2025 JOINT MID-ATLANTIC SECTION ASPB & UMD PLANT SYMPOSIUM – PROGRAM

UNIVERSITY OF MARYLAND, COLLEGE PARK

MAY 28-29, 2025

Rm 0224, Edward St. John Learning and Teaching Center (Bldg #226) University of Maryland, College Park (campus map link)

SPONSORS

DEPARTMENT OF PLANT SCIENCE & LANDSCAPE ARCHITECTURE

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Schedule	pp 2-5
Talk Abstracts	pp 6-22
Poster Abstracts	pp 23-80
Organizers	p 81
Parking Info	p 82

2025 Joint MAS-ASPB & UMD Plant Symposium Schedule

Day 1: Wednesday (May 28)	
8:30–9:00 am	**Registration; Light breakfast; Set up presentations for Session I**
9:00–9:03 am	Welcome Remarks: **Dr. Vijay Tiwari**
9:03–9:05 am	Keynote Speaker Introduction: **Dr. Vijay Tiwari**
9:05–9:55 am	**Keynote address – Dr. Sheng-Yang He** (Duke University) *Climate impact on plant immunity and microbial interactions*
9:55–10:10 am	**Coffee Break**
10:10–11:45 pm	**Session I: Plant Genetics and Genomics** **(session chairs Dr. Mark Zander and Joey Lagner)**
10:10–10:35 am	**Dr. Charles Seller** (University of Maryland, College Park) *Mechanisms enabling the regulation of the guard cell genome by a changing environment*
10:35–11:00 am	**Dr. Song Li** (Virginia Tech) *Orthologous marker groups reveal broad cell identity conservation across plant single-cell transcriptomes*
11:00–11:25 am	**Dr. James (Jack) Satterlee** (University of Wisconsin – Madison) *Evolution of plant prickles by repeated LOG gene co-option*
11:25–11:35 am	**Dr. Hong Ma** (Penn State University) *Genome-wide comparison of exon-intron structures across angiosperms and association with protein domain organization, gene expression and alternative splicing*
11:35–11:45 am	**Joshua Clem** (University of Maryland, College Park) *A novel Type I-F CRISPR system confers kilobase-scale genomic deletions at GC-rich PAMs in plants*
11:45–11:50 am	**Brief presentations from our Sponsors**
11:50–12:40 pm	**Lunch for all registered attendees**
12:40–2:00 pm	**Poster Session (Odd numbers present);** **Set up presentations for Session II**
1:00– 2:00 pm	**Webinar: “Meet the Candidates” for ASPB President**
2:00–3:25 pm	**Session II: Biotic Stress (session chairs Dr. Shunyuan Xiao and Joshua Clem)**

2:00–2:25 pm	**Dr. Hua Lu** (University of Maryland Baltimore County) *The flowering time regulator FLK controls defense response in Arabidopsis*
2:25–2:50 pm	**Dr. Aardra Kachroo** (University of Kentucky) *Regulators of the systemic acquired resistance signaling pathway*
2:50–3:15 pm	**Dr. Corlett Wood** (University of Pennsylvania) *Integrating mutualisms into the ecology and evolution of plant defense*
3:15–3:25 pm	**Dr. Jessica Allison** (University of Maryland Baltimore County) *Investigating the molecular basis underlying crosstalk between defense and the circadian clock in Arabidopsis*
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. Courtney Leisner and Weifeng Luo)**
3:40–4:05 pm	**Dr. Courtney Leisner** (Virginia Tech) *Physiological and transcriptomic responses of pepper to combined ozone and pathogen stress*
4:05–4:30 pm	**Alison Schulenburg and Nate Spicer** (University of Maryland) *Adapting to saltwater intrusion: Land management strategies and greenhouse gas implications during farmland-to-marsh transitions*
4:30–4:40 pm	**Moonia Ammari** (Rutgers University) *Elucidating stress-induced epigenome reprogramming in crop and medicinal plants* (Poster #616)
4:40–4:50 pm	**Dr. Brett Shelley** (USDA-ARS, WV) *Investigation of root phenotypes and drought stress responses associated with DEEPER ROOTING 1 overexpression in apple rootstocks*
4:50–5:10 pm	**Dr. Hong Ma** (ASPB President) and **Dr. Irma Ortiz** (ASPB Ambassador) *ASPB: Staying together makes all of us stronger*
5:10–5:25 pm	**Coffee Break**
5:25–6:15 pm	**Grant Development Workshop (Conveners: Drs. Nidhi Rawat and Charles Seller)** **Panelists:** **Dr. Diane Okamuro** (NSF PGRP) **Dr. Shin-Han Shiu** (NSF PGRP) **Dr. Aardra Kachroo** (University of Kentucky)

6:30–9:00 pm	**Reception/Dinner at The Hall CP for all registered attendees** 4656 Hotel Dr., College Park, MD 20742
Day 2: Thursday (May 29)	
8:30–9:00 am	**Registration; Light breakfast; Set up presentations for Session IV**
9:00–9:02 am	Keynote Speaker Introduction: **Dr. Caren Chang**
9:02–9:52 am	**Keynote Address – Dr. Robin Buell** (University of Georgia) *Complexity of plant natural product biosynthesis revealed through single cell multi-omics*
9:52–10:10 am	**Coffee Break**
10:10–11:45 am	**Session IV: Plant Development and Cell Biology (session chairs Dr. Xingyun Qi and Parva Sharma)**
10:10–10:35 am	**Dr. Ying Gu** (Penn State University) *Cell wall architecture and acid growth – an insight into the mechanism of ultra-fast auxin-induced shoot cell expansion*
10:35–11:00 am	**Dr. Kyle Swentowsky** (Cold Spring Harbor Laboratory) *Zea diploperennis perennial regrowth QTL Regrowth1 and Regrowth3 control life history traits*
11:00–11:25 am	**Dr. Bastiaan Bargmann** (Virginia Tech) *Deciphering Auxin Signaling Specificity Using a Synthetic Biology Approach*
11:25–11:35 am	**Evan Littleton** (Virginia Tech) *Antioxidant properties of dihydroxy B-ring flavonoids modulate circadian amplitude in Arabidopsis*
11:35–11:45 am	**Dr. Gen Li** (University of Maryland, College Park) *Simultaneous activation of multiple genes boosts poplar regeneration and growth using CRISPR-Combo* (Poster #636)
11:45– 12:30 pm	**Lunch for all registered attendees**
12:30– 1:45 pm	**Poster Session (Even numbers present)** **Set up presentations for Sessions V and VI**
1:45 – 2:45 pm	**Career Development Workshop (conveners: Drs. Hua Lu and Daniel Rodriguez Leal)** **Panelists:** **Dr. Murli Manohar** (CryoBio) **Dr. Elizabeth Cebul** (Leica) **Dr. Shab Vellani** (Ansa Biotechnologies)

	Dr. Yazmín Rivera (USDA)
2:45 – 3:00 pm	**Coffee Break**
3:00 – 5:20 pm	**Session V: Approaches for Crop Improvement (session chairs Drs. Edward Eisenstein and Gen Li)**
3:00 – 3:25 pm	**Dr. Gary Muehlbauer** (University of Minnesota) *Does barley contain resistance to Fusarium graminearum*
3:25 – 3:50 pm	**Dr. Vagner Benedito** (University of Maryland Eastern Shore) *Advancing tomato resistance to pests and diseases through harnessing genetic diversity from wild species*
3:50 – 3:55 pm	**Jordan Oxendine** (University of Maryland, College Park) *Developing a pipeline for Agrobacterium-mediated transformation in heirloom tomato cultivars* (undergraduate talk) (Poster #655)
3:55 – 4:05 pm	**Innocent Byiringiro** (University of Maryland, College Park) *Expanding CRISPR-Combo targeting scope for A and T rich PAM sites*
4:05– 4:15 pm	**Sunilda Frias** (Virginia Tech) *Sparse testing: a resource-efficient approach for enhancing genetic gain through early multi-environment trials*
4:15 – 4:30 pm	**Coffee Break**
4:30 – 4:55 pm	**Dr. Anne Simon** (University of Maryland, College Park) *Stabilizing inserts in virus-induced gene silencing vectors*
4:55 – 5:20 pm	**Dr. David Lowry** (Michigan State University) *Prospects for the improvement of yield and resilience in bioenergy switchgrass by genomic-enabled selection*
5:20 – 5:35 pm	**Poster and Oral Presentation Awards**
5:35 pm	**Closing Remarks; Poster take-down and Departure**

Please submit your nominations (including self-nominations) for the next Chair, Vice Chair, Secretary/Treasurer and Section Rep for the Mid-Atlantic Section of ASPB using the QR code at the right. These are two-year positions starting Oct 1, 2025. Elections will be held in Aug-Sept.

Current officers:
Chair: Vijay Tiwari (UMD)
Vice Chair: Daniel Rodriguez Leal (UMD)
Secretary/Treasurer: Nicholas Santantonio (Virginia Tech)
Section Rep: Caren Chang (UMD)

TALK ABSTRACTS

Wednesday, May 28

KEYNOTE TALK

Climate impact on plant immunity and microbial interactions

Sheng-Yang He

Biology Department, Howard Hughes Medical Institute, Duke University, Durham, NC 27708, USA.

Infectious disease outbreaks in plants require not only a genetically susceptible plant and a virulent pathogen, but also conducive environmental conditions. Molecular studies in the past four decades have made major strides in understanding the mechanistic bases of plant resistance and pathogen virulence. However, less effort has been devoted to addressing an increasingly important question – why climatic conditions, such as temperature, humidity and salinity, have a profound effect on host susceptibility and disease development. Moreover, current studies often ignore the potentially pervasive effect a plant's endogenous microbiome may have on host-pathogen interactions. In this talk, I will give an example of interplay between plant, pathogen and environment during *Pseudomonas syringae* infection of host plants. Results suggest that future studies should increasingly consider the multi-dimensional nature of "plant-microbe-environment" interactions, which are likely more reflective of what occur in natural ecosystems.

SESSION I – Plant Genetics and Genomics

Mechanisms enabling the regulation of the guard cell genome by a changing environment

Charles Seller

University of Maryland, College Park

Plants evolved sophisticated mechanisms to sense and respond to environmental stresses, however climate change has intensified many of these stresses by changing patterns of water availability, elevating average temperature, and increasing atmospheric carbon dioxide (CO2) concentration. We use the Arabidopsis guard cell as a model system for investigating the mechanisms that connect environmental conditions to plant genome structure and activity. Guard cells are specialized cells that integrate multiple environmental cues to optimally control the size of microscopic pores on the leaf surface known as stomata. For example, changes in the concentration of the plant hormone abscisic acid (ABA), a key regulator of drought responses, and changes in atmospheric CO2 concentration are well known signals that trigger adjustments in stomatal pore size. Stomatal regulation is essential for minimizing water loss while allowing CO2 uptake from the atmosphere for photosynthesis. We have uncovered cell-type specific genome regulatory programs deployed in guard cells by the hormone ABA.

During drought stress, ABA triggers extensive and long-lasting remodeling of chromatin structure. By isolating and analyzing guard cells from different higher order mutant plants we identified three distinct families of transcription factors that are responsible for controlling ABA-mediated chromatin dynamics and transcriptome reprogramming. Our research indicates that the distinct ABA and CO2 signaling pathways are integrated in guard cells by transcriptional regulation. Collectively, our results support a model wherein chromatin structure acts as a platform where different physiological signals are integrated in the guard cell leading to long-term and protective adjustments to stomatal function.

Orthologous marker groups reveal broad cell identity conservation across plant single-cell transcriptomes

Song Li

Virginia Tech, Blacksburg, VA

Single-cell RNA sequencing (scRNA-seq) is widely used in plant biology and is a powerful tool for studying cell identity and differentiation. However, the scarcity of known cell-type marker genes and the divergence of marker expression patterns limit the accuracy of cell-type identification and our capacity to investigate cell-type conservation in many species. To tackle this challenge, we devise a novel computational strategy called Orthologous Marker Gene Groups (OMGs), which can identify cell types in both model and non-model plant species and allows for rapid comparison of cell types across many published single-cell maps. Our method does not require cross-species data integration, while still accurately determining inter-species cellular similarities. We validate the method by analyzing published single-cell data from species with well-annotated single-cell maps, and we show our methods can capture majority of manually annotated cell types. The robustness of our method is further demonstrated by its ability to pertinently map cell clusters from 1 million cells, 268 cell clusters across 15 diverse plant species. We reveal 14 dominant groups with substantial conservation in shared cell-type markers across monocots and dicots. To facilitate the use of this method by the broad research community, we launch a user-friendly web-based tool called the OMG browser, which simplifies the process of cell-type identification in plant datasets for biologists.

Evolution of plant prickles by repeated LOG gene co-option

Jack Satterlee

University of Wisconsin at Madison

Convergent evolution, in which similar traits evolve independently in different lineages, often reflects adaptation to shared environmental pressures. While phenotypic convergence among closely related species may involve similar genetic mechanisms, the extent of genetic repeatability across deeper evolutionary timescales remains unclear. Prickles—sharp, epidermal outgrowths that deter herbivores—have evolved multiple times across flowering plants and are often lost during domestication due to their agricultural disadvantages. Focusing on the genus *Solanum*, which includes

tomato, eggplant, and numerous wild species, we investigated the genetic basis of prickle loss in domesticated lineages. Through interspecific crosses between cultivated eggplant (*Solanum melongena*) and its prickled wild relative (*S. insanum*), we identified mutations in a member of the *LONELY GUY* (LOG) gene family, involved in cytokinin biosynthesis, underlying domestication-associated prickle loss. High-quality genome assemblies from multiple domesticated eggplants, including African eggplant, enabled rapid discovery of additional, independent *LOG* mutations. By integrating genomic and herbarium data across the genus, we identified 16 independent *LOG* mutations that account for 14 of 31 known prickle loss events in *Solanum*. Strikingly, similar *LOG* mutations were also found to underlie prickle loss in distantly related plant species such as rose and Chinese date. Using genome editing, we engineered *LOG* mutations in wild *Solanum* species and successfully suppressed prickles without affecting other traits. These findings reveal that the repeated co-option of *LOG* genes underlies convergent prickle loss across angiosperms and demonstrate the potential for predictable, targeted trait modification in crop improvement.

Genome-wide comparison of exon-intron structures across angiosperms and association with protein domain organization, gene expression and alternative splicing

Hong Ma (1), Taikui Zhang (1), Jun Wang (1,2), and Lin Zhang (3)

(1) Department of Biology and the Huck InsEtutes of the Life Sciences, The Pennsylvania State University, PA, USA; (2) School of Life Sciences, Fudan University, Shanghai, China; (3) School of Life Sciences, Southwest University, Chongqing, China

Most genes in plants contain introns, which may contain cis elements for regulation of transcription but must be removed to produce mature messenger RNAs via splicing. Alternative splicing provides a step for regulation of gene expression levels and can generate alternative transcripts with differential protein sequences, potentially increase the functional repertoire of proteins encoded by a single gene. Genome-wide patterns of alternative splicing have been reported for several plant species and alternative transcripts of specific genes have been analyzed for impact on functional expression level and protein activity. However, little is known about genome-wide patterns of conservation and divergence of exon-intron structures across angiosperms, and possible association of such patterns with protein domain organization and possible functions, differential gene expression among major organs, and alternative splicing involved exons of different sizes. We have performed an extensive comparison of exon-intron structures with 46 angiosperm species covering a broad diversity and 4 gymnosperms as an outgroup with the following sets of results: (1) distribution of exons in five size groups; (2) conservation of exons among seed plants, angiosperms, and major subgroups of angiosperms; (3) patterns of correspondence between exon(s) and protein domain(s), including the number of exons that encode (portions of) a single domain; (4) relationship of exon size with differential gene expression, and (5) patterns of alternative splicing, including exon skipping and other forms, for different size categories, with potential for changing protein sequences. These results and their evolutionary implications will the discussed.

