whereas Moschata and Maxima showed marked declines. ICP–MS profiling revealed the highest As accumulation in Maxima roots (1789 µg/g DW), followed by Lagenaria (1253.46 µg/g DW) and Pepo (926.13 µg/g DW), while Cucumber exhibited the greatest shoot (11.28 µg/g DW) and fruit (2.19 µg/g DW) accumulation, indicating inefficient translocation control. OG-based PCA separated species according to molecular strategy: those with enhanced peroxidase activity, thiol-peptide transport, and ROS detoxification maintained physiological stability, whereas others relied mainly on stress signaling and proteostasis. Candidate gene analysis further highlighted RING E3 ligases (Lsi11G008250), aquaporins (Cp4.1LG00g00200), and TFs (WRKY, bZIP, MYB) as key detox regulators in tolerant species, contrasting with stress-inducible genes (MAPKKK18, HIPP7, HSP70) in susceptible species. This integrative framework delineates physiological and molecular coupling in As tolerance and identifies genetic targets for breeding As-resilient cucurbits and minimizing As accumulation.

493 **A Mobile P-loop NTPase Transcript (Lsi1G012550) from Lagenaria siceraria Coordinates Whitefly Defense Responses in Grafted Watermelon Scions**

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Silverleaf whitefly (Bemisia tabaci) is a major global pest causing severe yield losses in watermelon. Current management strategies rely heavily on insecticides, leading to resistance development, environmental contamination, and toxicity to non-target organisms. Grafting has emerged as a sustainable approach to improving crop resilience to biotic stresses. In this study, five watermelon graft combinations were evaluated under whitefly infestation to investigate grafting-mediated resistance mechanisms. Compared with ungrafted (UG) plants, grafted combinations exhibited significantly enhanced tolerance to whitefly stress. Among the evaluated rootstocks, LG3 (Lagenaria siceraria) showed the highest membrane stability index (82%), followed by MM30 (Cucurbita moschata × C. maxima) (80%), Carolina Strong Back (CSB) (68%), self-grafted (S/S) plants (67%), and UG plants (54%). Histochemical and physiological analyses revealed excessive accumulation of reactive oxygen species and increased cell death in UG plants, whereas grafted combinations, particularly LG3, displayed substantially reduced oxidative damage. Whitefly nymph density also significantly reduced in the heterograft combinations, with the highest

infestation observed in S/S plants (536 nymphs/plant), followed by UG (378), MM30 (355), CSB (281), and LG3 (131). To elucidate the molecular mechanism of this resistance, integrative transcriptomic and metabolomic analyses of the LG3 combination revealed a coordinated reprogramming of pathways associated with cell wall modification, lipid metabolism, hormone signaling, and defense responses. Furthermore, mobile transcript analysis identified several L. siceraria-derived coding RNAs translocated into the watermelon scion. Notably, Lsi1G012550, encoding a P-loop-containing nucleoside triphosphate hydrolase superfamily protein, emerged as a potential mobile signal associated with defense signaling and cell wall fortification under whitefly infestation.

495 **Identification of translationally active mobile mRNAs**

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Plant phloem transports a diverse group of molecules including carbohydrates, proteins, and nucleic acids. Previous research has identified numerous messenger RNAs (mRNAs) as phloem mobile, however, most of these are known only through high-throughput sequencing studies. This has raised concerns that such mRNAs are products of the identification process and not functionally relevant to the plant. To address this issue, we used a Translating Ribosome Affinity Purification (TRAP) technique to capture and sequence ribosome associated mobile mRNAs. Ribosome loading indicates the mRNA is actively translating, and thus more likely to have a functional impact on cell physiology. A tomato - Nicotiana benthamiana heterograft system with two phloem-specific promoters (pSUC2 and pSULTR2;2) and one ubiquitous promoter (cauliflower mosaic virus 35S) was used to express a TRAP – tagged ribosomal protein. Ribosome capture and sequencing of associated mRNAs identified approximately 60 – 90 genes with significant reads from mobile mRNAs that had crossed the graft boundary from root to shoot. Preliminary gene ontology analysis showed an enrichment of genes predominantly related to regulation of gene expression. Combined, this study represents the first comprehensive identification of translationally active mobile mRNAs and their association with target tissues.

497 **Characterization of a Novel Link Integrating Stomatal Development with ABA-Mediated Germination and Environmental Resilience**

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A plant's lifecycle is defined by its ability to manage water relations, beginning with the hydraulic trigger of seed imbibition. While germination initiates active metabolism, the foundation for post-germination transpirational control is established even earlier, as stomatal pre-patterning occurs during embryogenesis prior to seed maturation. These two stages, the intake of water to start life and the regulated water release to sustain it, are strictly governed by the integration of endogenous hormonal signals and exogenous environmental cues. Abscisic acid (ABA), acting as a hydraulic safeguard, is a well-known repressor of both seed germination and stomatal formation, yet the regulatory intersection between these developmental programs remains largely unexplored. Here, we identify a novel link between these processes through the characterization of a stomatal-associated mutant. We demonstrate that this mutant displays hypersensitivity to ABA during germination, evidenced by a significant delay in testa rupture compared to the wild type. Genetic analyses utilizing ABA metabolic mutants, aba2 and cyp707a1/a2, confirm that this gene functions within the ABA-mediated inhibitory framework. To elucidate the underlying mechanism, we performed time-series RNA-Seq analysis across four distinct germination stages. Transcriptomic profiling revealed extensive expression shifts in the ABA signaling pathway, positioning this regulator as a key coordinator of seed germination and stomatal development. Furthermore, the significant enrichment of drought- and cold-responsive genes among the differentially expressed genes further reinforces the role of this regulator in ABA-mediated stress signaling and environmental resilience. Collectively, this work reveals the multi-functional nature of a stomatal development regulator that integrates seed germination with stomatal formation via the ABA signaling pathway to determine plant environmental plasticity.

501 **Boosting plant organelle genome editing with a highly efficient TALE-Combo system**

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Heritable and efficient genome editing remains a key limitation in plant organelle engineering, largely due to low editing frequencies and regeneration bottlenecks. Here, we present TALE-Combo, a dual-function platform that integrates a TALE-linked cytidine base editor (hA3A-Y130F) with a TALE-based transcriptional activator (TALE-Act), enabling simultaneous chloroplast base editing and transcriptional activation of select nuclear gene(s) to enhance both editing efficiency and regeneration. First, we apply TALE-Combo in monocots in hormone-free treatment. In rice (Oryza sativa), TALE-Combo achieves >80% C-to-T conversion, outperforming a standalone TALE-CBE (~60%), at the OsPsaA target site. Moreover, activation of the morphogenic gene OsBBM1 facilitates efficient regeneration under hormone-free conditions, with the TV activators yielding a ~10-fold increase compared to the positive control. Together, these results establish TALE-Combo as a versatile toolkit for next-generation organelle engineering in plants, enabling enriched heritable, targeted edits, with strong potential to overcome the current barrier to plastid engineering in recalcitrant crop species.

503 **A Novel Type I-F CRISPR System Confers Kilobase-scale Genomic Deletions using a Cas2-Cas3 Fusion in Plants**

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CRISPR-Cas systems have provided remarkably capable tools for RNA-guided genome editing in eukaryotes prokaryotes. Type II and Type V CRISPR-Cas nucleases, Cas9 and Cas12a, respectively, are commonly used to generate small,

site-specific indels. However, achieving large genomic deletions with high efficiency in plants remains challenging using these canonical CRISPR-Cas systems. Type I CRISPR systems encode the multi-effector Cascade machinery known to generate large-scale deletions, yet these systems have received comparatively little attention for plant genome editing. Several Type I subtypes have been completely unexplored for use in plants, leaving untapped potential for novel variants possessing nuclease activity at alternative PAMs and enhanced editing efficiency. Aided by genome-database discovery tools, we uncover a Type I-F CRISPR system derived from Methylomonas methanica (MmeCascade). Harboring the unique Cas2-3 fusion characteristic of Type I- F systems, MmeCascade results in large genomic deletions on the order of several kilobases at two genomic target sites containing GC-rich PAMs in rice plants. Strikingly, deletions elicited by MmeCascade are bidirectional and occur with high editing efficiency. To complement this potent genome editor, we adapt a genome editing analysis tool for compatibility with Oxford Nanopore DNA sequencing of long-range PCR amplicons, complete with target site interrogation and data visualization capabilities. Finally, long-read whole genome sequencing reveals on-target genomic deletions exceeding 10 kilobases in size with no gRNA-dependent off-target nuclease activity, suggesting high specificity. Equipped with this novel Type I-F CRISPR system, engineering large-scale genomic perturbations in plants is achievable with high efficiency, enabling investigations into the functional roles of long non-coding RNAs, cis- regulatory elements, intergenic space, and gene clusters in plants.

509 **Microribonucleic acid (miRNA) mediated gene regulation in Musa spp. under cadmium stress**

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MicroRNAs (miRNAs) are important post-transcriptional regulators that control gene expression through messenger RNA (mRNA) degradation or translational repression and play critical roles in plant growth, development, and stress adaptation. Musa spp. are highly susceptible to abiotic stresses that significantly reduce productivity; however, the regulatory role of miRNAs under heavy metal stress remains poorly understood. This study investigated the involvement of miR528 in Musa spp. exposed to different concentrations of cadmium (Cd). Physiological analyses assessed root growth inhibition, leaf chlorosis, and chlorophyll degradation through chlorophyll a and b quantification. Biochemical analyses, including hydrogen peroxide (H_2O_2) quantification, and peroxidase (POD) activity were performed to evaluate oxidative stress and antioxidant responses. Raman spectroscopy and

infrared (IR) imaging were employed to characterize pigment degradation and biomolecular alterations, while quantitative real-time PCR (qRT-PCR) was used to examine MamiR528-5p expression patterns. Cadmium exposure significantly reduced chlorophyll a and b concentrations to 13 µg/mL and 7 µg/mL, respectively, and increased H_2O_2 accumulation to 33 mmol/g of total protein, whereas POD activity showed no significant variation. IR spectral analysis revealed weakening of the 1730 cm^{-1} carbonyl band and shifts within the 1000–1250 cm^{-1} region, while Raman spectra exhibited reduced intensity at 1525 cm^{-1}, indicating Cd-induced biochemical alterations. Gene expression analysis demonstrated significant upregulation of MamiR528-5p, with 1.5–5-fold increases in leaves and 7.5-fold induction in Cd75-treated roots. Putative miR528-5p target genes, including polyphenol oxidase, serine/threonine protein kinase, and mavicyanin, exhibited tissue and Cd concentration-dependent expression patterns, suggesting that miR528 contributes to oxidative stress regulation under cadmium toxicity.

511 **Molecular Analysis of Defense-Related Gene Expression through Optimized RNA Isolation and qPCR in Pomegranate**

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Bacterial blight caused by Xanthomonas axonopodis pv. punicae is one of the most destructive diseases affecting pomegranate (Punica granatum L.) cultivation. Molecular investigations in pomegranate are often challenged by the high accumulation of polyphenols and polysaccharides in plant tissues, which adversely affect RNA quality and downstream analyses. The present study aimed to standardize an efficient RNA isolation method for pomegranate leaf tissues and to assess the expression of selected defense-related genes in bacterial blight-infected and healthy plants using quantitative real-time PCR (qRT-PCR). Fresh leaf samples collected from greenhouse-maintained plants were subjected to four different RNA extraction methods, namely CTAB + TRIzol, TRIzol alone, phenol–chloroform extraction, and a modified CTAB protocol followed by DNase treatment and cDNA synthesis. Gene expression analysis was carried out through RT-PCR using SYBR Green chemistry targeting PR1, PAL, PPO, multiple unigene markers, and WRKY transcription factor genes. Relative gene expression was estimated using the 2^-ΔΔCt method. Among the tested protocols, the modified CTAB method produced high-quality RNA suitable for downstream molecular applications. qRT-PCR results

demonstrated significant upregulation of defense-responsive genes, including PR1, PAL, PPO, and specific WRKY genes, in infected tissues compared to healthy controls. In addition, successful amplification of the XopQ effector gene and unigene markers confirmed the specificity and reliability of the primers used. These findings validate the effectiveness of the modified CTAB protocol for RNA extraction in pomegranate and highlight the activation of multiple defense pathways in response to X. axonopodis pv. punicae infection. The optimized workflow will support future molecular investigations on host–pathogen interactions and aid in the development of diagnostic markers and disease-resistant pomegranate cultivars

513 **Morphogen activation enables genotype independent regeneration of gene edited carrot**

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Carrot (Daucus carota) is important vegetable crop grown worldwide and rich source of pro vitamin A. Recent advances in genome engineering have enabled rapid development of varieties with desired traits. However, genotype dependent regeneration and low transformation efficiency presents limit the application of these technologies in carrot. We have developed CRISPR-Combo platform for simultaneous multiplex activation and knockout of key morphogenic and allergen genes in carrot respectively. Key morphogenic regulator genes including DcBBM1, DcAGL15, DcWOX5, DcFUS3, DcPLT5, DcWIND1, and DcSERK1 were targeted for transcriptional activation, while allergenic genes Dau c 1.01 and Dau c 1.02, encoding pathogenesis-related protein-10 (PR10) isoforms were targeted for editing to develop hypoallergenic carrot plants. The transcriptional activation and editing at target genes was successfully validated by PEG-mediated transfection of carrot protoplasts. Where targeted morphogenic genes showed 2 to 16 fold increase in expression. While genome editing analysis revealed 1-, 2-, and 12-bp deletions in both PR10 genes. In parallel, protocol was established for morphogen activation assisted hypocotyl based transformation and regeneration in carrots. This study

demonstrates the potential of morphogenic gene activation using CRISPR-Combo in carrot regeneration to enable efficient genome engineering.