A Novel Type I-F CRISPR System Confers Kilobase-scale Genomic Deletions at GC-rich PAMs in Plants

Joshua Clem[1], Simon Sretenovic[2], Micah Dailey[3], Robin Schendel[4], Marcel Tijsterman[4], Thomas DuBois[5], Bastian Minkenburg[5], Rodolphe Barrangou[6], Yiping Qi[1,7].

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CRISPR-Cas systems have provided remarkably capable tools for editing plant genomes. 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, which encode the multi-effector Cascade machinery, are known to generate large-scale deletions but have received comparatively little attention for plant genome editing. Moreover, several Type I subtypes have been 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). When expressed in transgenic rice plants, the MmeCascade, harboring a unique Cas3-Cas2 fusion, results in large genomic deletions on the order of several kilobases at two genomic target sites containing GC-rich PAMs. 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. 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, and gene clusters in plants.

SESSION II – Biotic Stress

The flowering time regulator FLK controls defense response in *Arabidopsis*

Hua Lu

Department of Biological Sciences, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250, USA.

Plant defense and development are intricately interconnected. In a genetic screen for suppressors of a defense-sensitized Arabidopsis mutant *accelerated cell death 6-1* (*acd6-1*), we identified an allele of the canonical flowering regulatory gene *FLOWERING LOCUS K HOMOLOGY DOMAIN* (*FLK*), encoding a putative RNA protein. In addition to late flowering, the *flk* mutants exhibit altered disease resistance to pathogens. RNA-seq

analysis show that *FLK* regulates expression abundance of some major defense- and development-related genes as well as gene alternative splicing. Among the genes affected by *FLK* is *ACD6*, whose transcripts have increased intron retentions influenced by the *flk* mutations. The *flk* mutants are also impaired in defense signaling mediated by salicylic acid (SA) and reactive oxygen species (ROS) homeostasis. Mutant analysis reveals complex genetic interactions between *FLK* and several major SA pathway genes. In addition, *FLK* requires the primary catalase gene *CAT2* in regulating ROS homeostasis. Interestingly, the SA- and ROS-regulatory roles of *FLK* are independent of *FLK* function in flowering time control. Thus, our data provide a mechanistic support for *FLK*'s defense role and decouple the multifunctionality of *FLK* in defense and development in Arabidopsis.

Regulators of the systemic acquired resistance signaling pathway

Aardra Kachroo

University of Kentucky, Lexington, KY

Systemic acquired resistance (SAR) is a highly desirable form of resistance that protects against a broad-spectrum of pathogens. SAR involves the generation of a mobile signal at the site of primary infection, which arms distal portions of a plant against subsequent secondary infections. Several diverse chemical signals contributing to SAR have been isolated and characterized. Among these, salicylic acid (SA) functions in parallel to azelaic acid (AzA) and glycerol-3-phosphate (G3P) and both AzA and G3P function downstream of nitric oxide and reactive oxygen species. Both SA and G3P regulate the stability of trans-acting small interfering RNA (tasi-RNA), which function as an early mobile signal in SAR. We show that phloem loading of AzA, G3P and tasi-RNA occurs via the symplast, whereas that of SA occurs via the apoplast. The symplastic transport AzA, G3P and tasi-RNA is regulated by plasmodesmata localizing protein (PDLP) 5, which together with PDLP1 also plays a signaling role in SAR. Apoplastic transport of SA is regulated by the water potential as well as cellular pH. Distal transport of SA and G3P regulates de novo biosynthesis of pipecolic acid (Pip), which is catabolized to N-hydroxy Pip (NHP) or piperideine-6 carboxylic acid (P6C). P6C regulates vitamin B6 homeostasis, which in turn plays an important role in SAR, with both high and low levels compromising SAR. Our findings suggest that Pip catabolism may have evolved through the horizontal acquisition of specific metabolic enzymes from diverse bacterial sources during evolution.

Integrating mutualisms into the ecology and evolution of plant defense

Corlett Wood

University of Pennsylvania

Nearly all plants and animals form symbiotic partnerships with beneficial microbes. However, these symbioses don't take place in a vacuum. Hosts are simultaneously attacked by antagonists like parasites, pathogens, predators, and herbivores. My lab studies the ecology and evolution of conflict between beneficial symbioses and defense against these antagonists. We use the mutualism between legumes in the genus

Medicago and nitrogen-fixing rhizobia bacteria as our model system. In my talk, I will share an new story from my lab's research on conflict between the rhizobia mutualism and defense against antagonists. We recently discovered that rhizobia suppress production of leaf defensive hairs (trichomes). We reproduced this result in field experiments with a wild weed as well as across nine species the genus *Medicago*. We are currently exploring the physiological mechanisms that might underlie the suppressive effect of rhizobia on trichomes. Our work implicates beneficial microbes as major mediators of the host's response to infection and attack.

Investigating the molecular basis underlying crosstalk between defense and the circadian clock in Arabidopsis

Jessica Allison[1], Amelia Hallworth[1], Benjamin Sparklin[1], Chong Zhang[1], Yubing Yang[1], Nigar Fatima[1], Min Gao[1], Jose Pruneda-Paz[2], Rodrigo Gutierrez[3], and Hua Lu[1]

[1]University of Maryland, Baltimore County; [2]University of California, San Diego; [3]Pontificia Universidad Católica de Chile

Plant diseases caused by pathogens and pests result in billions of dollars of loss each year. It is crucial to understand how the defense systems work in order to mitigate disease. The circadian clock is an internal time measuring mechanism vital for many biological processes. Recent studies have implicated crosstalk between the circadian clock and plant defense. The molecular mechanisms underlying this crosstalk remains largely unclear. Using systems biology and machine learning approaches, we identified the previously known defense gene TGA3 as a potentially critical connection between plant defense and the circadian clock. TGA3 is expressed in a circadian manner and inducible by the defense signaling molecule salicylic acid (SA). A promoter deletion analysis narrowed down the cis element(s) responsible for this expression pattern within 543 bp of the TGA3 promoter. From a high-throughput yeast-one-hybrid screen, we identified a TGA3 promoter binding transcription factor, DEWAX. We showed that the DEWAX gene also exhibits a circadian expression pattern. Loss of function in DEWAX affects TGA3 circadian expression under SA treatment in addition to the circadian expression of the core clock gene CCA1. Together, this study revealed that TGA3 and its regulator DEWAX are important in mediating defense-clock crosstalk.

SESSION III – Abiotic Stress

Physiological and transcriptomic responses of pepper to combined ozone and pathogen stress

Collin Modelski[1], Guillian Hernández Casanova[2], Alvaro Sanz-Saez[3], Neha Potnis[4], **Courtney P. Leisner**[2]

[1]Department of Biological Sciences, Auburn University, USA
[2]School of Plant and Environmental Sciences, Virginia Tech, USA
[3]Department of Crop, Soil and Environmental Science, Auburn University, USA
[4]Department of Entomology and Plant Pathology, Auburn University, USA

Tropospheric ozone (O_3) is a harmful air pollutant that enter plants through their stomata, generating reactive oxygen species leading to oxidative stress in plants. Elevated O_3 can also lead to secondary impacts on plants by altering the incidence of pests or pathogens, or by mediating the ability of a plant to respond to these pressures. In this study we aim to understand the effects of climate variability on host physiology and, in turn, host susceptibility towards pathogens. We utilized the pepper-*Xanthomonas* pathosystem since this pathogen is endemic to the southeastern US, causing bacterial leaf spot disease. Two genotypes of the Solanaceous bell pepper (*Capsicum annuum*) were grown; one susceptible to *Xanthomonas* (cv. Early Calwonder) and one resistant (cv. Seminis 10X line PS09979325) and exposed to elevated O_3 to understand the interaction between biotic stress and O_3 tolerance. Utilizing a unique field site, we can grow plants in near-field settings inside open-top chambers while elevating the atmospheric O_3 concentration around the plants. Findings from this study indicate that elevated O_3 levels can compromise host immunity through impacts on plant stomatal conductance. The transcriptional response was also strongly driven by elevated O_3 as opposed to pathogen infection in the disease-resistant cultivar. Outcomes from this work will help guide future breeding efforts for plant responses to multiple stresses.

Adapting to Saltwater Intrusion: Land Management Strategies and Greenhouse Gas Implications During Farmland-to-Marsh Transitions

Alison Schulenburg, Nate Spicer, and Kate Tully

University of Maryland, College Park

As sea levels continue to rise and high tide flooding events increase in frequency, researchers and farmers alike are looking for solutions to adapt to the effects of saltwater intrusion (SWI). Landowners may respond by altering their management practices: 1) planting salt-tolerant crops, 2) using trap crops to remediate soils, 3) restoring native marsh grasses, or 4) abandoning fields altogether. Our research examined how these practices impact soil and porewater phosphorus (P) concentrations. Both remediation and restoration strategies effectively reduced P levels through biomass uptake, likely decreasing P loss to nearby waterways. These two practices can reduce pollution to waterways as fields transition, but the shift from farmland to marsh is inevitable and the environmental impact needs to be quantified. While salt marshes are significant carbon sinks, their anaerobic soils can also promote methane (CH_4) production. CH_4 is a potent greenhouse gas, and its emissions could offset some of the carbon sequestered in marsh soils. Although sulfate from seawater can suppress methanogenesis, soil heterogeneity and multiple methane production pathways may allow methanogenesis and sulfate reduction to occur simultaneously. Marsh plant species also significantly influence greenhouse gas fluxes through gas transport via aerenchyma and differences in root exudate material. As farmland transitions into marsh, plant species' effects on GHG fluxes must be carefully considered during field abandonment or restoration efforts.

Elucidating stress-induced epigenome reprogramming in crop and medicinal plants

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 SIENA (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.

Investigation of root phenotypes and drought stress responses associated with *DEEPER ROOTING 1* overexpression in apple rootstocks

Brett Shelley and Lisa Tang

Appalachian Fruit Research Station, 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 drier 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. However, the association between increased *DRO1* expression

and root growth morphology is always consistent between lines. 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.

Thursday, May 29

KEYNOTE TALK

Complexity of plant natural product biosynthesis revealed through single cell multi-omics

C. Robin Buell

University of Georgia

Plants and their natural products have been harnessed by humans for millennia for their health promoting activities, insecticidal and anti-microbial activities, as well as fragrance and food additive properties. These natural products, or specialized metabolites, have diverse functions in plants as pollinator attractants, defense and signaling molecules, and components of cell walls. The sheer diversity of specialized metabolites in plants is attributable to the dynamics of gene and genome evolution which are catalysts of species diversification. The advent of genomics technologies has revolutionized the discovery of the genes encoding specialized metabolites revealing a myriad of genome features and evolutionary mechanisms that underlie extant chemodiversity. For example, nearly the entire pathway for vinblastine and vincristine biosynthesis in *Catharanthus roseus* (Madagascar perwinkle) leaves has been resolved using gene expression profiling coupled with transcriptome and genome sequences over the last 15 years. Access to single cell -omics technologies, has revealed exquisite cell type specificity in the biosynthesis of monoterpene indole alkaloids in Madagascar perwinkle and aided in the identification of additional regulatory and structural sequences involved in the biosynthesis, transport and storage of vinblastine and vincristine biosynthesis thereby providing the foundation for understanding the evolution of cell-type specific gene expression.

SESSION IV – Plant Development and Cell Biology

Cell wall architecture and acid growth – an insight into the mechanism of ultra-fast auxin-induced shoot cell expansion

Donghui Wei, Zhihai Chi, Shundai Li, **Ying Gu**

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Auxin-induced cell elongation relies in part on the acidification of cell wall, a process known as acid growth that presumably triggers expansin-mediated wall loosening via

altered interactions between cellulose microfibrils. Cellulose microfibrils are a major determinant of anisotropic growth and provide the scaffold for cell wall assembly. However, little is known about how acid growth depends on cell wall architecture. To explore the relationship between acid growth-mediated cell elongation and plant cell wall architecture, we analyzed *csi1-3* mutants, which are defective in cellulose biosynthesis and cellulose microfibril organization. *csi1-3* is a null mutant of CELLULOSE SYNTHASE INTERACTIVE1 (CSI1), a linker protein between cellulose synthase complex and cortical microtubules. By examining cellulose microfibril structure and organization using various spectroscopic tools, we found that auxin-induced cell elongation was not strictly correlated with either the overall crystalline cellulose content or the transverse organization of cellulose microfibrils in the innermost layer of cell walls. Instead, we discovered that acid growth depends on a specific cell wall architecture known as crossed-polylamellate wall. Loss of the crossed-polylamellate wall in *csi1-3* mutants resulted in the loss of both auxin- and fusicoccin-induced cell elongation. Further exploration of *csi1-3* revealed a novel conditioned system, through which we discovered an ultra-fast auxin signaling pathway in shoot cells. This pathway is dependent on auxin-TIR1 binding and AUX/IAA degradation. The TIR1/AFB-AUX/IAA signaling pathway leads to plasma membrane proton secretion and cell wall acidification. However, cell wall acidification alone is not sufficient to trigger this ultra-fast auxin-induced shoot cell expansion. The existence of such a bifurcated auxin signaling pathway suggests that auxin-regulated plant cell growth may exhibit a dynamic and robust nature that extends beyond the classical acid-growth theory.

Zea diploperennis Perennial Regrowth QTL Regrowth1 and Regrowth3 Control Life History Traits

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Perennial plants regrow for multiple years and perennial crops are sustainable agriculture systems. The teosinte species *Zea diploperennis* is perennial and forms fertile hybrids when crossed with maize. Using *Z. diploperennis*/maize mapping populations, perennial regrowth was mapped to three dominant loci called Regrowth1 (Reg1), Reg2, and Reg3 on chromosomes 2, 7, and 8, respectively. Perenniality is a complex trait thought to involve the convergence of many loci that independently affect phenology, physiology, and meristem traits. We measured several traits in introgressed populations that were segregating Reg1 and Reg3 and determined that *Z. diploperennis* alleles of Reg1 and Reg3 significantly delay flowering time, Reg3 instills stay-green, and neither locus influences tiller number. We collected axillary meristems (AMs) and leaf tips of WT, Reg1, Reg3, and Reg1 Reg3 individuals at around the V10 stage for RNA-seq analysis. Consistent with our measurements of stay-green, we observed senescence-associated marker genes were downregulated in Reg3 but not Reg1 leaves. We also used this dataset to look for differentially-expressed genes in the QTL intervals. Our preliminary analysis has led to several candidate genes that are involved in flowering, sugar transport, and hormone signaling. Overall, these data support the idea that perenniality is controlled in part by altering phenology traits and may lead to interesting candidate genes or gene modules that will aid in perennial maize breeding. We are also testing a classical hypothesis that Indeterminate1 (Id1) and Grassy tillers1

(Gt1) contribute to perenniality. Using knowledge of cis-regulatory element locations provided by Conservatory, we have generated id1 promoter-edited alleles that have weaker flowering-time phenotypes compared to the id1 null allele. We will combine weak alleles of id1, gt1 with Reg1 and Reg3 alleles from *Z. diploperennis* to test if this combination is sufficient to reproduce perennial regrowth.

Deciphering Auxin Signaling Specificity Using a Synthetic Biology Approach

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Although the auxin signal transduction pathway is one of the best-studied processes in phytohormone perception, many questions remain. Predominantly, how the same signal can elicit such a wide variety of responses depending on where and when it is perceived is a puzzle that is yet to be completed. The canonical auxin signal transduction pathway is composed of TIR1/AFB receptors, Aux/IAA repressors, and ARF transcription factors, each encoded by extensive gene families in flowering plants. It is assumed that the makeup of the expressed modular pathway component isoforms in a particular cell determines the transcriptional output specificity. However, testing this assumption is hampered by the promiscuous interaction between Aux/IAA repressors and ARF transcription factors. Here, we show that we are able to assay the effects of signal transduction through specific family members in isolation by replacing the PB1 protein-interaction domains in Aux/IAAs and ARFs with orthologous, distantly related domains.

Antioxidant properties of dihydroxy B-ring flavonoids modulate circadian amplitude in Arabidopsis

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Flavonoids are abundant specialized metabolites produced by plants for a range of functions, including pigmentation, hormonal signaling, UV protection, and drought tolerance. We previously showed that flavonoids also influence the circadian clock in Arabidopsis. Here, we report that the antioxidant properties of dihydroxy B-ring flavonoids is responsible for regulating the amplitude of the core clock gene luciferase reporter, TOC1:LUC. We found the amplitude of TOC1:LUC rhythms correlate with the cellular H2O2 content in flavonoid-deficient seedlings. Moreover, reducing production of reactive oxygen species rescued the elevated TOC1:LUC amplitude in flavonoid-deficient seedlings, whereas reducing auxin transport rate, a known function of flavonoids, had no impact on TOC1:LUC amplitude. Interestingly, Ca^{2+} levels in the chloroplast, but not the cytosol, were also altered in flavonoid-deficient seedlings, hinting at retrograde signaling as a possible mechanism of flavonoid-mediated changes in clock amplitude. This study advances our understanding of the relationship between flavonoids and the circadian clock as well as the mechanisms underlying this interaction.

Simultaneous activation of multiple genes boosts poplar regeneration and growth using CRISPR-Combo

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Tissue culture regeneration is the major bottleneck for most of tree species recalcitrant to genetic transformation, which hinders the studies on functional genomics and plant breeding. Poplar (*Populus trichocarpa*), the first genome-sequenced forest tree, has been recognized as a viable and sustainable biofuel source and significant carbon sequester. Improving poplar growth is a major approach to boost biomass production and carbon sequestration. In this study, we used CRISPR-Combo system to simultaneously activate morphogenic genes (*WOX11* and *WUS*) to boost poplar regeneration and growth and knockout *4CL1* (a lignin biosynthesis gene) for tree engineering. Activation of *WUS* accelerates root initiation and shoot growth in tissue culture. Moreover, *WUS* activation also enhance shoot and root growth in soil growth condition. In addition, *WOX11* activation promotes de novo shoot regeneration from callus and root growth in soil growth condition. In both activation cases, the genome editing efficiency of *4CL1* gene is comparable with Cas9 control plants. To further investigate the synergistic effects of these two genes on poplar tissue culture regeneration and vegetative growth, we simultaneously activate *WOX11* and *WUS.* Surprisingly, double activation lines show accelerated root initiation and shoot regeneration under hormone-free conditions compared to single activation lines and wild-type plants, which enhances the enrichment of highly efficient genome-edited plants and also significant shortens the tissue culture process. Taken together, our results demonstrated CRISPR-Combo empower adventitious shoot and root regeneration as well as plant growth in soil growth by activation of *WOX11* and *WUS* genes together with accelerated tissue culture and high gnome editing efficiency of a trait gene.

SESSION V – Approaches for Crop Improvement

Does barley contain resistance to *Fusarium graminearum*?

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Fusarium head blight (FHB) of barley and wheat, caused by *Fusarium graminearum*, results in reduced yield and grain quality. Trichothecene mycotoxins (e.g., deoxynivalenol, DON) accumulate during *F. graminearum* infection and inhibit protein synthesis and act as virulence factors. FHB resistance in all barley accessions tested

exhibit type II resistance (resistance to spread of disease symptoms). Numerous barley genetic mapping studies have identified QTL associated with FHB resistance; however, most of these QTL are also associated with morphological or physiological traits, indicating that reduced FHB severity is associated with escape mechanisms and not true genetic-based resistance. Using transcriptomics and yeast complementation, we isolated a barley gene encoding a UDP-glucosyltransferase (HvUGT13248) that conjugates DON to the less toxic DON-3-O-glucoside (D3G). Transgenic barley overexpressing *HvUGT13248* exhibit reduced FHB severity and increased tolerance to DON due to rapid conjugation of DON to D3G. Mutations in the active site of *HvUGT13248* result in increased FHB susceptibility, sensitivity to DON, and reduced conjugation of DON to D3G. In addition, we found that *HvUGT13248* is required for type II resistance in barley. Resequencing a large barley germplasm collection revealed only a few non synonomous nucleotide changes that do not impact the function of the gene, indicating that *HvUGT13248* is highly conserved. Since *F. graminearum* produces numerous trichothecene mycotoxins, we tested our mutant and transgenic HvUGT13248 genotypes against a spectrum of trichothecenes and found that HvUGT13248 can conjugate and detoxify a broad range of trichothecenes. Taken together, our results show that HvUGT13248 provides type II resistance via detoxifying a broad range of trichothecenes.

Advancing tomato resistance to pests and diseases through harnessing genetic diversity from wild species

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Exploiting natural genetic variation underpinning key defense traits in tomato offers a powerful strategy to enhance pest and disease resistance. We focused on two targets for introgressing traits of interest and gene discovery: constitutive type-IV glandular trichomes for acylsugar-based defense against arthropod herbivory and resistance to the disease *Septoria* leaf spot (SLS). Type-IV trichomes secrete acylsugars, which are natural insecticides that deter whitefly oviposition, induce mortality in caterpillars, starve aphids, and inhibit fungal spore germination. By introgressing a *S. galapagense*-derived locus (*Get02*) into the cv. Micro-Tom and pyramiding it with a *WOOLLY* allele that enhances the number of digitate trichomes, we developed lines retaining type-IV trichomes into adulthood. These lines develop the double number of type-IV trichomes per leaf area and accumulate acylsugars at wild-type levels and exhibit strong resistance to whitefly and hornworm feeding, all while maintaining agronomic performance on par with Micro-Tom. Fine mapping and RNA-Seq of the 500 kb *Get02* region on chromosome 2 have narrowed the candidate gene list for functional validation. *Septoria* leaf spot is a fungal disease that can devastate crop production if not treated. We screened and identified genetic resistance in some wild tomato accessions of *S. peruvianum* and *S. arcanum*. We followed by introgressing the trait into commercial varieties (‘WV63’, ‘Cherokee’) via *in vitro* ovule culture and a backcross scheme using speedy breeding. Inoculation assays using a virulent *Septoria* isolate from Long Island revealed high resistance levels in advanced backcross populations. QTL mapping on chromosome 8, supported by CAPS markers and comparative DNA-

Seq, defined an oligogenic architecture and identified three genomic regions with candidate resistance genes. Together, these efforts provide precise markers and novel genetic insights to accelerate breeding of tomato cultivars with broad-spectrum, durable resistance to insects and a fungal pathogen.

Developing a pipeline for *Agrobacterium*-mediated transformation in Heirloom Tomato Cultivars

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Heirloom tomato cultivars exhibit superior fruit quality and are very important for local and urban agriculture in Maryland. However, they are less productive and resilient compared to modern hybrid varieties. Efforts to improve heirlooms will provide added value to local and urban agriculture markets. Our group seeks to implement genome editing for improving heirloom tomatoes. We are currently implementing *Agrobacterium*-mediated transformation in four heirloom cultivars and in our reference M82. Our initial transformations used binary vectors containing *NPTII* gene for kanamycin resistance and CRISPR/Cas constructs assembled using Golden Gate cloning targeting genes previously associated with key agronomic traits. To optimize transformation and regeneration, we are also testing a binary vector with the morphogenetic regulator *GRF-GIF*, known to increase transformation efficiency in crops like wheat. The baseline transformation efficiency in M82 and Sunray was 32% and 20%, respectively. When these two cultivars were tested with *GRF-GIF*, both exhibited improved transformation efficiency (49% and 26%, respectively). Jubilee, Brandywine pink, and Amana orange were more recalcitrant for transformation efficiency (3.7%, 7%, 21.3%, respectively), and exhibited improvements when using *GRF-GIF* (10%, 10.5%, 20%). We are currently developing new CRISPR vectors containing a *GRF-GIF* expression cassette to test changes in editing efficiency. These plants will also be characterized for edits generated by the presence of the CRISPR/Cas system. This research emphasizes the potential to enhance transformation efficiency using gene-editing and tissue culture and develop improved heirloom tomato cultivars while preserving their unique and superior fruit flavor.

Expanding CRISPR-Combo targeting scope for A and T rich PAM sites

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CRISPR-Combo permits concurrent genome editing and transcriptional activation in plants, yet the NGG PAM constraint of SpCas9 limits its use in AT-rich promoter regions. To broaden PAM compatibility, we established Combo platforms based on iSpyMacCas9, its nickase cytosine base editor (CBE-iSpyMac), and AaCas12b, which recognize NAAR and VTTV PAMs, respectively. In rice, coupling each nuclease with activation of BABY BOOM 1 (OsBBM1) enabled hormone-free regeneration and enhanced agronomic traits. OsBBM1 activation significantly increased callus formation and editing efficiency, with iSpyMacCas9-Combo producing the highest proportion of heritable edits, while AaCas12b-Combo showed moderate improvement. Building on these results, we developed SpRY Cas9-Combo and CBE-SpRYn-Combo, providing near-PAM-less editing together with activation. Collectively, these advances greatly expand the targeting space of CRISPR-Combo and furnish a versatile toolkit for crop genome engineering and precision breeding.

Sparse Testing: A Resource-Efficient Approach for Enhancing Genetic Gain through Early Multi-Environment Trials

Sunilda Frias, Nicholas Santantonio

Virginia Tech

Plant breeding programs release new varieties by generating new individuals through the crossing of parents with favorable traits and through multiple rounds of selection of this new population. The first rounds typically involve many genetically distinct individuals that are evaluated only once, while later rounds allow for replicated testing across multiple target environments. For scale, the first round of selection may include over 1,000 unique individuals, whereas only a handful are ultimately selected for release and large-scale cultivation.

First-year yield trials are traditionally excluded from multi-environment trials (METs) due to constraints in seed availability, land, labor, and equipment. Sparse testing (ST) has emerged as an innovative solution to generate multi-environment data for individuals (lines) even in early stages of selection. By leveraging genomic prediction models—particularly Best Linear Unbiased Predictors (BLUPs)—sparse testing enables breeders to predict line performance across environments, even when not every genotype x environment combination is directly observed. BLUPs use the genetic relationships among individuals to estimate how a given line will perform in diverse environments based on its own data and that of related lines.

By applying sparse testing, breeders can sample more environments earlier in the breeding cycle, increasing the likelihood of identifying regionally adapted lines while shortening the overall selection timeline.

Stabilizing inserts in virus-induced gene silencing vectors

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Virus-induced gene silencing (VIGS) is a quick and simple tool for generating siRNAs in plants to control host gene expression and invading pathogens, making VIGS a staple in molecular plant laboratories for 25 years. However, a major issue with using VIGS for biotechnology applications in the field is the lack of stability of sequences inserted into viral genomes. Hairpins or other inserted sequence/structures are virtually always partially or wholly deleted after only a few weeks of infection. We determined that hairpins inserted into single-stranded, functionally unimportant locations in the umbra-like virus CY1 were not maintained because the thermodynamic properties of the artificial hairpins did not match those of the natural virus hairpins. We found that natural CY1 hairpins ranging up to 200 nt were completely stable when duplicated and inserted into non-functional locations. We determined that CY1 natural hairpins have low average positional entropies and specific ΔG's relative to their size. Of 50 hairpin inserts that were designed with the same thermodynamic properties of these natural hairpins, 49 were stable for the life of the infected *Nicotiana benthamiana* plants and several are still stable after more than 4 years in citrus. Importantly, the same thermodynamic properties also applied to maintain stability of hairpin inserts in unrelated plant and animal RNA viruses. These results suggest that RNA structures in RNA virus genomes have evolved similar parameters that allow for un-interrupted transcription by their RNA-dependent RNA polymerases, and hairpin inserts that mimic these parameters should be stable. The ability to construct stable VIGS vectors means that VIGS can now be applied both in the laboratory and in the field to control gene expression and target invading pathogens in long-lived plants.

Prospects for the improvement of yield and resilience in bioenergy switchgrass by genomic-enabled selection

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The bioeconomy's expansion depends critically on developing high-performing perennial feedstocks for biofuels and bioproducts. Switchgrass (*Panicum virgatum*) stands out as a leading candidate among these perennials. Despite significant progress in yield improvement and agronomic techniques over the past decade, challenges persist in developing cultivars that consistently deliver both high yields and efficient conversion to valuable end products. Our research utilizes extensive common-garden experiments across the central United States to identify mechanisms for targeted improvement. We integrate cutting-edge approaches, including remote sensing phenotyping, transcriptomics, and in-field physiological assessments, to comprehensively characterize switchgrass performance. This multi-faceted strategy has enabled us to establish a genome-enabled breeding program specifically focused on maximizing freezing tolerance and biomass production. Simultaneously, we are developing methods to enhance downstream fermentation efficiency by reducing plant-

derived inhibitory compounds. Importantly, these compounds likely evolved as defense mechanisms against fungal pathogens, requiring careful balance in breeding to maintain pathogen resistance while improving processability. Our integrated approach aims to deliver robust switchgrass cultivars optimized for large-scale deployment in the bioeconomy, addressing both agricultural performance and downstream processing challenges.

POSTER ABSTRACTS

ODD NUMBERED POSTERS # 513-703

Wednesday, May 28

513 **EPFL8 negatively regulates embryonic stomatal pre-patterning independent of TMM in Arabidopsis thaliana**

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Plants regulate gas exchange through stomata --- specialized valves on the aerial epidermis. Stomatal development involves tightly controlled transitions orchestrated by basic-helix-loop-helix (bHLH) transcription factors, including SPEECHLESS (SPCH). These factors coordinate cell division and differentiation during stomatal development. Recent studies have underscored the importance of cell-cell communication in this process, mediated by secreted peptide ligands from the EPIDERMAL PATTERNING FACTOR (EPF) family and a Leucine-Rich-Repeat (LRR) Receptor complex that includes the ERECTA-family receptor kinases and the receptor protein TOO MANY MOUTHS (TMM). Although mature stomata do not form during embryogenesis, stomatal cell fate is determined at these early stages. In this study, we discovered that EPFL8 is highly expressed during early embryogenesis and inhibits stomatal development by negatively regulating SPCH. Furthermore, TMM, a target gene of SPCH, interferes with EPFL8-mediated signaling. In the absence of TMM, EPFL8 expression extends into later embryogenesis, which aligns with the reduced number of stomatal precursors observed in *tmm* mutants. These findings reveal that EPFL8 functions as an embryonic peptide ligand that suppresses stomatal formation via SPCH regulation, highlighting the diversification of EPF family members in fine-tuning plant epidermal patterning.