517 **Investigating the role of DEWAX Transcription Factors in regulating defense and circadian clock in Arabidopsis**

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Plant diseases pose a significant threat to agricultural sustainability, causing billions of dollars in annual global yield loss. This necessitates the elucidation of defense mechanisms essential for improving crop health and productivity. Recent studies have highlighted the crosstalk between innate immunity and the circadian clock, the endogenous oscillator that coordinates vital biological processes. The Lu lab recently identified TGA3, a known defense regulator, as a critical link between the circadian clock and innate immunity in Arabidopsis thaliana. A high-throughput yeast-one-hybrid screen identified DEWAX as a transcription factor that binds directly to the TGA3 promoter. While TGA3 and DEWAX exhibit circadian expression patterns, the specific role of DEWAX in coordinating defense and clock function in Arabidopsis remains unclear. This project aims to investigate the functional roles of DEWAX in pathogen defense and clock regulation. To do this, we generated DNA constructs: transcriptional fusions of the clock gene CCA1 and TGA3 promoter to luciferase reporters (CCA1:LUC and TGA3:LUC) and introduce them to Col-0 and a dewax mutant. Our data shows that the dewax mutant had reduced rhythmic expression on the CCA1 clock gene both in 1mM salicylic acid (SA)-treated and untreated groups relative to wild type (Col-0). In TGA3:LUC, dewax shows reduced amplitude on the TGA3 rhythmic expression in the untreated group, but showed no significant difference in the 1mM SA-treated group. In addition, we tested the dewax mutant for defense response against the bacterial pathogen Pseudomonas syringae DG3 and ROS burst elicited by flg22. Our data show that dewax significantly increased the ROS burst and decreased the bacterial load of Pseudomonas syringae DG3, suggesting that DEWAX is a negative regulator of defense. In addition, a

translational fusion of the DEWAX genomic sequence to a green fluorescent protein (DEWAX-GFP) was generated and we detected the DEWAX-GFP protein in the nucleus. In summary, our data demonstrate a critical role of DEWAX in influencing defense and circadian clock.

519 **Racing Pollen Tubes: Exploring Competition through in vitro Growth Patterns**

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Sexual selection by competition, usually between males, is an essential component of Darwinian evolution, in which the most fit individuals pass on their genetic information. In angiosperms, this competition can be seen in the form of racing pollen tubes, the male gametophyte of plants, along the style, an elongated tube-like organ that connects the external surface of the stigma, where pollen adheres to the ovary. The slowest germinating pollen tubes may be outcompeted either by (1) physical barriers to the number of pollen tubes reaching the ovary (e.g. the obturator) or (2) previous fertilization of available ovules. Here we show that pollen tube competition can occur in a completely artificial environment: an agar germination medium-filled microcapillary. We show evidence that in this space limited system, sized in the order of magnitude of real styles, pollen tubes of species with both open (Lilium longiflorum, Agapanthus umbellatus), or solid styles (Nicotiana tabacum, Arabidopsis thaliana) spontaneously compete with a conserved pattern affected by pollen density, pollen tube shank pH, and long-distance electric sensing. We are optimizing the system for A. thaliana to perform reverse genetic screens for putative signaling mechanisms responsible for this self-organizing pattern that determines the increased fitness of ONE successful competitor.

523 **Maize Traditional Varieties: Conservation, Characterization, and Beyond for Harnessing Genetic Diversity in Crop Improvement**

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Maize traditional varieties are native populations of cultivated maize (Zea mays L.) with a historical origin, distinct identity, and cultural and heritage value to their users, which have been developed, maintained, and transmitted through families and local communities rather than through commercial seed trade. Before the advent of formal breeding techniques, maize traditional varieties evolved through generations of incidental selection by local, independent farmers, allowing adaptation to specific environmental conditions and cultural and culinary preferences, and thereby generating extensive genetic diversity and district characteristics within and among populations. Traditional maize varieties are increasingly acknowledged as promising genetic resources for contemporary breeding programs providing unique alleles and adaptive traits that contribute to the genetic enhancement of modern maize, particularly in meeting the growing need for crop resilience to emerging biotic and abiotic stresses under the increasing frequency of extreme weather events. To fully realize this potential in breeding, an integrated framework is required, including conservation (ex situ and in situ) to maintain long-term access to genetic diversity, comprehensive phenotypic and genetic characterization to identify accessions with traits of interest, and fundamental research to discover and validate novel variants and adaptive mechanisms for crop improvement, thereby converting conserved diversity into practical breeding value. In this context, this presentation provides an overview of the current global status of ex situ and in situ conservation efforts for maize traditional varieties, synthesizes recent advances in their phenotypic and genetic characterization, reviews their utilization in genetics, genomics, and breeding research aimed at enhancing the agronomic performance and quality of modern maize, and discusses future directions and breeder-oriented recommendations to facilitate the effective use of traditional varieties in maize genetic improvement.

525 **CRK38 Positively Regulates Aluminum-Dependent Root Growth Inhibition in Arabidopsis thaliana**

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Acidic soils, which constitute nearly half of the world's arable land, severely limit crop productivity due to enhanced metal toxicity, particularly from aluminum (Al). Understanding the molecular mechanisms underlying plant adaptation to acidic soils is essential for developing stress-resilient crops. In this study, we investigated the role of CRK38 (Cysteine-Rich Receptor-Like Kinase 38) and related CRK homologs identified through genome-wide association studies (GWAS) in regulating aluminum stress responses in Arabidopsis thaliana. GWAS analysis of root architectural traits under Al stress identified multiple CRK family members, including CRK37, CRK38, CRK39, and CRK40, as candidate loci associated with Al tolerance. Functional characterization of CRK38 was performed using T-DNA insertion mutants, overexpression lines, and CRISPR/Cas9-generated knockout lines under varying pH and Al stress conditions. Preliminary results demonstrate that loss of CRK38 function enhances Al tolerance, as indicated by improved root growth and altered root architecture compared to wild-type and overexpression lines, suggesting that CRK38 acts as a positive regulator of Al-dependent root growth inhibition. To investigate potential functional redundancy among closely related CRK homologs identified through GWAS, CRK artificial microRNA (amiRNA) lines targeting CRK37, CRK38, CRK39, and CRK40 were generated, and simultaneous knockdown of these genes was confirmed through RT-qPCR analysis. In parallel, additional CRK homologs identified through GWAS are being considered for independent functional validation using corresponding T-DNA insertion mutants. Transcriptomic (RNA-seq) analysis of CRK38 lines under aluminum stress revealed differential regulation of genes associated with organic acid-mediated detoxification pathways, including MATE and ALMT1. Based on these findings, CRK38-mate and CRK38-almt1 double mutants were generated and compared with their respective single mutants to

investigate potential epistatic interactions and determine whether CRK38-mediated signaling converges with established aluminum detoxification pathways. Collectively, this study provides new insights into CRK-mediated regulation of aluminum stress responses and establishes a framework for understanding functional redundancy and signaling networks involved in acidic soil adaptation.

527 **Evaluating performance and stability of diverse maize germplasm under waterlogging stress using reaction norm model and multi trait stability index**

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Given the rising incidence of extreme rainfall events, improving waterlogging tolerance in maize is a priority, yet effective field-based phenotyping remains limited by the inability to quantify spatial heterogeneity in stress intensity. To address this limitation, 50 diverse maize genotypes were evaluated under waterlogging and non-stress conditions, and LiDAR was used to quantify waterlogging as a continuous field-scale stress gradient (relative depth). This enabled reaction-norm based modeling of genotype-specific responses, revealing pronounced phenotypic

plasticity along the stress gradient and substantial trait-dependent differences in sensitivity and genotypic variation. Integrating these responses through multi-trait stability analysis distinguished waterlogging-tolerant and susceptible genotypes across nine agronomic and phenological traits. These results demonstrate that waterlogging stress in field conditions is best quantified and interpreted as a continuous spatial gradient, as doing so reveals biologically meaningful plasticity and improves the accuracy of selection for waterlogging tolerance in maize.

529 **Combating Climate Change in Agriculture: Challenges and Innovations in Crop Production**

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Driven by post-industrial greenhouse gas emissions, climate change has accelerated global warming and erratic precipitation patterns, severely threatening sessile plant life. As the planet's primary producers, plants form the foundation of global food security through agriculture. This review examines the devastating impacts of climate-related stressors, most prominently drought and heat, on crop production. Drought restricts crop yield by altering water relations, while heat disrupts fundamental metabolic processes. To safeguard the global food supply against a growing population and accelerating climate threats, modern agriculture deploys multiple strategies, including phenotypic selection in traditional breeding, targeted gene editing biotechnology, and smart agronomic field management. Notably, Artificial Intelligence (AI) technologies, specifically Machine Learning and Deep Learning, emerge as a master facilitator of these strategies through integrating massive biological datasets with high-throughput phenotypic data. Overall, this multi-disciplinary approach transforms how we adapt agriculture to climate change, ensuring stable crop production for a growing global population.

531 **Echinacea purpurea, Native Plant Species of the Mid-Atlantic Region as a Source of Bioactive Therapeutics: Extraction Strategies, Pharmacological Potential, and Emerging Role of Echinacoside (ECH)**

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Echinacea purpurea is a widely used medicinal plant recognized for its diverse phytochemical profile and therapeutic potential. This study examines reported extraction methods, major bioactive compounds, and pharmacological applications of E. purpurea, with specific emphasis on echinacoside (ECH), a phenylethanoid glycoside of growing research interest. This review compares peer-reviewed literature on extraction efficiency, compound distribution, and biological activity. Studies show that the extraction strategy significantly influences phytochemical yield and extract quality. Conventional methods such as maceration and Soxhlet extraction are common for extracting bioactive compounds such as echinacoside, anthocyanins and flavonoids, while advanced techniques including ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) have been shown to improve recovery efficiency and reduce processing time. Major compounds identified from E. purpurea include echinacoside, polysaccharides, flavonoids, chicoric acid, and alkamides. Literature suggests that ECH demonstrates antioxidant, anti-inflammatory, neuroprotective, hepatoprotective, and anti-diabetic activities through multi-target mechanisms. These findings support the continued value of E. purpurea as a source of natural therapeutics and highlight echinacoside as a promising candidate for future pharmaceutical development in the Mid-Atlantic region. Further standardized extraction studies and clinical investigations can clarify efficacy and expand translational applications.

533 **American Ginseng (Panax quinquefolius) as a Source of Bioactive Therapeutics: Phytochemistry, Extraction, and Pharmacological Potential**

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Panax quinquefolius is a medicinal plant valued for its diverse phytochemical composition and significant therapeutic potential. This study investigates reported extraction methods, main bioactive compounds, and pharmacological applications, with particular emphasis on ginsenosides, the primary active constituents. A dry-lab approach was employed through systematic review and comparison of peer-

reviewed studies to evaluate extraction efficiency, compound distribution, and biological activity. The analysis indicates that extraction techniques play a critical role in determining ginsenoside yield and overall extract quality. While conventional methods remain widely used, advanced techniques such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction demonstrate improved efficiency and reduced processing time. Key bioactive compounds identified include ginsenosides Rb1, Rc, Rd, Re, and Rg1. These compounds have been associated with a wide range of biological effects, including neuroprotective, anti-inflammatory, antidiabetic, cardioprotective, anticancer, antimicrobial, and anti-aging activities. Overall, the findings reinforce the importance of Panax quinquefolius as a promising source of natural therapeutics. Further standardization of extraction methodologies and clinical validation studies are required to support its translation into pharmaceutical applications.

535 **American Elderberry (Sambucus canadensis) as a Source of Bioactive Therapeutics: Phytochemistry, Extraction, and Pharmacological Potential`**

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Sambucus canadensis is a medicinal plant widely recognized for its diverse phytochemical profile and therapeutic potential. This study evaluates reported extraction techniques, main bioactive constituents, and associated pharmacological activities, with particular focus on anthocyanins, flavonoids, and phenolic compounds. A dry-lab methodology was employed, involving comparative analysis of peer-reviewed literature to assess extraction efficiency, phytochemical distribution, and biological activity. Findings indicate that extraction methods significantly influence compound yield and overall extract quality. While conventional extraction approaches remain prevalent, emerging techniques demonstrate improved efficiency and reduced processing time. Key bioactive compounds identified include anthocyanins, quercetin, rutin, gallic acid, and neochlorogenic acid. These compounds are associated with a wide range of biological activities, including antioxidant, antiviral, anti-inflammatory, antibacterial, antidiabetic, and anticancer effects. Overall, the evidence supports the continued relevance of Sambucus canadensis as a promising source of natural therapeutics. Further standardization of extraction protocols and clinical validation studies are necessary to advance its pharmaceutical applications.

537 **Cutting Out the Waste: CRISPR-cas9 to Maximize Antimalarial Artemisinin Production**

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Malaria kills over 600,000 people each year, and the best treatment found is based on the molecule artemisinin, which comes from a single plant species known as Artemisia annua, or sweet wormwood. The issue is that the Artemisia annua does not naturally produce very much of this artemisinin, and a lot of its resources are wasted on a very similar molecule, arteannuin B, which has no medical benefits. This project aims to use the gene editing tool CRISPR-Cas9 to fix both of these problems by eliminating the production of arteannuin B and also activating the production of artemisinin. According to computer simulations of the internal chemistry of the plant, this should increase production of artemisinin by three to five times and reduce production of arteannuin B by more than 90 percent. This research project has a very strong connection to plants found locally in the Mid-Atlantic region since Artemisia annua is a common wild plant species found throughout Maryland, and its close relatives, mugwort and wormwood, are very common local plants used by herbalists.

POSTER ABSTRACTS
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276 **Cracking the Code of Resistance: SNP-Based Marker Development for Orobanche cumana Race G Response in Sunflower**

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Sunflower (Helianthus annuus L.) stands as one of the most fundamental oilseed crops, holding strategic importance for vegetable oil production both globally and in Turkey. Sunflower production is severely impacted by the holoparasitic weed broomrape (Orobanche cumana Wallr.), with highly virulent lines like Race G now effecting the major production areas including Turkey and Spain. Because existing resistance mechanisms are frequently overcome by the emergence of new virulent races, developing molecular tools for marker-assisted selection (MAS) is a critical objective for crop improvement. This study aimed to identify and validate single nucleotide polymorphism (SNP) markers linked to the Race G resistance. A wide panel of 358 sunflower breeding lines from MAY Seed was phenotypically evaluated under both field conditions in Adana/Cihadiye and greenhouse conditions in Bursa. To map the genetic locus, the most resistant (MAYorG_R) and most sensitive (MAYorG_S) lines were selected to generate F_1, F_2, F_3, and $BC1F_1$ populations. Phenotypic assessments confirmed that resistance depends on a monogenic dominant inheritance pattern, supports the proof of control by a single major gene. By genotyping leaf samples from the F_2 and BC_1F_1 populations using a 12K SNP microarray platform, the resistance gene was successfully localized to a defined genomic location on chromosome 7. Subsequent association analysis identified eight SNP markers situated within a 0.7–8.8 cM range of the resistance locus. These markers shown robust amplification profiles, clear allele differentiation, and strong segregation patterns corresponding to the observed phenotypic resistance traits. Validated across diverse genetic backgrounds, these highly accurate markers eliminate the reliance on environmentally variable phenotypic field screenings. Ultimately, integrating these tools into sunflower breeding pipelines allows for rapid early-generation selection, efficient backcrossing, and the accelerated deployment of durable, and resistant cultivars.

288 **Morphological and biochemical responses of cactus pear (Opuntia engelmannii) adventitious buds to salt stress induced in vitro**

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Climate change represents a severe threat to agricultural development and global food security. Extreme phenomena, such as soil salinization, droughts, and floods, exert increasing pressure on agricultural ecosystems, compromising production and threatening global agricultural productivity. Salinity is frequently considered one of the most influential abiotic factors affecting soil health and crop yields, particularly in arid and semi-arid regions. In this context, stress-tolerant species, such as the Cactaceae family, can contribute to the development of sustainable agriculture. The cactus pear (Opuntia spp.) is a plant of significant economic, agronomic, and ecological importance. In vitro culture is a powerful technology that enables rapid mass propagation and the evaluation of tolerance to various abiotic stresses, notably salinity. In this work, the effect of sodium chloride (NaCl) on the morpho-biochemical and physiological parameters of adventitious buds of a cochineal-resistant Moroccan cultivar (Opuntia engelmannii) in vitro was investigated. Adventitious buds were cultured on MS medium supplemented with various concentrations of NaCl (0, 2.5, 5, 7.5, and 10 $g.L^{-1}$). Following stress exposure (1 to 3 weeks), cultures were transferred to a NaCl-free medium and maintained for a three-month recovery period at 26°C under a 16 h/8 h light/dark photoperiod. Based on statistical analysis, there were significant differences between the control and other treatments at different stages of shoot bud proliferation, shoot elongation, and rooting. The results showed that tolerance to salt stress depends on the stage of development, intensity, and duration of stress. Salt stress reduced morphological parameters, such as the average number of shoot buds per explant, shoot length, and fresh biomass, significantly compared to control shoots. However, almost all treatments showed a high survival rate (100%) during the shoot bud proliferation phase, with only two concentrations (7.5 and 10 g/L) showing a significant difference in dry biomass, while the rest of the treatments showed no significant differences. During the elongation and rooting phase, all treatments resulted in 100% rooting, with significantly better results than in the previous phase. This suggests that while salt stress adversely affected growth during shoot proliferation, it did not simultaneously affect shoot rooting capacity. Indeed, some treatments revealed an increase in shoot length,

number of roots per explant and root length, which could suggest an adaptive response to salt stress during rooting. Regarding physio-biochemical parameters, proline concentrations increased significantly at 2.5 $g \cdot L^{-1}$ after 21 days, indicating a robust osmotic adjustment. Conversely, glycine-betaine, soluble sugars, and total protein levels decreased or remained similar to the control, suggesting a transient metabolic disturbance. Furthermore, chlorophyll a decreased progressively with salinity, while chlorophyll b dropped significantly from 2.5 $g \cdot L^{-1}$, reflecting the degradation of the photosynthetic process. Notably, all regenerated plants were successfully established in the greenhouse with 100% survival and normal growth, highlighting the physiological plasticity and agricultural potential of this cochineal-resistant cultivar. Keywords: Adventitious buds; Biochemical parameters; Cochineal resistance;In vitro culture; Osmoprotectants; Opuntia engelmannii; Salt stress.

364 **Establishing the Role of Hair Cells in Coleochaete breb.**

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Coleochaete is a multicellular streptophyte green alga that is closely related to embryophytes (land plants). Coleochaetophytes form specialized seta-bearing cells that produce distinctive sheathed hairs. These hairs are subcellular structures formed by every cell in the genus Chaetosphaeridium, but only a subset of cells in Coleochaete. The hairs of Coleochaete have received some significant attention since the 19th century, but their exact function remains unclear. The structure is complex, with a sheath of modified cell wall and a concentric bristle composed of plasma membrane and extensions of both the chloroplast and mitochondrion, with the chloroplast rotating around the base of the hair. We used confocal microscopy to explore the structure of hairs in several cultured species of Coleochaete, and investigated the effect of environmental conditions on hair formation. Our work from last year confirmed the presence of mitochondria within the hairs via mitotracker staining and also showed that environmental factors such as nitrate availability can cause variation in the production of these hair cells within the thallus. This led us to conclude that the hairs of Coleochaete may be involved in nutrient uptake or sensing. This year we have isolated several new strains of Coleochaete from Wisconsin that are more sensitive to pH levels than those previously available in culture. We found

that the Coleochaete actively alters the pH around the hairs, and that this activity changes in the presence or absence of light. This is reminiscent of processes documented in another related group of streptophyte algae, the Characeae, which are known to alter the pH of the surrounding water in response to light. From this, we infer that Coleochaete performs nutrient acquisition via a mechanism similar to that documented in Characeae, but with different cellular structures performing the process.

368 **Neocaryaflavonoside A from Neocarya macrophylla Exhibits Potent Antiproliferative Activity Against TNBC: Insights from Molecular Docking and ADMET Profiling**

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Triple-negative breast cancer (TNBC) remains a highly aggressive malignancy lacking effective targeted therapies. Natural products continue to serve as valuable sources of novel anticancer scaffolds. This study reports the isolation, characterization, and biological evaluation of Neocaryaflavonoside A (F4a), an Acylated flavonoid glycoside from Neocarya macrophylla. The compound was isolated using preparative thin-layer chromatography and structurally elucidated by NMR spectroscopy. Its antiproliferative activity was assessed against HCC 1806 TNBC cells after 72 hours of treatment. F4a exhibited significant dose-dependent cytotoxicity with an IC□□ of 2.11 µg/mL. Molecular docking studies revealed strong binding affinity toward EGFR (−7.9 kcal/mol), surpassing the native ligand (−6.9 kcal/mol), suggesting potential inhibitory activity. In contrast, binding to PI3K (−8.2 kcal/mol) was weaker than the reference ligand (−9.9 kcal/mol), indicating moderate interaction. ADMET profiling revealed high molecular weight, poor solubility, low permeability, and limited oral bioavailability. The compound was predicted to be a P-

glycoprotein substrate with poor metabolic stability and potential CYP inhibition. Toxicity risks, including hepatotoxicity and hERG inhibition, were also identified. Overall, F4a demonstrates promising anticancer activity but requires structural optimization to improve drug-likeness and safety. Keywords: Neocaryaflavonoside A; Neocarya macrophylla; TNBC; In silico; ADMET profiling

370 **A galling insect builds a 'pseudo-seed' by hijacking floral identity genes in blueberry**

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Insect galls are novel plant organs whose development is directed by the insect to provide nutrition and protection for its offspring. How galling insects co-opt specific host developmental programs to build these structures remains poorly understood. We used stage-resolved RNA-seq to trace gall development by the blueberry stem gall wasp (Hemadas nubilipennis) on highbush blueberry (Vaccinium corymbosum), profiling three developmental stages and two mature tissue zones. The host mounts a broad pattern-triggered immunity response at oviposition, but defense pathways are progressively suppressed and completely abolished by maturity. In their place, the gall activates MADS-box floral identity genes (CAULIFLOWER, SEEDSTICK, AGAMOUS, and AGL6) in vegetative stem tissue, accompanied by full cell cycle induction and lateral organ boundary domain gene activation. Auxin and cytokinin biosynthesis genes are systematically suppressed, yet signaling remains active, suggesting replacement by insect-derived hormones. At maturity the gall differentiates into two functionally distinct zones. The outer tissue lignifies into a protective shell while the inner tissue shuts down photosynthesis, secondary metabolism, and cuticular lipids, producing a soft, chemically undefended nutritive tissue sustained by membrane biogenesis and an expanded endomembrane system that drives controlled self-digestion. A SWEET10/SWEET11 sucrose relay channels sugar from phloem to the larval chamber, recapitulating seed coat-to-endosperm transport, while polyol transporters exploit Vaccinium-specific sorbitol as a second carbon stream. The cell cycle shifts to endoreduplication. The gall thus recapitulates key features of seed development (tissue architecture, ovule identity gene deployment, SWEET-mediated sugar relay, endoreduplication, and fruit identity

suppression) while the larva substitutes for the embryo. We propose the term 'pseudo-seed' to describe this convergence.