543 **The importance of small herbaria for revealing tropical biodiversity patterns**

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Herbaria, collections of dried and preserved plants, are present in many research institutions around the world. Herbarium specimens often include collection location, species name, and collection date. But the largest and best-known herbaria are at Western institutions, such as Kew Gardens (UK) and the New York Botanic Gardens (US).

This "size and prestige" disparity accompanies a disparity in the number of specimens from specific geographic regions. One of the many uses of herbarium specimens is the creation of species distribution models (SDMs); specimens usually include coordinates, which enable researchers to map the range of a particular species. Large herbaria may have the funding for more collecting trips to a particular country. However, they are also likely to have samples from all over the world. Conversely, smaller herbaria are likely to have more local samples due to a limited travel radius. So, while researchers may be inclined to rely on samples from famous herbaria in making SDMs, using data from smaller herbaria is also essential to generate accurate models.

This project aimed to test two hypotheses: 1) there will be notable differences between *Begonia* SDMs constructed using only data from Brazilian herbaria, using only data from non-Brazilian herbaria, and using all data, and 2) there will be notable differences between *Begonia* SDMs if herbaria are excluded from the models in descending order. Models were constructed with R's Maxent package; SDMs were compared using the Continuous Boyce Index (CBI) and the I statistic. The results indicate that, for 1), there is a difference between the models, but the answer to 2) is more complex. From these findings, we can conclude that smaller herbaria must be used in SDM creation to obtain more accurate models.

609 **Establishing CRISPR-based Detection Techniques for DNA/RNA-based Plant Pathogens**

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In the face of a changing climate and growing global population, strengthening agricultural systems is more critical than ever to ensure food security. While much research emphasizes increasing yield, my work focuses on protecting the crops we are already cultivating. Biological pests—including bacteria, fungi, and viruses—account for an estimated 30% of yield loss globally, totaling over $220 billion annually. Early and accurate detection is key to mitigating these losses. In this presentation, I will introduce CRISPR-based diagnostic tools designed to identify both DNA and RNA pathogens with high sensitivity and specificity. Our modular detection systems, based on Cas12a and Cas13a enzymes, are easily reprogrammable to target a wide array of biotic threats. Using Cas12a, we developed an assay that successfully detects the widespread yet underrecognized phytopathogen *Candidatus phytoplasma*, achieving a tenfold improvement in sensitivity over a benchmark "wild-type" CRISPR detection platform. Additionally, we have established a Cas13a-based method capable of rapidly detecting and genotyping three quarantine-significant ssRNA viruses affecting sugarcane, directly from plant tissue extracts. Together, these tools represent a scalable, flexible approach to crop protection. With the simple redesign of crRNA sequences, our system can be adapted to detect virtually any pathogen threatening agricultural systems today and in the future.

615 **Uncovering Blast Resistance in *Triticum monococcum*: From Phenotyping to Functional Genomics**

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Wheat blast, caused by *Magnaporthe oryzae* pathotype *Triticum* (MoT), is an emerging threat to global wheat production that threatens food safety and security. Directly striking the wheat ear, wheat blast can shrivel and deform the grain in less than a week from the first symptoms, leaving farmers no time to act. There is no knowledge about any broad-spectrum wheat-blast resistance gene identified in wheat except major locus on 2NS introgression in wheat, but there is no information about genetic basis of this resistance. We utilized a sequence indexed Einkorn wheat GWAS panel. The panel was phenotype for blast resistance under controlled infection conditions, revealing substantial variation in disease response. A k-mer-based genome-wide association studies (GWAS) was performed using the four available reference genomes (*T. monococcum* TA299, *T. monococcum* TA10622, *T. monococcum* TA391 and *T. monococcum* TA10868), enabling the identification of resistance-associated loci on chromosome 2AS with six genes. Additionally, haplotype analysis of the GWAS panel refined these loci and pinpointed candidate genes, including RGAs and other defense-related genes. These candidates are now being tested for functional validation. This study highlights the potential of Einkorn wheat as a genetic resource and provides a foundation for developing blast-resistant wheat cultivars.

619 **Investigating ACC-Responsive Gene Expression in the liverwort *Marchantia polymorpha***

Priyanka S. Bharadwaj[1], Ria Khare[2], Joseph J. Kieber[2] and Caren Chang[1]

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1-Aminocyclopropane carboxylic acid (ACC), the ethylene precursor in flowering plants, is often used to induce ethylene responses. However, recent studies show that ACC and ethylene can elicit distinct responses, suggesting ACC may act as a signal independent of ethylene. In most flowering plants, it is difficult to study ACC-specific phenotypes due to rapid conversion to ethylene. In contrast, the non-flowering plant *Marchantia polymorpha* does not convert ACC to ethylene, making it a valuable system to study ACC's independent role. The Chang lab showed that ACC treatment inhibits growth and development in Marchantia, unlike ethylene, supporting a signaling role for ACC (Li et al., 2020). However, the molecular mechanisms remain unknown.

Using RNA-seq, we analyzed differential gene expression in 6-day-old wild-type Marchantia at 2h and 7h post-ACC treatment. We found that ACC begins to visibly inhibit thallus growth by 7 hours, and included the 2-hour time point to capture early transcriptional responses that precede visible effects. The analysis revealed early upregulation of genes involved in stress responses, cell wall organization, and receptor signaling, alongside downregulation of genes related to the cell cycle, and meristem function.

We are now investigating early transcriptional regulators and signaling components underlying this response, focusing on candidate transcription factors and putative receptors. Several 7h DEGs overlap with ACC-responsive genes identified in Arabidopsis roots (Mou et al., 2025). These candidates will be studied through localization and functional assays to understand their roles in ACC signaling. This work aims to uncover components of ACC-specific signaling, offering insights into how ACC may act as a signal in early land plants.

639 **Title: Species-specific Responses of Deciduous Trees to Experimental Inundation with Fresh and Saline Water**

Kendalynn A. Morris,[1,2] Ben Bond-Lamberty[1,2], Nate McDowell[2], Stephanie C. Pennington[1,2], Roy Rich[3], Radha Srinivasan[1,4], Alice Stearns[3], Patrick Megonigal[3], Vanessa Bailey[2]

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Coastal sea level rise and altered precipitation regimes result in increasing rates of 'ghost forest' formation. Visual evidence of tree stress is often the first indicator of this transition from upland forest to wetland, but the underlying changes in tree physiology are poorly understood. To better understand the impacts of inundation frequency and estuarine vs fresh-water chemistry on coastal forests, the Terrestrial Ecological Manipulation to Probe the Effects of Storm Treatments (TEMPEST) experiment imposes extreme, disruptive, hydrologic events in a deciduous coastal forest in Maryland, U.S.A. Three tree species—*Liriodendron tulipifera* (tulip poplar), *Acer rubrum* (red maple) and *Fagus grandifolia* (American beech)—are annually exposed to inundation with fresh or estuarine water, and an extensive sensor network monitors a variety of tree and soil responses. *L. tulipifera* is generally considered the least tolerant of saturated conditions and salt-exposure, which is supported by these early experimental results. After three years of TEMPEST treatments and accumulating salt in the soil, the relationship between sapflow and soil electrical conductivity has reversed for the saltwater treatment plot. Notably, *L. tulipifera* in the estuarine-water treatment plot prematurely dropped their leaves then re-leafed late in the 2024 growing season. Manipulative, ecosystem-scale experiments such as TEMPEST are crucial for understanding species-specific resilience to disturbance in order to predict the timing and magnitude of coastal forest loss.

643 **Localization of Two Putative RPW8-Interacting Powdery Mildew Effector Proteins**

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We present preliminary characterization of two effector proteins from *Golovinomyces cichoracearum* UCSC1, which we have termed R8IE1 and R8IE2. These effectors were first identified by our lab using proximity labeling of RPW8.2-interacting proteins. We have expressed these genes transiently in *N. benthamiana* both alone and together with RPW8.2 to assess their expression and localization during powdery mildew infection. We have additionally expressed these effectors under the control of the RPW8.2 promoter, which is specific to powdery-mildew infected cells, in *Arabidopsis thaliana* ecotype Col-0, which lacks RPW8.2, and in transgenic Col-0 expressing RPW8.2. We first evaluate the effects of these effector proteins on *A. thaliana* without infection, using Salicylic Acid to induce transcription from the RPW8.2 promoter. We then assess whether increasing the gene dose of these effector proteins increases the disease severity and/or host range with adapted and poorly-adapted powdery mildews.

649 **Metabolite Profiling and Antimalarial Potential of *Vernonia ambigua* Leaf Extract: An *In Silico* and ADMET Insights**

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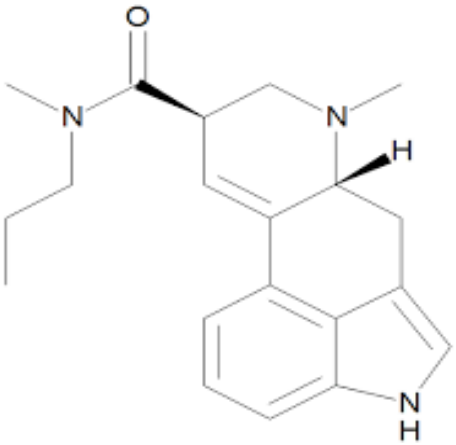
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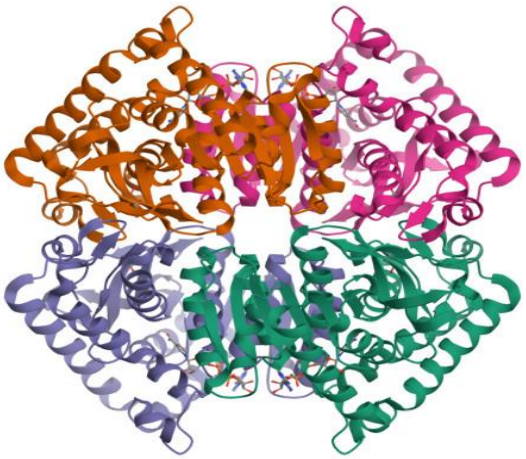
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Abstract

Malaria remains a major global health concern, especially in low-income regions. *Vernonia ambigua* is traditionally used to treat malaria and related ailments. This study evaluated the antimalarial activity of the ethylacetate leaf extract (ELE) of *V. ambigua* through *in vivo* efficacy and *in silico* docking and ADMET studies. Acute toxicity and curative antimalarial activity were assessed using Lorke's and Peters & Ryley's methods. The chemical compounds were profiled using GC-MS. ELE showed no toxicity ($LD_{50} \geq 5000$ mg/kg). *In vivo*, the extract exhibited significant ($p<0.05$), and dose-dependent parasite clearance, with % cure of 71.9, 69.4, and 56.4 % at 500, 250, and 125 mg/kg. GC-MS analysis identified 23 bioactive compounds, which were docked against *Plasmodium falciparum* lactate dehydrogenase (PfLDH) using AutoDock Vina. ADME and toxicity properties of these compounds were predicted via SwissADME and ProTox-3. Docking scores ranged from −7.7 to −4.2 kcal/mol, compared to −10.1 kcal/mol for the reference ligand. Lysergic acid methylpropylamide emerged as a promising lead based on its binding affinity and favorable ADMET profile. These findings support the antimalarial potential of *V. ambigua* extract, warranting further pharmacological and clinical investigation.

Keywords: *Vernonia ambigua,* anti-malaria, bioactive compound, molecular docking.





651

The Impact of Telomere Length Variation on Plant Fitness under Stress

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Telomeres are conserved structures at the ends of eukaryotic chromosomes that guard genome stability. The length of telomeric DNA varies substantially between different plant species and even between genotypes of the same species. Previous studies using T-DNA mutants of *Arabidopsis thaliana* with long or short telomeres suggested that telomere length may be an adaptive trait that contributes to better plant survival in one environment over another. This study tested the hypothesis that natural telomere length variation can differentially impact Arabidopsis fitness depending on soil quality (poor or control). Six Arabidopsis genotypes with long or short telomeres were grown in control soil or in sand supplemented with ½ (moderate stress) or 1/10 Hoagland (more severe stress) solution. We then measured several plant life history traits and vegetative (biomass) and reproductive (total seeds produced) fitness parameters. As expected, plants responded to stress in a treatment-dependent manner, with significant decreases in chlorophyll leaf content, vegetative and reproductive fitness under moderate and more severe stress compared to control conditions. Interestingly, Arabidopsis genotypes with long telomeres generally produced more biomass under all growth conditions compared to genotypes with short telomeres. However, while long-telomere genotypes produced fewer seeds under control conditions, their seed counts under the more severe stress were higher than in genotypes with short telomeres, suggesting a potential trade-off between vegetative and reproductive fitness. Overall, our results may provide important insights into whether telomere length is a plant trait that can be manipulated to achieve higher crop yields in poor growth conditions.

653

Using Golden Gate assembly to build modular constructs for trait development in the tomato crop model

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Genetic engineering is an important tool for providing novel traits in breeding programs. Notably, the CRISPR/Cas system has been extensively used as a tool for gene discovery and trait development in both academia and the private sector. Several cloning methods for building vectors are available, some allowing for easy and modular assembly of multigene constructs than others. Our research group has implemented a cloning strategy using Golden Gate and the Molecular Cloning (MoClo) toolkit to build vectors to deliver Genome Editing constructs in tomato. The Golden Gate assembly relies on type IIS restriction enzymes, allowing for the specific use of restriction sites for directional cloning using the MoClo vectors. Using this "semi-scarless" method, we have successfully built over 100 vectors containing single expression cassettes (e.g., *NPTII*) and higher-order assemblies containing up to 10 genes (*NPTII*, Cas12a or Cas9, and up to 12 guide RNA arrays). We confirmed the proper assembly of our vectors using long-read sequencing (Azenta). This method is highly efficient, cost-effective, and allows for very modular integration of multiple genes in a single vector. We are currently assembling higher-order constructs containing developmental regulators (*GRF-GIF*), a seed fluorescent marker (*pFAST*), and building 12+ gRNA arrays to simultaneously target promoters of multiple genes involved in disease resistance and plant architecture traits for improving locally-grown heirloom tomato varieties using genome editing.

655

Developing a pipeline for *Agrobacterium*-mediated transformation in Heirloom Tomato Cultivars

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Heirloom tomato cultivars exhibit superior fruit quality and are very important for local and urban agriculture in Maryland. However, they are less productive and resilient compared to modern hybrid varieties. Efforts to improve heirlooms will provide added value to local and urban agriculture markets. Our group seeks to implement genome editing for improving heirloom tomatoes. We are currently implementing *Agrobacterium*-mediated transformation in four heirloom cultivars and in our reference M82. Our initial transformations used binary vectors containing *NPTII* gene for kanamycin resistance and CRISPR/Cas constructs assembled using Golden Gate cloning targeting genes previously associated with key agronomic traits. To optimize transformation and regeneration, we are also testing a binary vector with the morphogenetic regulator *GRF-GIF*, known to increase transformation efficiency in crops like wheat. The baseline transformation efficiency in M82 and Sunray was 32% and 20%, respectively. When these two cultivars were tested with *GRF-GIF*, both exhibited improved transformation efficiency (49% and 26%, respectively). Jubilee, Brandywine pink, and Amana orange were more recalcitrant for transformation efficiency (3.7%, 7%, 21.3%, respectively), and exhibited improvements when using *GRF-GIF* (10%, 10.5%, 20%). We are currently developing new CRISPR vectors containing a *GRF-GIF* expression cassette to test changes in editing efficiency. These plants will also be characterized for edits generated by the presence of the CRISPR/Cas system. This research emphasizes the potential to enhance transformation efficiency using gene-editing and tissue culture and develop improved heirloom tomato cultivars while preserving their unique and superior fruit flavor.