422 **Complex Regulation of Plant Immunity Against Adapted and Non-adapted Powdery Mildew Fungi by the F-box Protein MAX2 in Arabidopsis**

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Powdery mildew (PM) fungi are obligate biotrophic pathogens that constrain plant productivity worldwide. Host resistance to pathogens including powdery mildew fungi is well characterized, whereas nonhost resistance (NHR) to non- or poorly adapted pathogens, a phenomenon intrinsically related to host range determination, is not well understood. Here, we show that MORE AXILLARY GROWTH 2 (MAX2) plays an important role in NHR of Arabidopsis against a non-adapted powdery mildew species infecting strawberry. Despite being super-susceptible to the adapted powdery mildew fungus Golovinomyces cichoracearum (Gc) UCSC1, the immuno-compromised eds1/pad4/sid2 (eps) triple mutant remains completely resistant to the strawberry powdery mildew Podosphaera aphanis (Pa) UMSG4. Forward genetics studies demonstrated that loss of MAX2 in eps led to compromised NHR against Pa UMSG4. Further genetic analyses of single mutants revealed that the strigolactone (SL) and karrikin/KAI2-ligand (KL) signaling pathways, which converge on the shared F-box protein MAX2, exert opposing roles in resistance to Gc UCSC1. Loss of the KL receptor KARRIKIN INSENSITIVE 2 (KAI2) enhanced resistance, whereas loss of the SL receptor DWARF14 (D14) conferred enhanced susceptibility, together explaining that max2 mutants displayed wild-type-like resistance to Gc UCSC1. Interestingly, we found that the expression of a degradation-resistant form of

SUPPRESSOR OF MAX2 1 (SMAX1), the primary substrate of the KAI2–MAX2 SCF ubiquitin ligase complex, promotes PM resistance in both wild-type (Col-0) and eps backgrounds. Together, these genetic data support a scenario that SL and KL co-regulate immunity possibly via crosstalk with the salicylic acid (SA)-dependent and SA-independent immune responses and suggest that stabilizing SMAX1 may be used as a new strategy for boosting plant immunity.

428 **Simultaneous activation of multiple genes boosts poplar regeneration, growth and genome editing using CRISPR-Combo**

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Tissue culture regeneration remains a major bottleneck for many woody plant species recalcitrant to genetic transformation, limiting functional genomics studies and molecular breeding. Poplar (Populus trichocarpa), the first genome-sequenced forest tree, is an important model for biomass production and carbon sequestration, yet its regeneration efficiency remains suboptimal. The objective of this study was to enhance poplar regeneration and genome editing efficiency by simultaneously activating key morphogenic regulators while engineering a lignin biosynthesis gene. We employed a CRISPR-Combo system to co-activate WOX11 and WUS and knock out 4CL1, a central gene in lignin biosynthesis. Following Agrobacterium-mediated transformation, tissue culture regeneration was conducted entirely under hormone-free conditions to assess synergistic effects on regeneration capacity. Dual activation of WOX11 and WUS significantly accelerated shoot regeneration and root initiation compared to single-activation and control lines, reducing shoot regeneration time from 8–10 weeks to approximately 4 weeks. In addition, biallelic editing efficiency of 4CL1 reached up to 75% in dual-activation lines, markedly higher than in single-activation or control lines. Correlation analysis revealed that high 4CL1 editing efficiency was enriched in lines exhibiting strong activation of both WOX11 and WUS. Taken together, these results demonstrate that co-activation of WOX11

and WUS synergistically enhances poplar tissue culture regeneration while improving genome editing efficiency under hormone-free conditions, providing a robust and scalable strategy for accelerating genetic engineering in woody plants.

442 **Integrated Metabolomic and Genetic Analysis Reveals Defense Pathways in Anthracnose-Resistant Tomato**

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Anthracnose, caused by Colletotrichum species, is a major constraint to tomato (Solanum lycopersicum) production, particularly during fruit ripening. To investigate the metabolic and genetic basis of resistance, we performed widely targeted metabolomic profiling of the anthracnose-resistant accession PI 272636 and the susceptible cultivar ‘Rio Grande’ at mature-green and red-ripe fruit stages. Across developmental stages, more than 1,100 metabolites accumulated differentially between genotypes. Although both genotypes remained symptom-free at the green stage, PI 272636 exhibited elevated accumulation of steroidal alkaloids, flavonoids, and phenylpropanoids, indicating constitutive metabolic differences prior to visible infection. At the red-ripe stage, resistance was associated with increased levels of glycosylated steroidal alkaloids, including lycoperoside C and acetoxytomatidine-related derivatives, as well as phenylpropanoids and flavonoids with established roles in plant defense. KEGG pathway enrichment highlighted flavonoid, phenylpropanoid, and sterol-derived metabolic pathways as key differentiators between resistant and susceptible genotypes. Integration of metabolomic profiles

with QTL-seq data identified candidate loci associated with steroidal alkaloid metabolism, including genes encoding phosphomevalonate kinase, squalene synthase, a 2-oxoglutarate-dependent dioxygenase, and an ethylene-responsive transcription factor, as well as genes involved in hydroxycinnamic acid amide and flavonoid biosynthesis. Collectively, these results indicate that anthracnose resistance in PI 272636 is associated with coordinated differences in specialized metabolite composition across fruit development, supported by genetic variation in pathways contributing to steroidal alkaloid and phenylpropanoid metabolism, and provide candidate biomarkers and targets for breeding improved resistance in tomato.

450 **Elucidating the impact of natural variation on chromatin dynamics underlying thermomorphogenesis**

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Plants are sessile so their traits are not only influenced by biotic but also abiotic factors including environmental cues e.g. light, water and temperature. Exposure to long periods of elevated ambient temperature (EAT) has profound effect on plant growth, development and immunity. Morphological hallmarks of this phenomenon are referred to as thermomorphogenesis (TM). TM can promote leaf cooling and maintain photosynthetic efficiency because of changes in morphology in Arabidopsis thaliana. EAT-induced epigenome reprogramming is driven by complex interactions between transcription factors (TFs) and chromatin remodelers which modulate gene expression, thereby facilitating plant adaptation. To explore how natural genetic variation influences the thermo-responsive epigenome, we generated an EAT diversity panel by assessing EAT-induced hypocotyl elongation of the entire A. thaliana 1001 accessions collection. This panel consists of hyper- and hypo EAT responsive accessions which we are currently subjecting to a high-throughput multiome pipeline consisting of various epigenome features, PIF4 (PHYTOCHROME-INTERACTING FACTOR 4) abundance and gene expression. In addition, we used our EAT phenotyping data to perform GWAS which led to the discovery of various SNPs in genes that are potentially involved in TM. Validation data from knockout mutants will also be presented to show their potential role in TM. Collectively, our integrative approach, combining large-scale phenotyping with high-throughput genomics, will provide a deeper understanding of EAT responses in plants.

460 **Assay toolkit for comparing abiotic stress function of AtLEA2 proteins**

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Late embryogenesis abundant proteins promote abiotic stress resistance in plants by stabilizing other proteins and membranes under denaturing conditions. Arabidopsis thaliana has 51 known LEA genes representing multiple protein families, expression patterns, and localizations. While many of the LEA proteins are intrinsically disordered, LEA2 proteins contain one or two LEA2 domains, which fold into beta-barrels. One of the three A. thaliana LEA2 genes, At1g01470, is expressed in leaves and roots predominantly under cold and high-salt conditions. We aim to differentiate the physiological effects of the three A. thaliana LEA2 proteins. To establish a new lab with an undergraduate LEA protein research lab, the lab initially used root-length assays to measure stress response in lea knockout mutants. However, unexpected results highlighted the need for complementary, literature-supported stress-phenotype assays. We conducted a structured PubMed searches for “Cold stress Arabidopsis,” “heat stress Arabidopsis,” and “salt stress Arabidopsis” limited to the last 10 years and recorded stress-response assays used in the top 12 best-match papers in each category. The total matches were 281, 282, and 521 papers, respectively. In the 12 selected papers, electrolyte leakage was the most common cold-stress assay (7/ 12), seedling survival was the most common heat-stress assay (9/12), and growth-based measures including root-length and biomass were the most common salt-stress assays (10/12). Seedling survival and germination rates were also frequent proxies for salt tolerance. Electrolyte leakage was also used to measure heat and salt stress (2/12 papers each). Using these results, we have begun setting up an electrolyte leakage assay for phenotyping lea2 knockout lines under cold treatment.

464 **Using Golden Gate cloning and gene synthesis for complex multi-gene and muti-guide RNA vector assembly for tomato transformation and CRISPR/Cas9 mutagenesis**

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Multiplex gene editing requires increasingly complex vector architectures, but efficient assembly of such constructs remains a challenge. GoldenGate Assembly has a modular framework that is well-suited for cloning of complex vectors. Our

group wanted to create a vector that included a selectable marker, the Cas9 gene, and a series of guide RNAs that target the promoter regions of four candidate disease susceptibility-associated genes (XSP10, AMT, TPL1, and TPL2) using CRISPR/Cas9 mutagenesis. With a total of 32 guides, and limited by the 7 possible positions in GoldenGate Assembly, we performed a hierarchical cloning assembling Level 1 vectors into Level M and then Level P before a final assembly into a binary vector (Level 2). Although we successfully cloned 32 entry gRNA vectors, assembling higher order constructs proved challenging due to low cloning and transformation efficiency. To optimize and reduce time and costs of cloning, we instead synthesized arrays of 8, 16 and 32 gRNAs using an external provider (ANSA Biotech). Using these synthesized gRNA arrays, we were able to assemble multi-gene constructs containing up to 37 expression cassettes (32 gRNAs plus 5 genes). To further test these new vectors, we cloned 2 morphoregulators for direct in planta tomato transformation. Our results demonstrate that gene synthesis is a cost-effective strategy to assemble multiplex CRISPR/Cas9 arrays for tomato mutagenesis. We are currently performing infiltration and tissue culture experiments to compare the transformation and editing efficiency between conventional tissue culture and in planta transformation protocols.

468 **Genetic and molecular characterization of brachytic-like, a novel locus controlling plant height in tomato**

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Reduced plant height without compromising fruit size or yield is an important breeding target for fresh-market tomato (Solanum lycopersicum). We acquired a brachytic (br) mutant line from the Tomato Genetics Resource Center (TGRC) that shows a short-stature phenotype. However, our initial characterization and genetic complementation tests indicate this line does not carry mutations in the Br locus. This line, which we renamed brachytic-like (brl), exhibits reduced plant height by reducing internode length. Interestingly, F1 individuals from a cross between brl and the heirloom tomato cultivar Eva purple ball exhibited intermediate height, suggesting the brl might be semi-dominant. Furthermore, Gibberellic acid (GA3) spraying significantly increased plant height of both brl and M82 (non-brl cultivar), suggesting this candidate gene might be involved in hormone pathways. To find the causative mutation at the brl locus, we analyzed plant height in a segregating (F2) population derived from the previous cross. Qualitative phenotypic characterization of the F2 population suggested that the segregation ratio of taller wildtype (WT) and short-stature phenotypes followed the expected 3:1 Mendelian ratio (χ^2 (df = 1, N = 128) = 2.38, p =0.12), suggesting the short-internode trait in tomato is controlled by

a single recessive gene. Subsequently, we performed bulked segregant analysis sequencing (BSA-seq) using two pools (WT and brl plants) of samples from the F2 populations. Our mapping uncovered a 1Mb interval in chromosome 5 containing several genes associated with hormone signaling. Our genetic characterization of the novel brl locus will allow breeding for short-stature tomatoes optimized for field and controlled-environment agriculture and for improving heirloom production for local markets in the Mid-Atlantic Region.

476 **Time-course RNA-seq identifies candidate genes associated with boxwood blight resistance in resistant and susceptible boxwood (Buxus spp.)**

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Boxwood blight, caused by Calonectria pseudonaviculata, is a major threat to ornamental boxwood production, yet the molecular basis of host resistance remains poorly understood. Time-course RNA-seq was used to characterize transcriptional responses associated with host resistance to boxwood blight from 0–96 hours following pathogen inoculation of resistant and susceptible Buxus species. Differential expression analysis identified 1,510 significantly differentially expressed genes (DEGs), including distinct temporal patterns associated with resistance. A coordinated early transcriptional response occurred from 2–24 hours post-inoculation, with the largest number of intersected resistance-associated DEGs detected at 8 hours post-inoculation. In contrast, 48–96 hours exhibited distinct expression profiles from earlier responses, suggesting a transition between early and late phases of host response. Gene Ontology (GO) enrichment analyses identified activation of immune and defense-related processes, including regulation of defense response, defense response to oomycetes and fungi, and kinase-associated signaling pathways. KEGG enrichment analyses further revealed temporal regulation of ribosome-associated pathways, ATP-dependent chromatin remodeling, plant–pathogen interaction pathways, and protein processing in the endoplasmic reticulum. Several candidate resistance-associated genes were identified, including MKK6, WRKY70, WRKY40, LECRK71, ZAR1-like receptor kinases, CNGC5, and MSL10, highlighting potential molecular regulators underlying boxwood blight resistance in Buxus.

478 **A Novel Negative Regulator and Its Crosstalk Shape Aluminum Stress Responses in Arabidopsis**

Vandana Thakral1, Arjun Ojha Kshetry1,, Shyam Gundu1, Sarai Cook1, Padma Nimmakayala1, Umesh K. Reddy1* 1. Gus R. Douglass Institute, Department of Biology, West Virginia State University, Institute, WV, 25112-1000, USA

Aluminum (Al) toxicity is a major factor limiting plant growth and productivity in acidic soils. To identify genes associated with Al stress tolerance, we performed a genome-wide association study (GWAS) using 203 Arabidopsis thaliana accessions under control and Al stress conditions. Eight root architectural traits were quantified, revealing significant natural variation in Al responses. GWAS consistently identified members of the RING/U-box (RUB) family and CRK genes as candidate regulators associated with multiple root traits under Al stress. Functional analysis using RUB knockout and overexpression lines demonstrated that RUB negatively regulates Al tolerance. Under Al stress, RUB knockout plants exhibited enhanced root growth, greater root volume, surface area, and branching compared to Col-0 and overexpression lines. In contrast, overexpression lines showed stronger root growth inhibition. Morin and hematoxylin staining revealed reduced Al accumulation in knockout lines and increased accumulation in overexpression lines. DAB staining further indicated lower oxidative stress in knockout plants under Al exposure. Combined stress studies showed that RUB-mediated responses were largely Al-specific, although Cd + Al treatment produced unique effects on root branching and chlorosis. GUS reporter analysis demonstrated root-specific RUB activity, suggesting its localized role in regulating Al responses. Transcriptomic analysis revealed that RUB influences pathways related to oxidative stress, hormonal signaling, MAPK cascades, and cell wall and root development. Overall, these findings identify RUB as an important regulator of Al toxicity and provide insights into the molecular mechanisms controlling root adaptation under acidic soil conditions.

480 **Elucidating How Rhizosphere pH Dynamics Regulate Arabidopsis Immune Signaling**

Runlong Dong, Shengyang He. Duke University

Rhizosphere pH (pHrhz) is increasingly altered by climate change and unsustainable agricultural practices, yet how plant immune systems respond to pHrhz shifts remains poorly understood. Here, we investigate how short-term pHrhz perturbations regulate local root and systemic leaf immune responses in Arabidopsis thaliana. Using a hydroponic system that enables precise manipulation of rhizosphere conditions, we found that both roots and leaves are responsive to short-term pHrhz perturbations at the levels of immune gene expression and/or pathogen defense. Transcriptomic analyses revealed that acidic and alkaline pHrhz shifts trigger distinct immune-related transcriptional programs in roots. Acidic pHrhz shifts preferentially enriched defense-associated pathways linked to innate immunity and salicylic acid signaling, whereas alkaline shifts were more strongly associated with ion homeostasis and metabolic remodeling. In addition, pattern-triggered immunity (PTI) marker genes exhibited enhanced flg22-induced expression following acidic pHrhz shifts in roots, whereas leaf PTI responses were strongest under the neutral pHrhz condition, suggesting that extracellular pH modulates early immune signaling outputs in an organ-dependent manner. Ongoing work focuses on identifying pH-sensitive cell-surface immune signaling components through plasma membrane-enriched phosphoproteomics. Together, these findings provide mechanistic insight into how environmental pH dynamics reshape plant immune responses and may inform future strategies to improve crop resilience under increasingly variable soil conditions.

482 **Elucidating Transcriptional Specificity of Auxin Response Factors**

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Auxin response factors (ARFs) are a family of transcription factors that respond to the plant hormone auxin, regulating the transcriptional output of the canonical auxin response pathway. In Arabidopsis thaliana, the ARF gene family comprises 23 paralogs, including five class-A members generally associated with transcriptional activation. ARFs activate auxin-responsive genes by binding repeated AuxRE motifs in promoters, typically spaced 1–20 bp apart (Boer et al., 2014). DNA affinity purification sequencing (DAP-seq) analysis of ARFs reveals the capacity of class-A ARFs to bind a wide range of AuxRE motif spacings and orientations, while class-B ARFs bind a narrower subset of AuxRE repeats (O'Malley et al., 2016). However,

motif models that explicitly encode AuxRE dimer configurations have limited power to predict class-A ARF binding, indicating that specificity among activating ARFs remains difficult to resolve (Stigliani et al., 2019). Additionally, distinct phenotypes in class-A ARF null mutants indicate functional divergence among class-A ARF paralogs. To address the unresolved basis of specificity among class-A ARFs, we identified genes upregulated by individual auxin-insensitive (PB1-deleted) ARF paralogs via RNA-seq and cloned their promoters into fluorescent reporter constructs. We then measured these reporters in the presence of overexpression of individual auxin-insensitive ARF paralogs in Arabidopsis protoplasts. This screening identified promoters that retain their ARF paralog specificity when removed from native genomic context. We also performed ARF domain swaps between active and inactive ARFs on a specific promoter to understand which portion of the TF may be conferring specificity against a promoter. Finally, this assay will also allow us to interrogate the promoter sequence content that leads to paralog-specific ARF activation. This work provides insight into the mechanism that mediates ARF-specific transcriptional activation.

484 **HEMP CURE - Using Genetics and Research to Cure Plant Blindness and STEM Retention**

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Hemp Experiences in Molecular biology and Population genetics (HEMP) CURE is a laboratory experience that allows students to gain experience and expertise with fundamental molecular biology skills while simultaneously helping to cure Plant Blindness and retain students in STEM majors/careers. HEMP CURE is a turn-key, semester-long research project that is adaptable and implementable in widely varying lab situations. During HEMP CURE, students will gain experience in plant DNA isolation, setting up PCR reactions, gel electrophoresis, and population genetic analysis. This CURE has been piloted for two years and have optimized student protocols and other lab experiences to not only build confidence in students but to also foster a sense of belonging and "being a scientist". The basis of the HEMP CURE research focus is to document the genetic structure of industrial hemp (Cannabis sativa) varieties using microsatellite amplification and scoring. If you are interested in implementing HEMP CURE within you lab course - contact David Puthoff (dpputhoff@frostburg.edu)

486 **Microgravity simulation reshapes microbial community structure and carbohydrate-active enzyme profiles in semi-hydroponic systems of Starbor Kale (Brassica oleracea L.)**

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Kale (Brassica oleracea L.) is a nutrient-rich crop whose production is increasingly explored through advanced soilless systems to address food insecurity under climate change. In this study, we characterized the phytomicrobiome of Starbor kale cultivated in coco coir under normal gravity and simulated microgravity using custom 2D clinostats. Seedlings were grown for 43 days in a controlled CONVIRON chamber, after which microbial DNA from coco-coir and root samples was extracted and subjected to shotgun metagenomic sequencing. Comparisons between components revealed significant differences in microbial community profiles. Coco-coir substrates were dominated by bacteria, whereas root samples exhibited higher proportions of Eukaryota and archaea. Across all samples, the phyla Pseudomonadota and Actinomycetota were consistently abundant, with elevated levels in coco-coir samples from horizontal clinostats (HCR) under simulated gravity and from rotating vertical (VCR) clinostats. The HCR group yielded the greatest number of biomarkers (n = 28). Functional profiling revealed gravity-dependent shifts in carbohydrate-active enzymes (CAZymes). Glycoside hydrolases and carbohydrate esterases were more abundant in coco-coir under normal gravity, while carbohydrate-binding modules were enriched in HCR and VCR samples. Root microbiomes displayed substantially higher levels of polysaccharide lyases (0.00088–0.00097) and carbohydrate esterases (0.030–0.033). These findings demonstrate that simulated microgravity alters both the taxonomic composition and functional potential of microbial communities associated with soilless kale cultivation. These insights provide a foundation for optimizing hydroponic production of kale and other leafy greens in controlled-environment agriculture.

488 **Elucidating stress-induced epigenome reprogramming in crop and medicinal plants**

Linkan Dash, Moonia Ammari, Aanchal Choudhary, Hyuk Sung Yoon and Mark Zander Waksman Institute of Microbiology, Department of Plant Biology, Rutgers State University of New Jersey

Plants have a highly dynamic relationship with their environment. Understanding the molecular mechanisms underlying these interactions holds great promise for the development of innovative strategies to enhance resilience to abiotic and biotic stresses. The epigenome has emerged over the past decade as a key regulatory layer governing plant-environment interactions. Its responsiveness to environmental

cues is orchestrated by complex interactions between transcription factors (TFs) and chromatin regulators, shaping the epigenomic landscape and influencing plant adaptation. Using the jasmonic acid (JA) defense pathway and its master regulator MYC2 as a model, I investigated JA-responsive gene regulatory mechanisms across diverse plant systems. Leveraging our newly developed high-throughput PHILO (Plant HIgh-throughput LOw-input) ChIP-seq platform, I demonstrate how JA signaling extensively reprograms the epigenome to drive robust defense gene expression across multiple species. I further show that MYC2 plays a pivotal role in shaping a highly permissive chromatin landscape at its target genes, an essential feature for the coordinated transcription of defense-related gene clusters. These Stimulus-Induced Enhancer Acetylation (SIENA) regions are characterized by a histone acetylation signature in regulatory regions. I will present evidence for their widespread occurrence across various crop and medicinal plant species, along with their potential role in transcriptional activation. Together, these findings provide a conceptual framework for understanding the coordinated regulation of stress-inducible gene clusters, opening new avenues for targeted genetic engineering of traits relevant to crop resilience and medicinal compound production.

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their potential role in transcriptional activation. Together, these findings provide a conceptual framework for understanding the coordinated regulation of stress-inducible gene clusters, opening new avenues for targeted genetic engineering of traits relevant to crop resilience and medicinal compound production.

494 **Optimization of Tissue Culture Regeneration and Transformation Frequency of Cucumis sativus for CRISPR/Cas9**

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The Cucurbitaceae family includes some of the most extensively cultivated vegetable crops worldwide and represents an important source of economic value in agriculture. However, cucumber (Cucumis sativus) production is frequently affected by numerous biotic and abiotic stresses. The emergence of CRISPR/Cas9 genome-editing technology has transformed cucumber biotechnology by enabling precise and targeted genetic modifications. However, cucumber remains one of the most recalcitrant species for genetic transformation, with the highest reported transformation frequency ranging from 1-10%. Therefore, the continuous optimization of explant selection, transformation efficiency, and plant regeneration protocols is critical to maximizing its genomic edition. In this study, an efficient protocol for shoot regeneration, their elongation and selection of transformed plants was developed using the cucumber genotype Poinsett 76. Agrobacterium LBA4404 mediated transformation was done under optimized conditions including the pre-culture of explants in pre-culture media enriched with hormones, and vacuum for infection, 3 days of co-cultivation in co-cultivation media, for its further placement into shoot induction media. We were able to induce shoot formation from the hypocotyl apical meristem and the cotyledonary nodal region using a BAP-zeatin

combination after 1 month, faster than in other protocols. Furthermore, the antibiotic kanamycin was effective for selection against non-transformed plants during the subsequent elongation and rooting stages. The amplification of CRISPR/Cas9 and NPTII genes, confirms the edited cucumber plants, with an average transformation frequency of 25% after 2.5 months of the infection, representing the highest transformation frequency reported for the Poinsett 76 genotype. This methodology offers an approach to overcome barriers in cucumber tissue culture, potentially generating CRISPR/Cas9 gene-edited plants for further application in editing key genes involved in biotic and abiotic stress by creating resilient crops.

496 **Maize unstable factor for orange1 coordinates primary metabolism and epigenetic regulation**

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Maize is an important agronomic crop used for food, feed, and biofuel purposes. Maize seed comprises an embryo and a nutrient-rich endosperm. As a storage tissue for starch and proteins, maize endosperm development holds potential to improve grain size and quality; therefore, identifying candidate genes governing endosperm development is critical for future crop improvement. Our research focuses on a key endosperm-specific gene, called unstable factor for orange1 (Zmufo1), in maize. Initially, Zmufo1 was discovered as an epigenetic modifier of the pericarp color1 (p1) gene, which is a MYB transcription factor regulating flavonoid pigment accumulation in the seed pericarp. Recent studies have shown that wildtype Zmufo1 is spatio-temporally expressed in the endosperm during kernel development. Gain- and loss-of-function Zmufo1 mutants have endosperm developmental defects, abnormal accumulation of soluble sugars and reactive oxygen species leading to oxidative DNA damage. Transcriptomics and proteomics data suggest that Zmufo1 expression is associated with mitochondria-localized oxidative phosphorylation, the TCA cycle, and the upstream glycolysis cycle, which alters the accumulation of primary as well as specialized metabolites. Results from ongoing experiments will be discussed to understand how Zmufo1 expression alters energy metabolism and epigenetic landscape to orchestrate transcriptional regulation during endosperm development in the maize kernel.