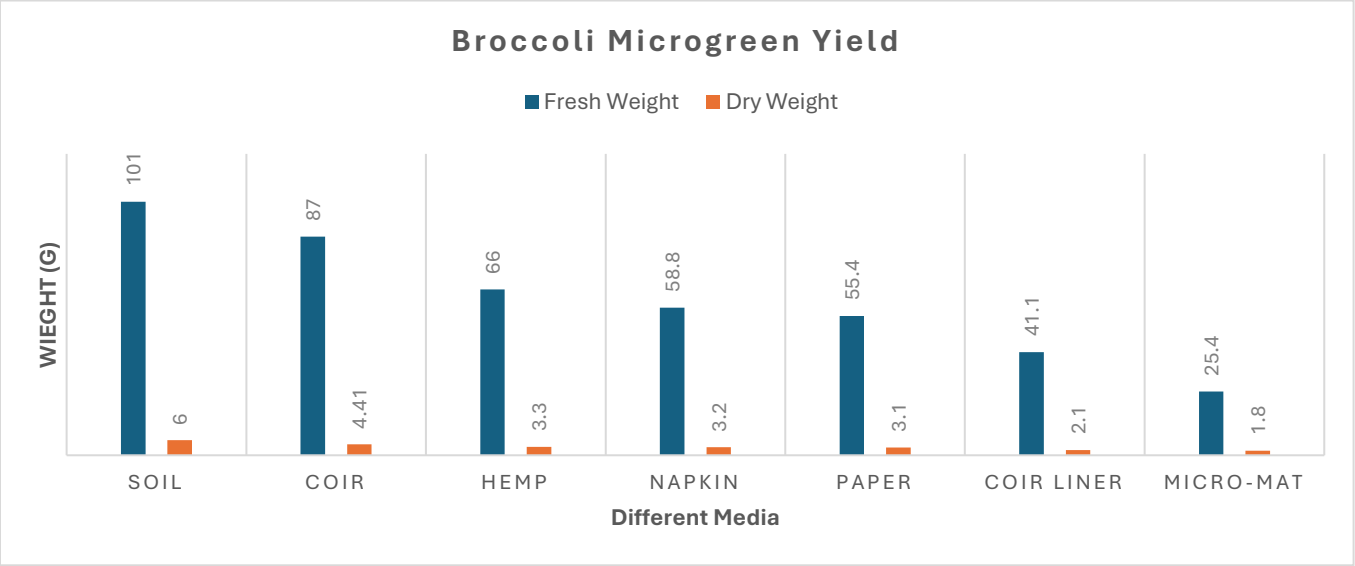
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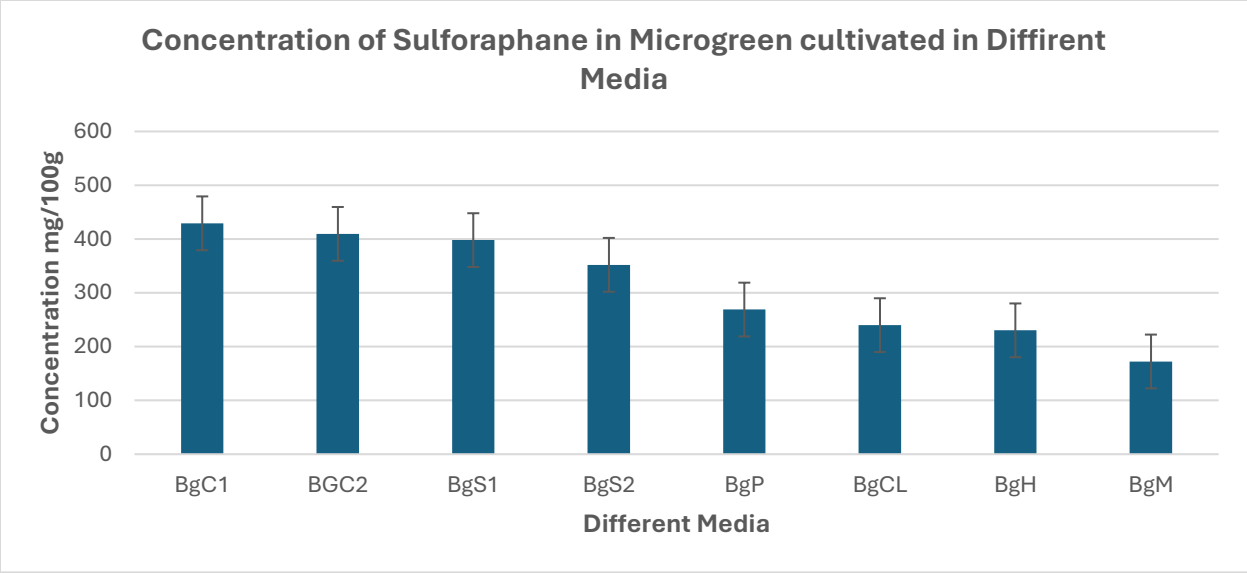
Comparative Analysis of Sulforaphane Levels in Broccoli Microgreens Cultivated on Various Media

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Abstract

Broccoli microgreens are emerging as a functional food due to their dense nutritional profile and significant levels of sulforaphane, a bioactive compound associated with cancer prevention. This study investigates the impact of different growing media on broccoli microgreens' fresh weight yield and sulforaphane concentration. Seven different media types were tested under controlled conditions. An optimized quantification method was established using HPLC. Coconut coir and soil were shown to promote high biomass and sulforaphane accumulation. The micro-mats and napkins showed limited capacity to support optimal growth and glucosinolate development. The findings suggest that the choice of growing media plays a pivotal role in modulating broccoli microgreens' physical yield and nutritional composition.




Concentration of Sulforaphane in Microgreen cultivated in Diffirent Media
Concentration mg/100g
600
500
400
300
200
100
0
BgC1
BGC2
BgS1
BgS2
BgP
BgCL
BgH
BgM
Different Media

661 A Natural Antibiotic Duo: Exploring the Microbial Inhibition of Fermented Garlic in Honey

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Department of Biology, Notre Dame of Maryland University

As global antibiotic resistance continues to rise, coupled with the side effects associated with conventional antibiotics, there is growing interest in natural, food-based alternatives with antimicrobial properties. Notably, certain bacterial strains such as *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Escherichia coli*, commonly associated with skin infections, surgical site infections, urinary tract infections (UTIs), neonatal meningitis, and enteric diseases, are increasingly exhibiting resistance to standard antibiotics.

Garlic (*Allium sativum*) and honey are well-documented natural remedies known for their distinct antimicrobial properties. Garlic contains allicin, a sulfur-containing compound with strong antibacterial activity, while diluted honey exhibits antimicrobial effects through the slow release of hydrogen peroxide and the presence of bioactive compounds such as flavonoids and phenolic acids. When combined and subjected to fermentation over three months, the mixture may demonstrate enhanced antimicrobial potency due to synergistic interactions between these components.

This study evaluates the antimicrobial activity of fermented garlic in honey against *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Escherichia coli* using the agar well diffusion method on Mueller-Hinton Agar (MHA). Standard microbiological procedures were employed to isolate and identify the bacterial strains. Antimicrobial efficacy was assessed by measuring the diameters of the zones of inhibition around each well on the MHA plates. Preliminary findings suggest that the fermented garlic–honey mixture exhibits enhanced inhibitory effects against bacterial growth. These results underscore the potential of functional fermentation to enhance the antimicrobial activity of natural substances, supporting the use of fermented garlic in honey as a sustainable and natural alternative to conventional antibiotics.

671 **Integrating UAS-Based Phenotyping and Machine Learning for Yield Prediction in Soybean**

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Abstract: Soybean breeding programs require precise phenotypic assessments for genetic improvement; however, traditional selection relies on labor-intensive yield measurements and subjective visual evaluations at the end of the season, limiting early-stage selection efficiency. This study aimed to develop a UAS-based high-throughput phenotyping pipeline for early and accurate yield estimation. In 2024, 558 soybean plots were planted in Suffolk, Virginia, and multimodal RGB, thermal, and multispectral images, were collected from the R3 to R5 growth stages, generating 10 vegetation indices (VIs), plant height, and canopy temperature data. Ground truth measurements, including plant height, chlorophyll content, foliar water content, and yield, were collected to establish predictive relationships between aerial and field-based traits. Results showed that moderate correlations ($r > 0.35$) were observed between nine aerial traits and ground truth data, while six VIs, aerial plant height, canopy temperature, and chlorophyll content from R4 to R5 exhibited moderate correlations with yield. After dimensionality reduction, five VIs, aerial plant height, and canopy temperature from R3 to R5 were selected to train predictive models. Among the machine learning approaches tested, stepwise linear regression (SLR), partial least squares regression (PLSR), artificial neural networks (ANN), support vector machines (SVM), random forests (RF), and k-nearest neighbors (KNN), RF achieved the highest yield prediction accuracy ($r = 0.81$, rRMSE = 10.46%). These findings highlight the potential of UAS-based multimodal data fusion and machine learning for early-season yield prediction, supporting standardized, data-driven breeding strategies to enhance selection efficiency and genetic gain in soybean.

673 **Forward Genetic Screening of an EMS Mutagenized Population of *Hordeum vulgare* Identifies Resistant Genotypes Against *Fusarium graminearum***

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Barley (*Hordeum vulgare*) is the fourth most produced grain in the world with 150.97 million metric tons harvested in 2023-2024. Fusarium Head Blight (FHB), caused by the hemi-biotrophic fungal pathogen, *Fusarium graminearum*, is a devastating disease of barley in the Mid-Atlantic region. FHB significantly reduces grain yield, as well as contaminates the grain with mycotoxins including Deoxynivalenol (DON), which can cause long-term health problems and liver damage upon consumption by human beings and animals. Genetic resistance is the most economical and environmentally sustainable approach to managing FHB, however current commercial barley varieties contain little resistance to FHB. This forward genetic screen in a mutagenized population of 706 lines of six-rowed barley cultivar "Thoroughbred" was performed to explore novel genes and alleles conferring FHB resistance. These mutagenized lines were tested under field and greenhouse conditions through corn inoculum spread and point inoculation respectively and scored for phenotypic and genotypic data. Three resistant mutant lines have been identified to exhibit strong resistance against FHB. Identification of FHB resistant variants in barley can be utilized in breeding programs, with further testing of the lines in this population to be continued.

675 **Identifying proteins that mediate ABA-induced chromatin remodeling**

Cindy Wang, Charles Seller
University of Maryland, College Park

In response to different stress environments, such as droughts, plants maintain homeostasis through guard cell-mediated opening and closing of stomatal pores. Stomatal closure is triggered by abscisic acid (ABA), a hormone that gets released during drought conditions. Previously, we found that ABA also triggers genome-wide chromatin remodeling in *Arabidopsis* guard cells thereby reprogramming gene expression during drought stress. Chromatin opening in response to ABA requires four related transcription factors known as ABF proteins. However, we do not know the molecular mechanism by which ABF proteins alter chromatin structure. To investigate this question we sought to identify proteins that interact with ABF4 during the ABA response. Using proximity labeling based proteomics (TurboID) we identified 253 candidate ABF4 interactors. To test these interactions and further explore the relationship between ABF4 and the identified proteins, we will use two approaches: 1) in-vitro pulldown and 2) yeast-two hybrid. Finally, we will test the contributions of these ABF4-interacting proteins to ABA and drought responses by obtaining or generating mutant plants. By better understanding plant genome regulation in stress environments, we can provide insight on improving drought tolerance in plants.

679 **Study on the quality index and mechanism of action of ZGCD in the treatment of arrhythmia based on network pharmacology combined with molecular docking**

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2. Beijing No.4 High School, Beijng 100034, China

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Abstract

Zhi-Gan-Cao Decoction (ZGCD) is a traditional Chinese herbal formula that has been widely used for arrhythmias. The aim of this study is to investigate the quality markers (Q-markers) and the mechanism of ZGCD on treating arrhythmia based on the network pharmacology combined with molecular docking. The potential targets of ZGCD were predicted by TCMSP, BATMAN-TCM, Swiss Target Prediction. The targets related to arrhythmia were searched using the OMIM, GeneCards, and DisGeNET databases. Subsequently, 206 targets were identified by Venny 2.1.0 platform as common ones of ZGCD and arrhythmia. The top 20 targets were identified as the key ones by Protein-protein interaction (PPI) network which was established with STRING platform. The top 20 pathways, Biological Processes, Cellular Constituents, Molecular Functions, were given according to P_{Value} in the Gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses which were performed in the DAVID database. Top 20 constituents were identified as key ones to treat arrhythmia by "herb-constituent-target-pathway-desease" (H-C-T-P-D) analysis, which was established using Cytoscape 3.10.3. By validation of molecular docking, which was

conducted by DS2019, finding that the CDOCKER energy of 11 key constituents with 14 key targets were less than -10.0 kcal/mol, showing excellent affinity. These results showed the mechanism of ZGCD to treat arrhythmia is the integrated effect of multi-constituent synergistic regulation of multiple -targets and -pathways. Therefore, the quality control of ZGCD formula and its related products should be based on multi-constituent active ingredient groups as Q-markers.

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683 **Phenylacetic acid (PAA): A Volatile Auxin?**

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Plants have numerous hormones regulating plant growth and development, such as auxins, gibberellic acids, and jasmonates. Plants also have volatile hormones such as ethylene and methyl-jasmonate. Among the myriad of auxins in the plant, indole-3-acetic acid (IAA) is the pre-dominate form. The auxin phenylacetic acid (PAA) has been measured in plants and appears to occur at low abundance but has been shown to be an active auxin in *Arabidopsis thaliana*. Method development to improve PAA extraction from rice tissues and quantitations via LS-MS^3, together the strong honey-like odor (unpleasant in concentrate amounts) led us to hypothesize that PAA may function as a volatile auxin. Preliminary data indicate that volatile PAA can function as auxin in physiological and auxin reporter assays. The goal of my project is to determine the mechanism of volatile PAA signaling using molecular genetics and physiological assays.