500 **Comparative Transcriptomic Analysis Reveals Strain-Specific Reprogramming in Wild-Type and Engineered Fremyella diplosiphon**

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Genetic engineering has emerged as a powerful strategy to enhance stress tolerance and metabolic productivity in Fremyella diplosiphon, a model cyanobacterium recognized for its pigment plasticity, environmental adaptability, and bioresource potential. While engineering interventions aim to improve specific traits, the molecular mechanisms underlying shared and divergent transcriptomic responses among engineered strains remain unclear. The objective of the present study was to investigate metabolic reprogra mming using comparative RNA -seq analysis in the F. diplosiphon wild -type (B481 -WT), engineered photolyase -overexpressing UV-tolerant (B481-ViAnSa), and sterol desaturase -overexpressing strains (B481-SD). Total RNA was extracted from three biological replicates of each strain grown to the exponential phase, and paired -end RNA sequencing was performed using the Illumina platform. Reference genome and gene annotation files were obtained from the Joint Genome Institute genome portal, and Bowtie 2 software was used to build the genome index and align clean reads for transcriptome analysis Differential mRNA expression analysis was performed using DESeq2, and mRNAs exhibiting an absolute $\log_2$ fold change ≥ 1 and an adjusted p -value < 0.05 were considered significa ntly differentially expressed in each comparison. Our results revealed downregulation of genes associated with carotenoid biosynthesis, carbon fixation, photoprotection, DNA repair, and redox regulation in B481 -ViAnSa compared to B481 -WT, suggesting coordi nated transcriptomic responses associated with UV -B stress adaptation. In contrast, B481 -SD showed strong upregulation of mRNAs related to lipid biosynthesis, pigment production, and central

carbon metabolism, reflecting its metabolic engineering orientation. Shared transcriptional changes across both engineered strains indicated coordinated regulation of core photosynthetic and stress-response pathways. Interestingly, genes involved in nitrogen metabolism were also significantly downregulated in B481-ViAnSa compared to B481-WT. These findings demonstrate that distinct genetic engineering strategies drive strain-specific transcriptomic reprogramming in F. diplosiphon, providing a foundation for rational strain optimization toward enhanced bioresource production. This work was supported by the National Science Foundation's Nanoscale Interactions Program Award #1900966

502 **DOF1.5 -mediated Sieve Element Differentiation in Arabidopsis thaliana**

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Environmental factors are critical for directing plant cell differentiation to optimize growth, yet the specific regulatory pathways by which iron deficiency influences sieve element enucleation in Arabidopsis roots remain unclear. Our findings indicate that iron deficiency disrupts sieve element differentiation by modifying the underlying gene regulatory network. The transcription factor DOF1.5 has been identified as essential for regulating sieve element differentiation in both iron-sufficient and iron-deficient conditions. The transcriptional and promoter analyses demonstrate that DOF1.5 is enriched in several root tissues, including the root phloem, phloem pericycle, protophloem, and metaphloem. Furthermore, qRT-PCR results indicate that DOF1.5 modulates the expression of phloem transcriptional regulators APL, NAC86, and NEN4. Phenotypic evaluations indicate that DOF1.5 loss-of-function mutants exhibit longer roots under iron deficiency compared to wild-type plants, while root growth in gain-of-function lines is consistently inhibited, regardless of iron conditions. Furthermore, we demonstrate that DOF1.5 modulates iron-deficiency-induced cell wall thickening and sap unloading in the roots. Ultimately, DOF1.5 functions as a vital intersection between root phloem transport and systemic signaling during iron deficiency. Madison et al. (2025) Iron deficiency changes regulatory mechanisms governing sieve element cell differentiation. Nat Commun 16, 10196.

504 **Investigating ABA Transcription Factors Through Higher-Order Group A bZIP Mutants**

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Plants are continuously exposed to environmental or abiotic stress that can disrupt cellular processes, impair physiological functions, and ultimately affect plant survival. Relevant environmental stressors include shifts in precipitation patterns that drive more frequent and severe droughts, as well as rising atmospheric CO_2 levels. ABA, also known as abscisic acid, is a well-known hormone that regulates plant responses to abiotic stress. During drought stress, ABA triggers closure of stomatal pore apertures thereby limiting plant water loss. ABA hormone signaling activates ABF (ABRE binding factor) transcription factor proteins, which then reprogram gene expression to promote stress tolerance. The ABFs are members of a subclade of bZIP proteins known as the group A bZIPS. The goal of this project is to create a higher-order group A bZIP mutant Arabidopsis thaliana plant lacking all nine ABF related genes. Presently, we have generated quintuple and sextuple ABF mutant plants using CRISPR-Cas9 mutagenesis. Higher order ABF mutants have distinct stomatal gas exchange phenotypes, supporting their involvement in guard cell regulation. We are working to mutate the last three group A bZIP genes to generate a nonuple ABF mutant plant. By elucidating the transcriptional networks that ABA signaling, this work could contribute to strategies for engineering crops with improved stress resilience.

506 **CRISPR–Cas9-Mediated Dissection of Stress-Responsive Genes Reveals Divergent Roles of PPR, F-Box, and NRAMP in Plant Growth and Arsenic Tolerance**

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Abiotic stress tolerance is a key factor influencing crop productivity, especially under escalating environmental challenges such as heavy metal toxicity and drought. This study utilized CRISPR–Cas9-mediated genome editing to generate targeted mutants of stress-responsive genes, including PPR, F-box, and NRAMP, to elucidate their roles in plant growth and stress adaptation. Phenotypic and physiological analyses demonstrated that PPR mutants exhibited approximately 45% greater plant height than wild-type plants and showed enhanced tolerance to arsenic toxicity and drought stress, indicating a positive regulatory role in stress-resilience pathways. In contrast, F-box mutants displayed pronounced stunted

growth, while NRAMP mutants experienced severe growth inhibition, with marked stunting even under non-stress conditions, underscoring their essential roles in normal growth and metal homeostasis. These results underscore the gene-specific and contrasting functions of these regulators in plant growth and stress tolerance, and identify PPR as a promising target for engineering crops with improved multi-stress resilience. The findings advance understanding of the genetic basis of stress adaptation and support the development of crops with superior performance under adverse environmental conditions.

510 **ERF17, an AP2/ERF transcription factor homolog, potentially regulates ACC responses in the liverwort Marchantia polymorpha**

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1-Aminocyclopropane-1-carboxylic acid (ACC) is best known as the precursor to ethylene in seed plants. However, studies in seed plants have suggested that ACC may also play roles distinct from those in ethylene signaling. While ACC responses have primarily been studied in seed plants, much less is known about ACC in non-seed plants such as liverworts. The liverwort Marchantia polymorpha provides a unique system to investigate ACC responses because it synthesizes ACC but does not use it for ethylene production. Previous work from the Chang lab demonstrated that ACC inhibits growth in Marchantia, although the molecular mechanisms underlying this response remain unclear. To identify ACC signaling components, we performed RNA-seq on 6-day-old wild-type Marchantia treated with ACC for 2h and 7h. We chose the 7h time point, because ACC begins to visibly inhibit the thallus area at 7h, and we included 2h as an earlier time point. Differential expression analysis revealed that ACC induces expression of stress-response, signaling, cell-wall, and transcriptional-regulation genes, while repressing cell-cycle and meristem genes. Among early ACC-responsive genes, we identified the AP2/ERF transcription factor homolog MpERF17, which was strongly induced at 2h, as a candidate regulator. Mperf17 CRISPR mutants growing on ACC-containing agar medium displayed a reduced response to ACC (i.e. less growth inhibition) compared to the wild type. In liquid treatment assays, Mperf17 mutant plants similarly showed reduced ACC-mediated growth inhibition while wild-type plants displayed significant growth reduction. From the 7h dataset, we identified the meristem-associated peptide gene MpCLE2 as ACC-repressed. In Arabidopsis, the expression of orthologs AtCLE5, 6, and 7 is also repressed by ACC treatment in roots, suggesting

conservation across land plants. In the one Mperf17 CRISPR mutant analyzed so far, MpCLE2 was elevated but unresponsive to ACC, suggesting that MpCLE2 is downstream of ERF17 in the ACC-responsive pathway. Ongoing work includes characterization of additional erf17 CRISPR mutant lines and further phenotypic analyses to better define the role of ERF17 in ACC signaling. Together, these findings support a role for MpERF17 in ACC-responsive growth regulation, thereby advancing our understanding of ACC-specific signaling in liverworts.

512 **Rootstock-Specific Mobile mRNAs (mb-RNAs) Mediate Dynamic Regulation of Sugar Accumulation and Carbon Partitioning in Grafted Watermelon Fruits**

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Watermelon (Citrullus lanatus) fruit sweetness and sugar composition are major determinants of consumer preference and fruit quality; however, the molecular mechanisms underlying rootstock-mediated sugar accumulation remain poorly understood. Here, integrated metabolomic, transcriptomic, and mobile mRNA (mb-mRNA) analyses were performed to elucidate the role of rootstock-derived mb-mRNA in regulating fruit sugar metabolism in grafted watermelons. Distinct rootstock-dependent fruit morphometric traits and sugar accumulation patterns were observed among self-graft (SG), Carolina Strongback (CSB), LG-3 (Lagenaria siceraria), and MM30 (Cucurbita maxima × C. moschata) combinations. The results demonstrated that CSB significantly improved fruit weight, fruit width, soluble solids content, and sucrose accumulation, whereas LG-3 markedly enhanced fructose and glucose accumulation relative to the self-graft. In contrast, MM30 improved fruit firmness and rind thickness but exhibited comparatively lower soluble sugar accumulation. Integrated sugar metabolomic and transcriptomic analyses further revealed the coordinated regulation of key genes in sugar metabolism, indicating rootstock-specific modulation of sucrose turnover, hexose phosphorylation, and carbon flux. Further analyses revealed that CSB- and LG-3 rootstock-derived mb-mRNAs, including major facilitator superfamily sugar transporter, mannose-6-phosphate isomerase, WRKY70, NAC-domain protein, and sugar carrier protein C-like, were strongly associated with enhanced sugar biosynthesis, starch degradation, and carbohydrate interconversion pathways. In contrast, MM30-derived mb-mRNAs, including MYB44-like transcription factor, glycerol-3-phosphate dehydrogenase, and FAD-binding berberine family protein, were associated with the

suppression of sucrose biosynthesis and enhanced downstream carbon utilization. Collectively, these findings establish that rootstock-derived mb-mRNAs function as systemic regulators of fruit sugar metabolism, where CSB and LG-3 promote sugar accumulation, whereas MM30 activates transcriptional repressors and carbon utilization pathways that restrict soluble sugar retention in grafted watermelon fruits.

514 **Aquatic System Contamination in Sugar-Industry–Impacted Pomegranate Cultivation Areas: Implications for Environmental Health**

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Industrial effluent discharge and intensive agricultural activities associated with sugar industries can adversely affect irrigation water quality and contribute to heavy metal accumulation in horticultural ecosystems. The present study assessed the physicochemical characteristics and heavy metal contamination of irrigation water sources in sugar-industry–influenced pomegranate-growing regions of Solapur District, Maharashtra, India. Water samples were analyzed for pH, electrical conductivity (EC), total dissolved solids (TDS), major cations and anions, salinity and sodicity indices, and selected heavy metals, and the results were compared with BIS, FAO, and WHO permissible standards. The analyzed water samples showed neutral to slightly alkaline pH ranging from 6.82 to 8.59, with a mean value of 7.59. Electrical conductivity varied considerably, indicating moderate salinity levels, with a mean EC of 1.86 dS m^{-1}. Total dissolved solids ranged from 305 to 1953 mg L^{-1}. Elevated concentrations of chloride (18.4 meq L^{-1}), bicarbonate (12.4 meq L^{-1}), sodium (214 mg L^{-1}), and residual sodium carbonate (7.40 meq L^{-1}) exceeded recommended FAO irrigation standards at several locations, suggesting potential salinity and sodicity hazards. Heavy metal concentrations were generally within permissible limits; however, iron (0.16–0.38 mg L^{-1}), nickel (0.008–0.018 mg L^{-1}), and cadmium (up to 0.002 mg L^{-1}) approached critical threshold values prescribed by BIS and WHO guidelines. Statistical analysis revealed considerable variability and positive skewness for several parameters, indicating localized and episodic contamination events. The findings demonstrate that prolonged use of such irrigation

water may contribute to gradual heavy metal accumulation and salinity-induced deterioration of orchard soils, potentially affecting crop productivity, environmental quality, and food safety. The study highlights the importance of continuous monitoring, sustainable irrigation practices, and remediation-oriented management strategies to minimize ecological and toxicological risks in agro-industrial horticultural landscapes. Keywords Heavy metals; Irrigation water quality; Sugar industry pollution; Pomegranate orchards; Salinity and sodicity; Environmental toxicology; Soil–plant–water interactions

516 **Anaerobic Enzyme Activity and Aerenchyma Formation in Maize Roots in Response to Waterlogging Stress**

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The increase in extreme precipitation events across the United States has intensified waterlogging stress in maize (Zea mays), leading to significant yield loss. Oxygen deprivation in the root system is the primary constraint during waterlogging, disrupting aerobic respiration and essential metabolic processes. Under hypoxic conditions, tolerant plants initiate structural and metabolic adaptations, including the formation of lysigenous aerenchyma to facilitate internal oxygen transport and the activation of anaerobic fermentation pathways. However, the temporal and spatial coordination between anatomical adaptations and anaerobic metabolism underlying waterlogging tolerance remains poorly defined. Using contrasting genotypes B73 (moderate tolerance) and Oh7B (susceptible), we investigate the temporal and spatial dynamics of lysigenous aerenchyma formation and alcohol dehydrogenase (ADH) activity in nodal roots under waterlogging stress. We hypothesize that ADH activity and aerenchyma formation will enhance tolerance while maintaining an inverse relationship by sustaining anaerobic metabolism until internal oxygen transport is sufficient. The objectives of this study are to 1) characterize the spatial distribution of aerenchyma formation and ADH activity within the root system, 2) determine the temporal onset of these responses under waterlogging stress, and 3) define the relationship between ADH activity and aerenchyma formation. Root anatomical changes will be quantified using confocal microscopy imaging, while ADH activity will be measured through enzyme kinetics assay across defined stress time points on fresh root tissue. By integrating anatomical and biochemical responses, this study will establish a mechanistic framework linking root structural

remodeling to metabolic adaptation, providing targets for breeding and functional validation of waterlogging tolerance in maize.

518 **A Two-Component RUBY Reporter System to Assess Plant Virus Infection**

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The use of viruses to express reporter genes has significantly enhanced the study of virus replication and movement. In this study, we expanded the list of virus vectored reporter systems to include the RUBY visible reporter. Betalain, a natural product responsible for the bright red coloration in beets is the result of systematic enzymatic conversion of tyrosine which was further utilized to develop a visual reporter RUBY. To adapt this system for virus expression, Nicotiana benthamiana plants were transformed with two enzymes P450 oxygenase CYP76AD1 and glucosyltransferase required for the betalain production while L-DOPA 4,5-dioxygenase (DODA) was engineered into the tobacco mosaic virus (TMV) and turnip mosaic virus (TuMV) vectors. Inoculation of transgenic plants with virus vectors expressing DODA produced bright red tissues that corresponded with the virus infections. The primary advantage of this system is it provides robust

visualization without the need for fluorescence, the addition of enzymatic substrates or dependence on invasive sampling.

520 **Expanding CRISPR-Combo's Scope: Targeting A-T Rich Sites for Advanced Genome Modification in Plants.**

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CRISPR-Combo enables concurrent genome mutagenesis and transcriptional activation in plants, but the original SpCas9-based platform is constrained by its PAM requirement, which can limit access to AT-rich genomic regions, including many promoter sequences used for gene activation. To broaden the targeting range of CRISPR-Combo, we evaluated Cas12b and iSpyMacCas9 as alternative nuclease platforms for simultaneous gene editing and transcriptional activation. These systems were tested in rice using hormone-free regeneration driven by activation of the endogenous morphogenic regulator OsBBM1, while simultaneously editing the target genes. The Cas12b-Combo system produced only modest OsBBM1 activation, approximately 3-fold, and did not noticeably improve editing outcomes. In contrast, iSpyMacCas9-Combo induced strong OsBBM1 upregulation, approximately 12-fold, and enabled efficient hormone-free plant regeneration at a rate of 42%. Coupling iSpyMacCas9 with OsBBM1 activation also improved overall

editing performance, including multiplex editing, compared with standard iSpyMacCas9 systems regenerated with or without hormones. Together, these results demonstrate that iSpyMacCas9-Combo is an effective platform for rice genome editing, particularly at targets with otherwise low editing efficiency. This work expands the CRISPR-Combo toolkit by increasing genomic targeting flexibility and showing that activation of an endogenous morphogenic gene can enhance recovery of edited rice plants.

526 **Comparative Transcriptomics of CRISPR/Cas9-Edited brachytic 1 Reveals Control of Internode Elongation in Maize**

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The brachytic 1 (br1) locus is a crucial regulator of the maize plant. It is associated with plant height and internode elongation. The br1 locus encodes an MYB transcription factor involved in internode development and growth regulation. To investigate the function of br1, a mutant line was generated by editing the third exon of the gene using CRISPR/Cas9. The br1 mutant showed significantly reduced plant height and shorter internode length, playing a crucial role in internode development and regulation. The process affects the architecture of the plant, stem strength, biomass accumulation, and overall crop productivity of maize. Although internode development is a major determinant of plant height, the molecular function of br1 in maize internodes, transcriptional changes, and biological pathways affected by br1, remains poorly understood. To address the gap, transcriptomic profiling of br1 mutant and wild-type maize internode tissues was performed. Tissue samples capturing the spatial variation from top, middle, and bottom internode tissues were collected at the V6 stage and subjected to RNA sequencing. Differential expression and Gene Ontology (GO) analyses identified genes and biological processes affected by the loss of br1 function. Comparative analysis revealed distinct gene expression changes between mutant and wild-type internodes, indicating that br1 regulates gene networks associated with stem growth, development, and elongation. Out of 140 differentially expressed genes, 94 genes were downregulated, and 46 genes were upregulated. 3 of the differentially expressed genes are common in all tissues, 70, 25, and 42 significant genes are expressed in the down, middle, and top tissue respectively. Differentially expressed genes were associated with cell wall organization, lignin biosynthesis, hormone signaling, regulation of primary metabolic processes, and stress-related responses. These findings indicate that br1 contributes to both structural and regulatory processes in maize internodes and provide insight into its role in coordinating gene expression during internode development. Overall, this study improves our understanding of the molecular mechanisms controlling maize stem development and establishes a foundation for future functional characterization of br1 and associated regulatory pathways.

528 **Engineering tomato disease resistance by targeting a host susceptibility factor to combat fungal pathogens**

Alex Broadway, Ruiting Wang, Daniel Rodriguez-Leal, and Nidhi Rawat

With nearly 190 million tons produced annually, tomato is a vital global crop. Fungal diseases such as Early blight, Fusarium wilt, Verticillium wilt, and Grey mold are major threats to tomato production. As emerging races evolve to overcome currently deployed resistance, developing new genetic defense strategies is critical for tomato breeders. We aim to engineer broad-spectrum disease resistance in tomato against multiple fungal pathogens with minimal negative pleiotropic effects on plant performance. We used multiplex gene editing to target *DMR6*, a susceptibility gene which confers resistance to multiple pathogens in tomato and other crop species. We are targeting multiple sites in the *DMR6* promoter to create a diverse array of *DMR6* expression mutants in tomato. We will use this array to identify variants with robust disease resistance and minimal negative effects on plant performance. We generated novel variations in the DMR6 promoter region in M82, a processing tomato variety. From this mutant panel, we identified homozygous edited events in mutant lines dmr6 pro-3 and dmr6 pro-10 which have small and large deletions in the promoter region respectively. Using these lines, we analyzed whether *DMR6* expression was affected by promoter editing and plants were challenged with foliar fungal pathogen Botrytis cinerea. Our findings will be shared in the presentation. Our research aims to develop a novel strategy to engineer disease resistance against fungal pathogens without negative pleiotropic effects, facilitating sustainable tomato production.

530 **A Comprehensive Web-Based Platform for Genome-Specific Primer and KASP Marker Design in Polyploid Species**

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Wheat is an important crop for global food security. Gene discovery and deployment are critical for wheat improvement and require gene or locus-specific markers. In light of new wheat genomes and pangenomes of wild and related species, there is a need to develop platforms that can identify species-and genome-specific primers to support marker-assisted selection and gene discovery, and validation pipelines that systematically utilize all available genomes and pangenomes. Polyploid species pose a fundamental challenge for molecular marker development: their multiple, similar subgenomes lead conventional primers to amplify homeologous loci, compromising genotyping accuracy and gene expression studies. This problem is especially problematic in hexaploid bread wheat (Triticum aestivum, 2n = 6x = 42, AABBDD), where three closely related subgenomes confound standard primer design approaches. Earlier, we addressed this problem by developing a web-based primer design tool that used wheat draft genome sequences from Chinese Spring to identify genome-specific markers. Here, we present GSP 2.0, an upgraded species- and wheat-subgenome-specific primer development platform that overcomes these limitations and provides a resource for primer development from 28 publicly available wheat genomes spanning diploid, tetraploid, and hexaploid species, enabling genome-wide specificity assessment. This platform is useful for developing genome- or species-specific PCR primers and allele-specific KASP primers. With more than 80/90% accuracy in developing genome-specific primers, GSP 2.0 provides a powerful, accessible resource for accelerating genome-specific marker development in wheat, and this system can be expanded to other polyploid or diploid crops.

532 **Combined effects of *Xanthomonas* and temperature stress in *Capsicum annuum***

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Bacterial spot disease caused by *Xanthomonas euvesicatoria* remains a major threat to *Capsicum annuum* production in the southeastern United States despite resistance breeding efforts. Current and future environmental conditions may favor pathogen growth while also suppressing host defense responses. Recessive resistance genes have been incorporated into pepper breeding programs to reduce disease severity; however, the durability of this resistance under elevated temperature is uncertain. This pilot study investigates the effect of elevated temperature and inoculation with *X. euvesicatoria* strain XEU 85-10 on growth and development in pepper cultivars are resistant to *X. euvesicatoria*. Both a susceptible (cv. Earl Calwonder) and resistant (cv. Early Calwonder with *bs*5/6 gene) cultivar of pepper were grown in growth chambers for 5 weeks in ambient temperatures before transitioning to elevated (35 °C) or ambient (25 °C) conditions for 72 hours prior to inoculation. Gas exchange measurements were taken mid-day to evaluate stomatal efficiency prior and impacts on plant productivity. Leaf tissues were flash frozen to evaluate bacterial colonization, callose deposition and defense hormone accumulation and any host RNA expression changes. We hypothesize that elevated temperature compromises resistance-mediated defense in *C. annuum*. This compromise would support increased bacterial proliferation, reduce stomatal efficiency, decrease callose deposition and reduce defense hormone accumulation compared to susceptible cultivars grown under the same conditions. Understanding the interactions of elevated temperature and *Xanthomonas* inoculation will provide insight into the stability of resistance genes under temperature associated stress and may inform future breeding strategies for durable disease resistance in pepper crops.

534 **Molecular indexing of *Tomato brown rugose fruit virus*, *Pepino mosaic virus* and DNA barcoding of tomato fruits from Maryland grocery stores**

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Tomatoes (*Solanum lycopersicum* L.) are widely consumed vegetables in the U.S. and globally due to their various benefits. Tomatoes are produced in the U.S. and supplemented by imports, but production is threatened by viral diseases, particularly Tomato brown rugose fruit virus (ToBRFV: family Virgaviridae, genus Tobamovirus) and *Pepino mosaic virus* (PepMV: family Alphaflexiviridae, genus Potexvirus). Early detection and host identification are critical for management strategies. This study indexed ToBRFV and PepMV in tomato fruits from local and international stores in Maryland and used DNA barcoding to identify susceptible tomato species. Symptomatic tomato fruits were collected for RNA and DNA extractions using RLT QIAgen buffer. RNA was converted to cDNA followed by RT-PCR and Sanger sequencing, while DNA was amplified with the rbcL marker for species identification. 61% tested positive to ToBRFV and showed 100% sequence identity with known isolates in the NCBI database. ToBRFV incidence was 53% in local stores and 80% in international stores. 53% had mixed ToBRFV and PepMV, while 86% tested positive to PepMV, highlighting concerns about their combined effects on fruit quality and yield. Mutations were detected at nucleotide and amino acid levels [position 131: Asparagine (N)-Serine (S)]. DNA barcoding identified several tomato-related species, including *Solanum lycopersicum*, *S. galapagense*, *S. chilense*, *S. pimpinellifolium*, among others, with *S. lycopersicum* being the most susceptible. This study provides important insights into the prevalence, diversity, epidemiology, and spread of these economically important viruses through retail supply chains. High-throughput sequencing, RT-qPCR, and ddPCR technologies are being explored with more samples from expanded locations for validation.