685 **AMSH1, a highly conserved deubiquitinase associated with ESCRT, contributes to nonhost resistance (NHR) and basal resistance in *Arabidopsis***

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*Presenter (email: wksexton@umd.edu)

Genetic engineering resistant crop cultivars to reduce losses caused by plant pathogens is a key strategy for improving food security. We can facilitate this by studying (NHR), a phenomenon in which all genotypes of a plant species are immune to all strains of a phytopathogen. While using NHR to guide crop improvement holds promise, poor understanding of its molecular basis impedes this effort. MAMP-triggered immunity (MTI) and effector-triggered immunity (ETI) have been shown to contribute to NHR against powdery mildew fungal pathogens, but further characterization is difficult due to the presence of multiple defense layers and lack of intraspecific variation. Thus, conventional genetic screens are ill-suited for studying NHR. To work around this, we created an immunocompromised *Arabidopsis* mutant, *eds1-pad4-sid2-pen1-pen2* (*epsp1p2*), that has compromised resistance against non-adapted dicot powdery mildews but is still mostly resistant to barley (a monocot) powdery mildew, *Blumeria graminis* f. sp. *hordei* (*Bgh*). We then conducted a forward genetic screen with *epsp1p2* for mutants susceptible to non-adapted *Bgh* (*snab*). The *snab1* mutant supports sporulation of *Bgh,* indicating breakdown of NHR. The causal mutation in *snab1* was mapped to *AMSH1* which encodes a highly conserved deubiquitinase that interacts with endosomal sorting complex required for transport (ESCRT) machinery. ESCRT is involved in several processes, including autophagy and intracellular trafficking. Our data indicate that AMSH1 is required for the hypersensitive response, a form of localized cell death associated with ETI that helps restrict pathogen growth. This work implicates ESCRT in the regulation of plant immunity and validates our approach for studying NHR.

687 **Functional Analysis of ABA-Regulated Stomatal Development in *Arabidopsis thaliana***

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Stomata are microscopic pores on the aerial surface of plants that enable gas exchange between the plant and the atmosphere. They play a crucial role in minimizing water loss. Abscisic acid (ABA), a key phytohormone in plant stress responses, triggers a variety of physiological changes to conserve water, including the regulation of stomatal conductance. ABA homeostasis is tightly controlled through a dynamic balance of biosynthesis and catabolism. In the model plant *Arabidopsis thaliana*, several genes are known to modulate ABA levels, such as *ABA2* involved in biosynthesis, and the *CYP707A* family involved in catabolism. In our study, we identified a mutant in which stomatal development is strongly inhibited by exogenous ABA treatment. Through genetic manipulation of ABA levels in the mutant, we demonstrate that it exhibits hypersensitivity to ABA during stomatal development.

689

The roles and mechanisms of G protein signaling in the polarized proliferation of plant cells

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In multicellular animals and plants, orientation of cell division is highly regulated. In animals, the conserved WNT (Wingless and Mouse Mammary Virus Interacting-1) signaling pathway regulates both the proliferation and polarity of stem cells. Briefly, the WNT ligand activates the receptor Frizzled, a 7TM G Protein Coupled Receptor (GPCR) that interacts with the polarity determinant Disheveled. Active Disheveled tethers the Regulator of G protein Signaling (RGS) domain-containing Axin protein to the membrane; thereby blocking the destruction of the transcriptional co-activator Armadillo and driving oriented cell division. Given the many connections between WNT signaling and the ancient eukaryotic heterotrimeric G (Gα, Gβ and Gγ) protein signaling pathway, I hypothesize there was convergent co-option of G protein signaling function in animals and plants, particularly in the evolution of WNT-like signaling pathways that regulate polarized proliferation. To test this, I decided to focus on the Frizzled plant ortholog RGS1, which contains both a GPCR-like domain and an RGS domain similar in structure to the WNT scaffolding protein Axin. My preliminary *in planta* BiFC data confirm the interaction of Arabidopsis RGS1 with the recently characterized Disheveled plant orthologs SOSEKIs (SOKs) that drive oriented cell division in Arabidopsis roots. Of interest, Arabidopsis RGS1 interacted with all five Arabidopsis SOSEKIs (SOK1-5), even showing polarized interaction with SOK3. These data are consistent with the hypothesis that G proteins might have been employed in the evolution of polarized cell proliferation in both plants and animals, with wide-reaching implications in our understanding of how polarized proliferation is regulated in multicellular organisms.

691

Investigating Male Sexual Competition in Pollen Tubes Using Novel *in vitro* Germination

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1. University of Maryland College Park 2. Instituto Gulbenkian de Ciência

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) by previous fertilization of all available ovules. Here we show that pollen tube competition can occur even in a completely artificial environment, an agar germination medium filled microcapillary. We show evidence that in this space limited system, with a volume and geometry 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: the majority halt in the first third of the capillary, and only (and always!) one gets to the end of the capillary. This pattern is affected by pollen density, P-type ATPase pharmacology, and long-distance electric sensing, but not by gravity of any secreted factor. We are optimizing the system for *A. thaliana* to which perform reverse genetic screens for putative signaling mechanisms responsible for this self-organizing pattern that determines the increased fitness of ONE successful competitor.

693 **Analyzing the validity of Cel-1 Endonuclease for genotyping CRISPR-Cas Edits**

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The CRISPR-Cas9 genome editing platform enables targeted genetic modifications with high precision, but efficient downstream screening remains a bottleneck in many workflows. Although high-throughput approaches like NGS are widely used for detailed analysis, they are often not feasible for routine or early-stage genotyping. Low-throughput methods, including mismatch detection using enzymes such as T7E1 and Surveyor, are available but can be inconsistent or expensive. Cel-1-based detection offers a cost-effective and accessible alternative that meets the needs of mid-throughput genotyping workflows.

In this study, we assess the performance of Cel-1 endonuclease, a mismatch-specific enzyme derived from celery (*Apium graveolens*), for early detection of CRISPR-induced edits in two different wheat genotypes for 4 different gene targets edited using multiplex gene editing. Cel-1 is a single-strand-specific endonuclease in the S1 nuclease family, with high activity against DNA heteroduplexes formed by insertions, deletions, or single-nucleotide mismatches. For this work, we extracted the Cel-1 enzyme in our lab from fresh Celery stalks. Protein extraction and purification was done using ammonium sulphate precipitation, followed by dialysis. The DNA of the edited plants was used to generate amplicons spanning the guide RNA sites. The PCR amplicons from the edited plants were either reannealed with/ without the amplicons from the wild type to form heteroduplexes in case of hetero edits, treated with Cel-1, and analyzed via agarose gel electrophoresis. Distinct cleavage patterns successful detection of editing across multiple gRNAs. The assays without the wild type DNA showing cleavages indicated heterozygous deletions, whereas cleavage obtained with samples having mutant plus wild type indicated homozygous events. Sanger sequencing validated the Cel-1 results, confirming the presence of SNPs at expected target sites. These findings demonstrate that homemade Cel-1 can be used as a reliable and cost-effective tool for early detection of CRISPR edits, enabling rapid genotyping.

697 **Transgenic expression of the Wheat pore-forming toxin-like protein provides a broad-spectrum resistance against fungal pathogens in Arabidopsis and tomato**

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Fusarium head blight (FHB), caused by the hemi-biotrophic fungus *Fusarium graminearum*, is a major threat to global wheat productivity. A wheat pore-forming toxin-like (PFT) protein was previously reported to underlie *Fhb1*, the most widely used quantitative trait locus in FHB breeding programs worldwide. In our previous study, heterologous expression of the wheat PFT gene in the dicot plant *Arabidopsis thaliana* provided a broad-spectrum quantitative resistance to hemi-biotrophic and necrotrophic fungal pathogens *F. graminearum, Colletotrichum higginsianum, Sclerotinia sclerotiorum,* and *Botrytis cinerea*. In this follow-up study, PFT transgenic Arabidopsis plants were tested for their resistance to powdery mildew fungus along with SA, JA mutants and wild type plants. Meanwhile, the wheat PFT gene was transgenically expressed in tomato (cultivar Money Maker). These transgenic plants were challenged with tomato root pathogens *Fusarium oxysporum* and *Verticillium dahliae*. After 4-5 weeks of inoculation, data on symptoms and disease severity were recorded and compared to the wild type Money maker plants. In both experiments, transgenic Arabidopsis and tomato plants expressing PFT showed significantly less disease severity as compared to their respective wild type plants against the tested pathogens. This study provides evidence on broad spectrum autonomous resistance of the wheat PFT gene to major fungal pathogens *in planta* irrespective of the plant background where it is being expressed. These experiments reveal the potential application of wheat PFT gene in efficient plant disease management against major fungal pathogens in crop plants.

Keywords: Fusarium head blight, Wheat PFT, Broad-spectrum resistance, Fungal pathogens, Arabidopsis, Tomato

699 # Discovery and mapping of FHB-resistant mutations in a susceptible wheat variety 'Jagger'

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Genetic resistance against Fusarium Head Blight (FHB) in wheat is quantitative in nature, with over 550 quantitative trait loci (QTLs) identified for FHB severity and DON content. Despite extensive breeding efforts, achieving high levels of FHB resistance remains a significant challenge due to the impact of numerous small-effect QTLs. This study investigates alternative strategies for improving FHB resistance in wheat. We focused on Jagger, a hard red winter wheat cultivar, known for its high yield, and better quality but, inherent susceptibility to FHB. A forward genetic screen was performed on 840 M4 mutagenized lines derived from an EMS-mutagenized Jagger population across multiple years in field conditions, followed by confirmation under controlled environment conditions. Ten mutant lines consistently exhibited significantly lower FHB severity, with seven of these lines also showing significantly reduced DON levels. The identified resistant mutants were crossed with FHB-susceptible Jagger wild type to develop MutMap populations. Screening of the F2 population derived from one of the mutants (JagMut-271) identified a 9.9 Mb interval on the short arm of chromosome 2D associated with FHB resistance Future work is underway to fine-map the candidate region to support breeding efforts for improved FHB resistance.

701 **Reconstituting Auxin Signaling in Yeast with the Auxin MoClo Toolkit**

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The core signaling pathway that mediates responses to the plant hormone auxin is composed of a small number of large, multi-member protein families. While the architecture of this pathway is relatively simple, functional redundancy among family members generates a wide range of outputs, complicating efforts to dissect how auxin regulates plant growth and development. To facilitate the study and prototyping of specific interactions within this pathway, we are developing the **Auxin MoClo Toolkit**—a modular cloning system designed for use in *Saccharomyces cerevisiae*. This toolkit includes coding sequences for diverse paralogs of key auxin signaling components: auxin response factors (ARFs), Aux/IAA transcriptional repressors, and TIR1/AFB auxin co-receptors. By enabling the expression of different combinations of these elements in yeast, the toolkit allows simulation of tunable auxin responses. This system will be particularly valuable for probing and perturbing known auxin signaling modules in a controlled, synthetic context.

703 Pathogenicity and movement of Umbra-like viruses in non-promiscuous hosts

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The ability of umbravirus-like viruses (ULVs) to systemically invade their host plants in absence of helper viruses was recently demonstrated (Ying et al., 2024). Lack of encoded movement proteins in ULVs was compensated by recruiting the host phloem protein 2 which binds the viral RNAs and facilitates phloem trafficking. To understand the movement mechanism of ULVs in plants, we needed to find a model host that can be systemically invaded. The ULVs citrus yellow vein associated virus 1 (CY1) and CY2 were inoculated via *Agrobacterium* infiltration into Arabidopsis, and several other cucurbit plants besides *Nicotiana benthamiana* which served as a positive control. As a result, no symptoms were detected on any plant during the month following the inoculations. The same experiment was repeated after adding viral RNA silencing suppressors (VSRs) p19 (tomato bushy stunt virus). Symptoms started to develop only on *N. benthamiana* after two weeks post infiltration (wpi), while other plants remained in a normal look and confirmed negative by RT-PCR. Then we added the VSRs 2b (cucumber mosaic virus), p14 (pothos latent virus), p24 (grapevine leafroll-associated virus 2) and HC-Pro (tobacco etch virus) to see if this will allow viruses to replicate. Infiltrated leaves of cucumber plants become symptomatic and confirmed positive by RT-PCR, but the virus was not detected at the systemic leaves. However, we detected CY1 & CY2 in the lower stem and the roots of inoculated plants. We confirmed the replication of both viruses by the strand-specific RT-PCR method that specifically detects the viral negative strand RNAs. We found a combination of p14, p24 and HC-Pro helps CY1 move systemically to the new leaves. We will use this model to study the components of virus movement complex.

POSTER ABSTRACTS

EVEN NUMBERED POSTERS # 526-700

Thursday, May 29

526 **Unraveling the stress granule (SG) transcriptome in Arabidopsis thaliana under heat, cold, and drought stress**

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Climate change threatens agriculture, with heat, drought, and cold impacting plant growth and yield. As sessile organisms, plants adapt to stress via responses like stress granule (SG) formation. SGs are membrane-less organelles formed by liquid-liquid phase separation, playing key roles in mRNA regulation and protein protection during stress. While proteomic and metabolomic profiles of heat-induced SGs are well studied, transcriptomic data, especially under cold and drought, remain limited. We investigated the SG transcriptome in Arabidopsis thaliana under heat, cold, and drought. SGs were isolated using anti-GFP beads targeting RBP47, an SG marker. After confirming SG formation under heat, RNA was extracted and sequenced. Preliminary results show SGs also form under cold and drought, showing patterns like heat-induced SGs. 10,918 transcripts were unique to heat-induced SGs, 258 to controls, and 3,092 were shared. Comparison with a prior proteomic study of heat-induced SGs revealed only 57 overlapping genes, suggesting that RNA and protein recruitment to SGs is governed by distinct regulatory mechanisms. We also found overlaps between heat-induced SGs-associated RNAs and m6A-enriched genes under drought (2,816), copper (439), salt (598), and cold (676), suggesting selective SG assembly and a role for m6A in guiding RNA localization. Future work will include the analysis of SG transcriptomes under cold and drought and integrate proteomic data to gain a more comprehensive understanding of SG composition and function across stress conditions.

546 **Phylogenetics of miR398-directed Targeting of Selenium Binding Protein Transcripts in Plants**

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MicroRNA 398 (miR398) is an ancient and well conserved microRNA (miRNA) in plants. Its most well-known function is targeting transcripts for Cu/Zn Superoxide Dismutase (CSD), a copper-containing protein involved in detoxifying Reactive Oxygen Species (ROS). This mechanism allows for efficient downregulation of CSD translation when ROS are low and/or when copper is in short supply. Additionally, miR398 targets transcripts for several other copper-utilizing proteins involved in ROS response. Our recently generated rice degradome libraries have validated Selenium Binding Protein (SBP), yet another copper-containing protein involved in ROS response, as a target of miR398. Because SBP is a largely underappreciated target of miR398, we sought to uncover potential conservation of a miR398-SBP regulatory module throughout plants by detecting miR398-complementarity regions in SBP transcripts, as well using degradome analysis to validate predicted cleavage sites. This analysis finds miR398-complementarity regions in SBP transcripts from several monocots, particularly those of the *Commelinids* clade. Additionally, plants from four distinct dicot clades had miR398-complementarity in SBP transcripts. Degradome evidence validated miR398-induced cleavage of the SBP mRNA in several *Commelinid*s and three of the four dicot clades with predicted miR398-complementarity in SBP transcripts. These findings suggest that the SBP mRNA is indeed a target of miR398 in several monocots as well as a few dicot clades. This means that, in some plants, SBP is another component of copper utilization and ROS response that is regulated by miR398, and future studies involving the analysis of miR398 and its effects should take this into account where applicable.

592 **A Forward Screen in an EMS Mutagenized Population of *Hordeum vulgare* Identifies Resistant Genotypes Against Fusarium Head Blight**

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Barley is one of the most produced crops in the modern agricultural world, with 151.62 metric tons being harvested in 2022 - 2023. Fusarium Head Blight (FHB) is a hemibiotrophic fungal pathogen found in the Mid-Atlantic region. Barley plants infected by FHB have significantly reduced yields and create mycotoxins known as Deoxynivalenol (DON), which can infect both humans and animals, potentially causing catastrophic symptoms such as liver damage and immune system suppression. There are many approaches to managing FHB, such as fungicide application and crop rotation. However, genetic resistance is the most economical and sustainable approach, as it provides a more consistent and longer lasting resistance to FHB. By utilizing a forward genetic screen in a population of the barley cultivar 'Nomini,' we aim to find sources of resistance against FHB, which were evaluated under greenhouse conditions for potential significant phenotypic data. In doing so, several mutants were found to show resistance to FHB. These findings are significant and important for identifying susceptibility genes in barley that can be used in future breeding programs for widespread usage of these resistant cultivars.

604

Efficient genome editing by CRISPR-Cas12a in common wheat via shoot apical meristem delivery

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CRISPR-Cas12a has been widely used for genome editing in crops. However, tissue culture is needed and the editing efficiencies are low when applying this technology in wheat (Triticum aestivum L.). Here, we report a new approach to create genome edited wheat by utilizing Cas12a variant and wheat shoot apical meristem. We delivered the gold particles coated with plasmids expressing LbCas12a components into the embryos of imbibed seeds, targeting shoot apical meristem (SAM). Mutations in the target gene were subsequently analyzed by using NGS analysis. We investigated various temperature conditions to identify the optimal treatment condition. Our results demonstrated that the average genome editing efficiency of the T0 generation was 9.3% at 30°C, compared to 4.5% at 26°C, 0% at 34°C. These findings indicate that 30°C is the optimal temperature for the SAM-based strategy. Additionally, we compared six different Cas12a variants, and found that the intron-containing Cas12a reagents exhibited higher genome editing efficiency compared to the non-intron versions. Taken together, this report demonstrated a new approach for CRISPR-Cas12a genome editing in wheat, utilizing a high temperature regime and a highly efficient Cas12a construct.

610

Trans-Species MicroRNAs from Parasitic Plant *Cuscuta campestris* Hijack Host AGO1 through *DCL1*-Dependent Processing

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The parasitic plant *Cuscuta* spp. (dodders) are vampires. Their “fangs”, or haustoria, pierce host plants and siphon nutrients from bridging vasculature. At the haustorial interface, *C. campestris* sends microRNAs (miRNAs) to manipulate host genes, but how *trans*-species miRNAs are produced remains unclear. Endogenous miRNAs are processed by Dicer-like 1, we suspected the same for *trans*-species miRNAs. If so, reducing *C. campestris Dicer-like 1* (*CcDCL1*) should hinder their accumulation. By infiltrating artificial miRNA into *Nicotiana benthamiana* and allowing *C. campestris* to parasitize it, we achieved a 40% knockdown of *CcDCL1* in 10 days. Strikingly, *trans*-species miRNAs were significantly repressed upon reduced *CcDCL1*, while host *DCL1* knockdown had no effect, confirming the processing occurs in the parasite, not the host. *CcDCL1* knockdown also reduced *C. campestris* attachment by 30%. These findings expand DCL1’s role in cross-species communication and parasitism. *Trans*-species miRNAs carry a 5′ uridine, favoring ARGONAUTE 1 (AGO1) binding. To see if they act through host AGO1, we immunoprecipitated AGOs from *Cuscuta*-infected *Arabidopsis*, where *Cuscuta* miRNAs were enriched in host AGO1, not AGO2. Using an sRNA protection assay, we found these miRNAs remain unbound to AGOs in *Cuscuta*. The results suggest that *Cuscuta* miRNAs evade self-targeting by sidestepping parasite AGOs, becoming functional only when selectively loaded into host AGO1. Together, we revealed the dicing, export, and protein binding of *Cuscuta trans*-species miRNAs. Halting their production could help protect crops from *Cuscuta*, creating a vampire-free zone. As the first to deliver RNA interference into *Cuscuta* via agroinfiltration, we open the door to rapid gene testing and more efficient parasite research.

614 **Title: Growth of *Arabidopsis thaliana* LEA Knockout Mutants Under Osmotic Stress**

Authors/Presenters: Ainsley Plume*, Arya Singh*, and Kristen Clermont.

*co-presenters

Affiliation: Department of Natural Sciences, La Salle University

Abstract:

Late Embryogenesis Abundant (LEA) proteins are crucial for plant tolerance of abiotic stresses such as drought, salinity, and osmotic imbalance. This study investigates the roles of LEA2 and LEA3 protein families in *Arabidopsis thaliana* using T-DNA insertion knockout (KO) mutants, specifically lines AT2G44060 (LEA2), AT2G44060 (LEA2), and AT4G02380 (LEA3). Genotyping was performed via PCR and gel electrophoresis to confirm successful disruptions of target genes. No wild-type (WT) genes were detected in our mutant lines. However, WT controls displayed inconsistent banding patterns across three gels, potentially due to experimental variability, DNA template issues, or biological differences. Under osmotic stress (100 mM mannitol) and control growth conditions, we analyzed phenotypic responses, particularly the root development. Root length was reduced under osmotic stress condition. Root length did not vary significantly from each genotype. Notably, several seeds exhibited stunted growth, an unusual outcome potentially linked to harsh seed sterilization protocols (such as bleach concentration) or variability in light exposure during early growth. A structural model of the LEA protein highlights potential functional domains involved in stress response. Our findings underscore the complexity of stress responses in plants and support further optimization of experimental conditions to reduce variability. This knowledge helps to advance our understanding of LEA proteins.

616

Elucidating stress-induced epigenome reprogramming in crop and medicinal plants

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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 SIENA (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.

636 **Simultaneous activation of multiple genes boosts poplar regeneration and growth using CRISPR-Combo**

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Tissue culture regeneration is the major bottleneck for most of tree species recalcitrant to genetic transformation, which hinders the studies on functional genomics and plant breeding. Poplar (*Populus trichocarpa*), the first genome-sequenced forest tree, has been recognized as a viable and sustainable biofuel source and significant carbon sequester. Improving poplar growth is a major approach to boost biomass production and carbon sequestration. In this study, we used CRISPR-Combo system to simultaneously activate morphogenic genes (*WOX11* and *WUS*) to boost poplar regeneration and growth and knockout *4CL1* (a lignin biosynthesis gene) for tree engineering. Activation of *WUS* accelerates root initiation and shoot growth in tissue culture. Moreover, *WUS* activation also enhance shoot and root growth in soil growth condition. In addition, *WOX11* activation promotes de novo shoot regeneration from callus and root growth in soil growth condition. In both activation cases, the genome editing efficiency of *4CL1* gene is comparable with Cas9 control plants. To further investigate the synergistic effects of these two genes on poplar tissue culture regeneration and vegetative growth, we simultaneously activate *WOX11* and *WUS.* Surprisingly, double activation lines show accelerated root initiation and shoot regeneration under hormone-free conditions compared to single activation lines and wild-type plants, which enhances the enrichment of highly efficient genome-edited plants and also significant shortens the tissue culture process. Taken together, our results demonstrated CRISPR-Combo empower adventitious shoot and root regeneration as well as plant growth in soil growth by activation of *WOX11* and *WUS* genes together with accelerated tissue culture and high gnome editing efficiency of a trait gene.

638

Scaffold Protein RACK1 mediates plant hormone Auxin and Salt stress Cross-talk pathway

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Plant signal transduction pathways, although seemingly distinct in their operation, are beginning to be understood to be governed by crosstalk coordinating the various hormone-based developmental and environmental stress signaling processes. The recent discovery of the growth hormone auxin signaling environmental stresses including drought and saline has garnered widespread interest due to its potential relating to food security under climate change. The foundational cellular mechanisms however remain poorly defined. In previous studies, through the application of our in-house developed RACK1 Y248 phosphorylation inhibitor compounds (pharmacological downregulation), and genetic knock-out approaches, our lab independently demonstrated that the RACK1 protein plays a positive role in auxin-induced lateral root development signaling, and concurrently negatively regulates salt stress signaling in the Arabidopsis. Here we aim to test our hypothesis that RACK1 serves as the mediator in the molecular crosstalk between these auxin and salt stress signaling pathways. Employing transgenic Arabidopsis plants with the auxin reporter construct pIAA5::GUS, we investigated the cross-talk mediating function of the RACK1 protein. While pIAA-GUS plants exhibited salt-induced auxin reporter gene expression, the use of a RACK1 inhibitory compounds significantly up-regulated pIAA5::GUS expression resulting in enhanced resistance to salt stress by down-regulating a yet-to-be-identified subset of auxin-regulated genes. Additionally, RACK1 deficient transgenic lines of *Oryza sativa,* have also shown alterations in the saline stress response at the molecular level shown by genome wide Poly-A RNA sequencing. This further supports RACK1 playing a key regulatory role in crosstalk between abiotic stressors and the plants hormonal response in two distantly related species.

642 **ASRF and Arsenic Tolerance in Plants: Genetic, Metabolic, and Population Insights**

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Arsenic contamination presents a global environmental challenge due to its highly toxic and carcinogenic nature. Plants manage arsenic uptake and metabolism through diverse mechanisms, yet further insights are critical. Here, we explore the ASRF (*Arsenic Stress-Related F-box* (*ASRF*)) gene, previously characterized by our lab (Yadira et al., 2021), for its role in arsenic tolerance. ASRF mutants demonstrated hypersensitivity to arsenic, while overexpression lines showed enhanced tolerance. Comparative RNA-Seq analyses among ASRF overexpression, mutant, and wild-type lines identified key differentially expressed genes (DEGs) and further homozygous mutants screened on arsenate. We used Weighted Gene Co-expression Network Analysis (WGCNA) and genome-wide alternative splicing to examine ASRF's regulatory role. Genetic interactions were analyzed with known arsenic-responsive genes (e.g., MYB40, ATPT1, ARS5). Protein-protein interactions were assessed through yeast two-hybrid (Y2H) assays to identify direct ASRF targets, affirming ASRF's function as an F-box protein involved in the ubiquitination system. Localization studies have been done using GUS to indicate ASRF's transcriptional and translational activities. ICP-MS and SEM analyses further demonstrated differences in arsenic accumulation among wild-type, mutant, and overexpression lines. Additionally, population studies have been done, a core collection has been developed from 1135 Arabidopsis accessions, revealing haplotype networks and significant phenotypic variation under arsenate stress. Our findings underscore critical genes and pathways in arsenic tolerance and highlight population-level variation in arsenic response mechanisms.

644

Potential local adaptation to zinc between serpentine and non-serpentine populations of *Arabidopsis lyrate spp. lyrata*

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Arabidopsis lyrata spp. *lyrata* has disjunct populations that are found in both serpentine and non-serpentine soils. Serpentine soils are particularly stressful due to low nutrient availability and high levels of nickel (Ni). Previous research demonstrated local adaptation to Ni in populations from serpentine soils, but differences in tolerance among the different serpentine populations and non-serpentine populations, which had very different concentrations of zinc (Zn) in the soil. The purpose of this research was to determine if there were differences in Zn response between which might result in co-tolerance to Ni. Seeds were gathered from two serpentine locations in Maryland: Lake Roland (LR), with low Zn soil, and Pilot Serpentine Barrens (PS), with high Zn soil. Seeds from Jug Bay Wetlands Sanctuary (JB) acted as the non-serpentine control from high Zn soil. When the seedlings were grown on a Zn gradient the roots of all three populations grew preferentially toward the lower Zn concentration with an increase in lateral root formation in the two populations from soils with higher Zn concentrations (JB and PS); however, the roots grew longer under high Zn conditions. During juvenile growth with high Zn exposure the LR serpentine population had reduced growth compared the Jug Bay and Pilot populations. The LR population also had greater root to shoot ratio in higher Zn concentrations compared to low Zn but accumulated more Zn in the root at under low Zn conditions compared to high Zn conditions. The PS serpentine population, which also has the high Ni tolerance, did not demonstrate any significant changes in growth in response to changes in Zn concentration indicating that there may be a possible link between Zn response and Ni tolerance in this population.

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648

Multidimensional Evaluation of *Vernonia ambigua* Leaf Extract for Antimalarial Therapy: From Bioactivity to Molecular Dynamics

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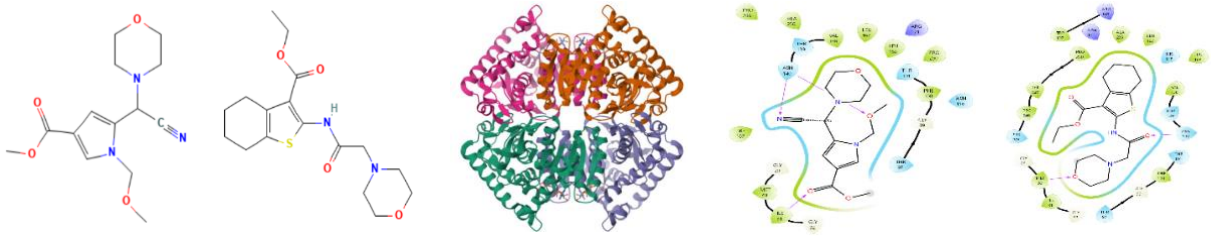
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Abstract

Vernonia ambigua, a medicinal plant traditionally used to treat malaria and fever, was investigated for its antimalarial efficacy. This study explored the bioactivity of the methanol leaf extract of *V. ambigua* (MLE) through acute toxicity testing, in vivo curative antimalarial assays, GC-MS profiling, and in silico studies. Acute toxicity evaluation using Lorke's method revealed an $LD_{50} \geq 5000$ mg/kg, confirming the extract's safety. *In vivo* curative assay demonstrated dose-dependent parasite clearance, with the highest dose (500 mg/kg) achieving a 43.36% cure rate. GC-MS analysis identified ten phytoconstituents. These compounds were subjected to molecular docking against *Plasmodium falciparum* lactate dehydrogenase (*pf*LDH). The native ligand, Adenosine 5'-(trihydrogen diphosphate) emerged as the most promising, with a Glide score of -11.656 kcal/mol and MM-GBSA binding energy of -62.25 kcal/mol, forming multiple hydrogen bonds with key residues, followed by compounds methyl 5-[cyano(morpholin-4-yl)methyl]-1-(methoxymethyl)pyrrole-3-carboxylate (MPC-01) and ethyl 2-[(2-morpholin-4-ylacetyl)amino]-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (MBTC-01) with glide scores of -1.595 and -3.872 kcal/mol and MM-GBSA binding energy of -58.62 and -50.21 kcal/mol, correspondingly, while reference drugs, Artemisinin and Chloroquine showed comparatively weaker binding and stability (-4.576 and -32.425) and (-4.574 and -40.584), respectively. These findings suggest that *V. ambigua*, holds potential as a source of novel antimalarial agents.