536 **Studying Cell-Type Specific Transcriptional Responses To Salt Stress In Pennycress (Thlaspi arvense) Roots Using Single Nuclei Transcriptomics**

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Soil salinization threatens over 800 million hectares of agricultural land globally, rendering vast areas unsuitable for traditional crops. Winter cover crops like pennycress (Thlaspi arvense) offer a promising solution for these marginal saline lands, providing oilseed production on otherwise unproductive acreage. However, the cellular mechanisms underlying pennycress salt tolerance remain poorly understood, limiting efforts to optimize this emerging crop for saline environments. To investigate cellular responses to salt stress in pennycress, we examined 6-day-old seedlings treated with 75 mM NaCl. Root tissues were harvested post-treatment and processed for both bulk RNA-seq and single-nucleus RNA-seq. Bulk RNA-seq differential expression analysis identified 1,183 differentially expressed genes (padj < 0.05), revealing enrichment of photosynthesis-related processes and water deprivation responses among upregulated genes, while downregulated genes were enriched in oxidative stress response, defense responses, and immune system regulation. Single-nucleus RNA-seq yielded 28,022 high-quality nuclei across four samples (control and salt-stressed, two biological replicates each), detecting approximately 1,351 genes per nucleus in control samples and 1,666 genes per nucleus in salt-stressed samples, providing a comprehensive resource for dissecting cell-type-specific salt stress responses in pennycress roots. Cross-species marker gene identification revealed 10 major root cell populations. Comparative analysis revealed that salt stress dramatically altered cellular composition, with a 39% reduction in actively dividing meristematic cells, a 23% increase in vascular tissue populations (phloem and xylem pole pericycle), and a 40% reduction in root endodermis cells. Cell-type-specific differential expression identified key regulators of these shifts, including upregulation of NRT1.5 (AT1G68570) in xylem pole pericycle and downregulation of auxin signaling genes ARF2 and AUX, linking reduced auxin transport to lateral root suppression. These shifts in cell-type proportions indicate that pennycress roots undergo defensive cellular remodeling under salt stress, prioritizing vascular expansion for ion exclusion and water management while suppressing growth-related processes. Furthermore, root growth measurements at 48 hours post-treatment revealed growth inhibition at 75 mM NaCl, directly correlating with the reduction in meristematic cells observed at 24 hours and demonstrating that early cellular compositional changes predict subsequent

developmental phenotypes. These findings reveal the cellular mechanisms enabling pennycress salt tolerance and establish a cell-type-resolved transcriptomic atlas that can guide breeding efforts to optimize this winter cover crop for productive oilseed production on marginal saline agricultural lands.

538 **Deciphering the Molecular Mechanisms of Abscisic Acid-dependent Gene Regulation in Plants**

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The plant hormone abscisic acid (ABA) plays a central role in drought responses by inducing stomatal closure in guard cells and reprogramming gene expression across different plant cell types. Chromatin accessibility is a key determinant of gene activation. Previous studies indicate that ABA triggers extensive remodeling of chromatin structure, and its opening requires four related transcription factors (TFs), known as ABF1-4 proteins. Current understanding lacks the mechanisms by which ABF TFs coordinate with other proteins to initiate chromatin remodeling during ABA/drought responses. This project explores the molecular mechanisms of ABA-dependent gene regulation in Arabidopsis thaliana. We employed TurboID-based proximity labeling, followed by streptavidin affinity purification and mass spectrometry to identify proteins that interact with the ABF4 TF. Notably, histone acetyltransferase 1 (HAC1) was the second-most-abundant protein detected, after ABF4 itself. Histone acetylation is known to play a major role in transcriptional activation. Yeast two-hybrid experiments indicated that HAC1 directly interacts with ABF proteins. Using cell-type-specific epigenomic approaches, we have discovered that ABA triggers genome-wide changes in histone acetylation. Interestingly, ABA-induced acetylation coincided with genomic regions with ABA-stimulated chromatin accessibility and transcription. Next, we will determine if this acetylation depends on ABF TFs and investigate the role of HAC1 in ABA responses. This research will deepen our knowledge of plant genome regulation under drought stress, aiding efforts to improve drought tolerance in plants.

540 **Wheat Pore-Forming Toxin-like gene provides broad spectrum resistance to fungal pathogens in transgenic tomato and strawberry plants**

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Wheat pore-forming toxin-like (PFT) gene was reported by Rawat et al. (2016) previously to provide Fhb1 mediated resistance to Fusarium graminearum infection in resistant wheat cultivar Sumai 3. To investigate the effect of PFT in another plant system, we ectopically expressed it in dicot plant model plant Arabidopsis thaliana, which does not have any PFT ortholog or homolog and observed a broad-spectrum resistance to several necrotrophic and hemi-biotrophic fungal pathogens. In this follow up study, the wheat PFT gene was transferred to a diploid tomato cv. Moneymaker and an octaploid strawberry cv. Camerosa as both of these varieties are susceptible to several pathogens. The PFT transgenic tomato plants were challenged with Fusarium oxysporum f. sp. lycopersici, Verticillium dahliae, Alternaria linariae, and Botrytis cinerea (T1 and T2) whereas the PFT transgenic strawberry plants (T0) were challenged with Botrytis cinerea and Colletotrichum fioriniae. In both the experiments, transgenic plants of PFT tomato and strawberry showed significantly less disease severity index and fungal biomass with significant disease resistance against the fungal pathogens tested. In an in vitro antifungal assay, the F. graminearum spores treated with the purified and Dylight594 labelled PFT protein retarded the spore germination during 24h incubation period when compared to the buffer control. Glycan binding kinetics assays of purified PFT with different carbohydrates exhibited strongest binding with chitin monomer N-Acetyl-D-Glucosamine (NADG). These results support a model in which PFT targets fungi via GlcNAc/chitin recognition through its lectin domains and suppresses infection through its pore-forming toxin-like domain. Collectively, this work establishes PFT as a transferable, broad-spectrum antifungal trait effective across diverse plant genetic backgrounds.

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Qi Mu (Assistant Professor, UD)
Sadanand Dhekney (Professor, UMES)
Behnam Khatabi (Associate Professor, UMES)
Purushothaman Natarajan (Assistant Professor, UMES)
Eloisa Vendemiatti (Assistant Professor, WV)

Undergraduate and Graduate Students and Postdocs organizers and volunteers:

Simran Goyal (Graduate Student, UMD-PSLA)

Alex Susannah Broadway (Graduate Student, UMD-PSLA)

Prem Kumar Ganesan (Postdoctoral Researcher, UMD-PSLA)

Parva Kumar Sharma (Postdoctoral Researcher, UMD-PSLA)

Edward George Haag (Research Assistant, UMD-PSLA)

Jenna Pugh (Undergraduate Student, UMD-CMNS)

Tara Dinh (Undergraduate Student, UMD-PSLA)

Innocent Byiringiro (Graduate Student, UMD-PSLA)

Aidan Michael Danoff (Graduate Student, UMD-CBMG)

Ruiting Wang (Graduate Student, UMD-PSLA)

Jordan Oxendine (Research Assistant, UMD-PSLA)

Thaihjla Pernell (Undergraduate Student, UMD-CMNS)

Blake Komisar-Bury (Undergraduate Student, UMD-PSLA)

Thomas Randall Young (Graduate Student, UMD-PSLA)

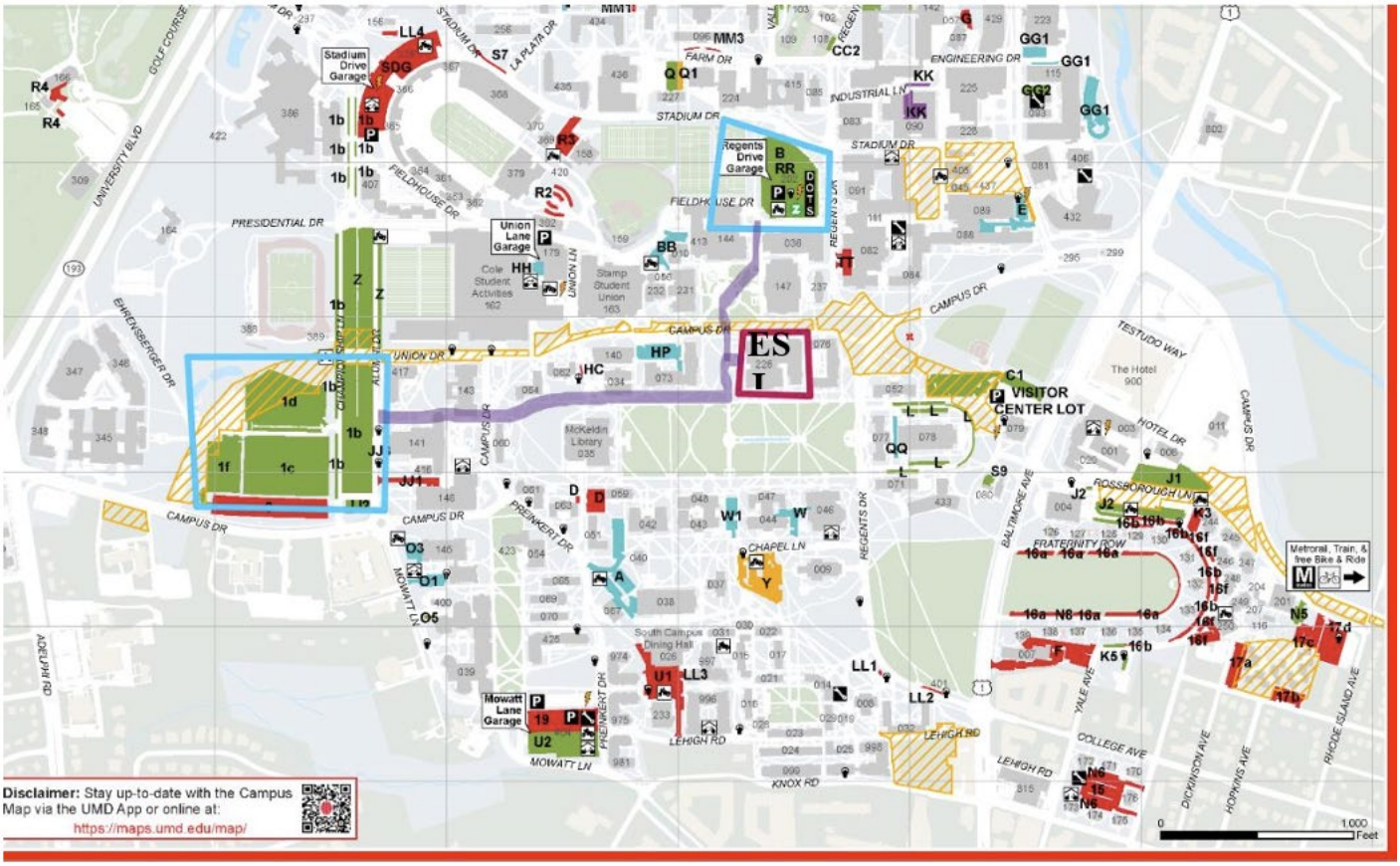
Gen Li (Postdoctoral Researcher, UMD-PSLA)

Meghan Shoger (Graduate Student, UMD-PSLA)

Joshua Scott Clem (Graduate Student, UMD-PSLA)

Parking

For those who will drive to UMD, we suggest two different parking locations: 1) Regents Drive garage is the closest option, however it is not free. Once you enter the garage, drive to the top floor and pay at the meters (cash or card) located at each corner of the floor or using a mobile app (e.g. Parkmobile). The cost of whole-day parking is ~$20. The other option for those who would like free parking is Lot 1, located about a 10-minute walk from the conference building. Note: There may be several local high school graduations that will be hosted on campus this week so traffic to and from campus might be higher than usual. Please plan accordingly.



In the map above, parking lots are marked with blue borders and the walking path outlined in purple. Directions from Regents Drive Garage after parking: take the elevator down to the 3rd floor, then walk the bridge over to Hornbake Plaza. Cross Street from Hornbake Plaza. The ESJ building is to your left. Enter the main doors. Direction from Lot 1 garage after parking: Walk towards main campus. Cross street. Walk along the border of McKeldin Mall. Take left at halfway point of McKeldin Mall. The ESJ building is on your right. Enter the main doors. The actual address of the ESJ Building is: 4131 Campus Dr, College Park, MD 20742.