Keywords: *Vernonia ambigua*, Antimalarial activity, GC-MS analysis, Molecular docking, Drug discovery



650

Anticancer Activity and Secondary Metabolites from *Lantana camara*

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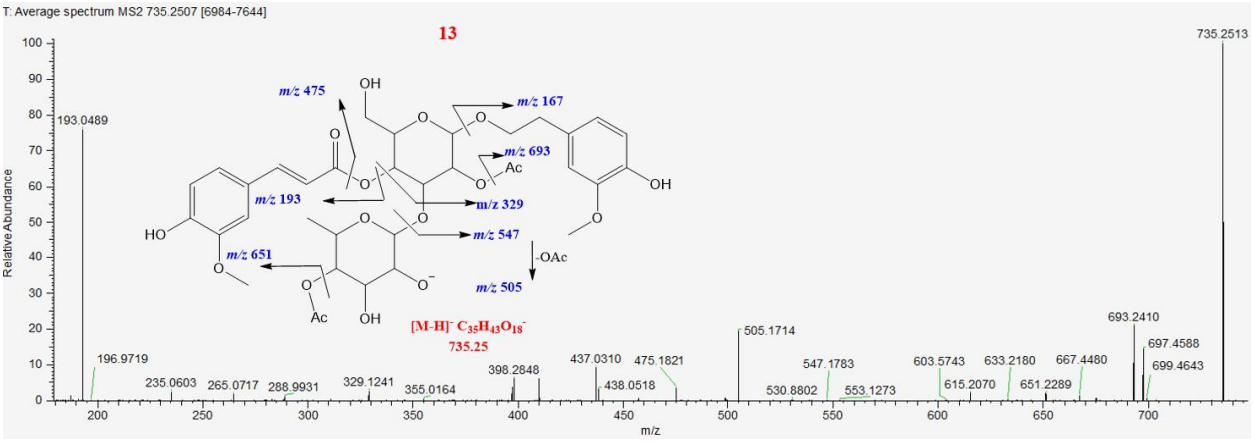
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Abstract:

Lantana camara, a tropical plant known for its medicinal properties, was studied for its potential anticancer effects on human breast cancer cells (MCF-7). Ethanolic extracts from different plant parts—leaves, stems, fruits, and roots—were tested using the MTT assay. Among these, the stem extract showed the most significant cytotoxic activity. Phytochemicals in the stem extract were analyzed using NMR and LC-MS/MS techniques. Twelve compounds (**1**–**12**), including phenylpropanoids, iridoid glucosides, and phenolics, were isolated and identified using 1D and 2D NMR. One of the key findings was the full characterization of cistanoside D (compound **5**), a phenylpropanoid, whose NMR data is reported for the first time. LC-MS/MS analysis revealed additional compounds such as terpenes, flavonoids, and phenylpropanoids. Notably, nine new phenylpropanoids (**13**–**21**) and three new iridoid glucosides (**22**–**24**) were tentatively identified. Among the isolated compounds, **1**–**4** showed cytotoxic activity.

Keywords: *Lantana camara*, LC-MS/MS, phenylpropanoid, breast cancer, MCF-7 cells



652 **Assessing the response to water deficit in a diverse panel of tomato genotypes**

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Drought is known to restrict growth and reduce yield potential in crops. Thus, developing drought-resistant varieties is an important breeding goal. In this study, we evaluated the responses to water deficit before the flowering stage in a panel of 31 genotypes composed of one commercial hybrid and multiple ex-PVP lines, one wild species, our reference cultivar (M82), US-grown heirlooms, Mexican landraces and genome edited lines. During the course of the experiment, we examined different morphological and biochemical traits to assess responses to water deficit. Seedlings at the 3-5 leaf stage were transplanted into 6 inch pots using conventional soil mixture and grown under controlled greenhouse conditions. Irrigation was done at field capacity (100%) and at water deficit (60%). Moisture was evaluated every 2-3 days using a soil moisture meter. Three weeks after transplanting, we measured plant height, stem diameter, leaf area, plant fresh and dry weight, root fresh and dry weight and other biochemical traits in genotypes grown in 100% and 60% irrigation. Our results indicate that most genotypes exhibited reduced growth under water deficit. When compared to full irrigation, plant height decreased by 7-41%, stem diameter decreased by 6-33%, leaf area decreased by 11-57%, plant fresh weight decreased by 7-57%, root fresh weight decreased by 12-65%, plant dry weight decreased by 26-64% and root dry weight decreased by 7-80% with water deficit. The wild species *Solanum pimpinellifolium* (LA1589) exhibited the highest tolerance to water deficit, followed by a commercial hybrid and few Mexican landraces collected from hot and arid environments. Intriguingly, we found that a genome edited mutant (*slcle9*) previously involved in stem cell regulation in shoot meristems exhibited moderate resistance, suggesting it may have an unknown role in abiotic stress.

656 **Sulforaphane Contents in Different Broccoli Parts**

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Abstract

Broccoli is well known for its high sulforaphane content. Sulforaphane, an isothiocyanate derived from the hydrolysis of glucosinolates, possesses chemopreventive and antioxidative properties. Despite its health benefits, its consumption predominantly comprises florets, with stems, leaves, and peels often discarded, representing substantial food waste. To address this, sulforaphane was quantified across four distinct parts of broccoli: whole heads, heads without peels, peels, and head leaves using an optimized HPLC method. The samples were purchased from five grocery stores, labeled as LP I, LP II, HP I (Organic), HP II, and AS. The results revealed that sulforaphane distribution varied significantly among different parts of broccoli and between grocery store sources. Broccoli peels generally exhibited the highest sulforaphane levels, particularly from the AS (126.21 mg/100g), followed by HP I (113.3 mg/100g). Broccoli heads with peels had notable concentrations, reaching up to 104.8 mg/100g in HP II samples. Leaves and heads without peels showed moderate sulforaphane content, varying by store origin. The findings suggest that the peels and leaves of broccoli represent a valuable source of sulforaphane. Utilizing these parts could increase the nutritional intake of sulforaphane and reduce food waste.

660 **TOMETO: Tomato Multiplex Editing for Trait Optimization**

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The primary goal of plant breeding is to develop improved crops for food, feed, and fuel. In vegetables, this requires several years of testing and validating traits introgressed from wild or exotic germplasm before deploying them into breeding programs. This is often hindered by the lack of genetic variation or linkage drag. Notably, trait development in tomato could be accelerated using genome editing, especially for yield components and disease-resistance traits with known or validated genes. Here, we propose the use of multiplex editing and transcriptional modulation as a reliable strategy for developing and stacking traits in the vegetable crop tomato, using locally-grown heirloom varieties. To achieve this, we have designed genome editing vectors targeting multiple genes at their coding (*SP5G* and *Br*) and *cis*-regulatory regions (*SP* and *DMR6*) regulating internode length, flower time and broad resistance to bacterial and fungal pathogens. We are currently generating genome-edited materials via *Agrobacterium*-mediated transformation in several heirloom tomatoes. We expect changes in inflorescence branching, shifts from indeterminate to semi-determinate growth habit, early flowering and increased yield potential due to increased disease resistance. Our approach and results will offer a transformative tool for modern plant breeding, particularly for locally-grown cultivars relevant for urban agriculture.

664 ***TWD1*, *TWISTED DWARF 1*, associates with enhanced resistance against thaxtomin A produced by *Streptomyces scabies***

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Thaxtomin A is a phytotoxin with a cyclic dipeptide structure, composed of 4-nitrotryptophan and phenylalanine residues. It is produced by various *Streptomyces* species, including *S. scabies*, *S. acidiscabies*, and *S. turgidiscabies*, and serves as the primary agent responsible for scab disease in crops like potato, radish, and turnip. Despite its agricultural relevance, the exact mode of action of thaxtomin A and the associated downstream signaling pathways remain poorly understood. TWD1 (TWISTED DWARF1), a member of the FK506-binding protein (FKBP) family, is known to influence the translocation of various proteins, including ABC transporters. A study indicates that TWD1 is involved in the BR1 and BAK1 signaling pathways, implying a possible role in pathogen resistance. In this study, we hypothesize that the *Arabidopsis* mutant *TWD1-Ct*, which carries a mutation in the C-terminal hydrophobic domain of *TWD1*, may confer resistance to thaxtomin A. This points to a potential strategy for improving resistance to *Streptomyces* pathogens in crops such as potato.

666 **Wheat genome assembly and glaucousness trait mapping: emerging traits and tools for wheat breeding and genomics**

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Adaptive physiological traits are becoming breeding targets to adapt crops to changing climates. Glaucousness is the bluish-white appearance of some plants resulting from the scattering effect of visible light on plant surface wax; it can contribute to tolerance of abiotic stresses in wheat (*Triticum aestivum*) such as drought, solar radiation, and heat. Trait mapping for flag leaf and spike glaucousness is presented in two biparental populations and a panel of elite winter wheat lines. A significant locus on the long arm of chromosome 3A (*QFlg.ncb-3A*) was identified in all populations, explaining up to 36.6 percent of phenotypic variation (PVE) for flag leaf glaucousness. For spike glaucousness, a major locus was identified (*QSpg.ncb-1B*, PVE = 22.8) within the t1RS·1BL translocation from rye (*Secale cereale* L.), demonstrating that glaucousness variation can be associated with an alien introgression in wheat. New technologies like high-fidelity (HiFi) long-read sequencing enable a new frontier in plant biology research. We present two chromosome-scale genome assemblies generated using HiFi sequencing for soft red winter (SRW) wheat cultivars ‘AGS2000’ and ‘Hilliard.’ These assemblies are orders of magnitude more contiguous than the current RefSeq reference genome for wheat (‘Chinese Spring’ RefSeq v2.1). Briefly, the development of these assemblies will be presented. These cultivars are well-represented in crosses for SRW wheat germplasm in the United States; further, the assemblies provide some of the most complete sequences of the wheat genome currently available. These assemblies will provide a foundation for genetic studies and bioinformatic tools in the represented germplasm.

668 **Actin Depolymerization Factors (ADFs) Moonlighting: Nuclear Immune Regulation by Interacting with WRKY Transcription Factors and Shaping the Transcriptome**

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Abstract
Remodeling of the actin cytoskeleton during the activation of plant immunity is a critical process for the transport and stabilization of immune-regulatory organelles and molecules. In this process, actin depolymerization factors (ADFs) function as key player through severing and depolymerizing actin microfilaments. However, recent evidence suggests that ADFs may possess non-canonical immune functions inside the nucleus, in addition to the canonic cytosolic role, a phenomenon not adequately explained by the traditional mechanistic model of ADFs and actin. In this study, we demonstrate that ADFs indeed have a moonlighting function in the nucleus, interacting with transcriptional machinery and regulating the transcriptome during both resting states and immune responses. Arabidopsis ADF2/3/4 exhibit redundant functions in defense against virulent and avirulent Pseudomonas, but not certain species of Pectobacterium and powdery mildew. Notably, nuclear – rather than cytosolic – ADFs contribute to defense against Pseudomonas and mediate pro-immune transcription. Mechanistically, we demonstrate that nuclear ADFs interact with transcription factors, histone complexes, and other components of the transcriptional machinery. Specifically, ADF2/3/4 can form a complex with WRKY transcription factors, such as WRKY22/29/48, thereby binding to promoters and directly regulating WRKY activity to shape the pro-immune transcriptome. In summary, our study reveals that ADFs moonlight as direct nuclear regulators of transcription factors, mediating a broad range of nuclear-cytoplasmic regulation of plant immunity and other processes.

670 **Determining the Function of MLO5, 10, and 11 in Relation to Powdery Mildew Pathogenesis**

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Powdery mildew is a widespread fungal disease that affects over 10,000 plant species, including many agriculturally important crops. In barley, resistance to this disease arises from a recessive mutation in the Mildew Locus O (MLO) gene, which encodes a GPCR-like protein required for fungal infection. In *Arabidopsis thaliana*, the loss of three clade V genes—*MLO2*, *MLO6*, and *MLO12*—confers complete resistance, suggesting that MLO2, MLO6 and MLO12 are host susceptibility factors of powdery mildew fungi. This study explores whether other MLO family members—MLO5 (clade III), MLO10 (clade III), and MLO11 (clade I)—possess similar molecular functions even though they exhibit distinct expression patterns. To test this, *MLOx-GFP* translational fusion constructs for the three *MLOs* were stably expressed from the *MLO2* promoter in the *eds1-pad4-sid2-mlo2-mlo6-mlo12* sextuple mutant background. None of the transgenic plants restored susceptibility to powdery mildew, indicative of no functional complement for *MLO2*, *MLO6,* and *MLO12*. Confocal microscopy was used to check the fungal development on the respective transgenic plants and examine if and where these fusion proteins are expressed in leaf epidermal cells. Results showed that except for limited hyphal growth detected on MLO10-GFP-expressing plants in rare cases, sporelings were arrested after spore germination in plants transgenic for either of the three transgenes. While MLO5-GFP signal was undetectable, MLO10-GFP was mostly found in puncta and MLO11-GFP exhibited plasma membrane localization. These observations suggest that the molecular functions of MLO5, MLO10, and MLO11 are largely (in the case of MLO10) or entirely distinct from those of MLO2, MLO6, and MLO12, highlighting functional divergence within the MLO protein family.

678 **Epigenetic Regulation of Flowering Time by the Enhancer of Zeste Ortholog *TdEz* in Durum Wheat (*Triticum turgidum ssp. durum*)**

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Wheat is a global staple crop, and optimizing its flowering time is critical for ensuring yield stability under climate stress. Polycomb Repressive Complex 2 (PRC2) mediates epigenetic silencing of key developmental genes through H3K27me3. The *Enhancer of zeste* (*Ez*) gene encodes PRC2's catalytic subunit. We investigated the function of the *TdEz* ortholog in tetraploid wheat (AABB genome) using the durum variety Kronos as a wild-type reference. We identified SNP mutations in *TdEz* homeologs on chromosomes 4A and 4B, including a neutral missense variant (4A-4236) and three independent loss-of-function stop-gained alleles (4A-4443, 4B-2087, 4B-3011). Through targeted crossing, we developed single mutants (*aaBB*, *AAbb*) and double mutants (*aabb*) to dissect gene dosage effects on flowering regulation. Wild-type plants (AABB) displayed early flowering, consistent with full PRC2 activity and high H3K27me3 at a floral repressor locus. Single-copy mutants of *TdEz* exhibited delayed flowering, suggesting that partial loss of PRC2 function leads to partial derepression of the repressor. Intriguingly, *aabb* double mutants, lacking PRC2 catalytic activity entirely, reverted to early flowering—likely due to chromatin instability and failure to maintain silencing. Our results indicate that *TdEz* controls flowering time in a dosage-dependent manner by modulating chromatin states. We hypothesize that loss of PRC2 activity leads to dynamic and unstable repression of flowering regulators. To test this, ongoing expression profiling and phenotypic analyses will further elucidate the underlying molecular mechanisms. These insights highlight the broader potential of *TdEz*-mediated chromatin regulation as a tool to fine-tune flowering time in wheat. By understanding and manipulating this epigenetic control, breeders can optimize developmental timing to escape terminal drought, synchronize crop cycles, and ultimately improve yield resilience and adaptability under changing climatic conditions.

680 Gene Discovery using a MutMap approach in *Triticum monococcum ssp. monococcum*

Carl Paulson[1], Anmol Kajla[1], Parva Sharma[1], Vijay Tiwari[1]

University of Maryland[1]

Bread wheat (*Triticum aestivum* 2n = 6x = 42, AABBDD genome) is an important crop contributing 21% of the world's caloric intake. With the growing global population and increasing pressure from biotic and abiotic stress, wheat yield improvements grow increasingly important. Due to the bottleneck effect resulting from all allopolyploid nature of wheat, there is need for genetic improvement to introduce genetic diversity into wheat germplasm to fortify it against developing environmental stresses. *Triticum monococcum ssp. monococcum* (2n = 14, A^mA^m genome) is a domesticated diploid wheat called Einkorn and has a wild form (*Triticum monococcum ssp. boeticum).* Triticum monococcum has a genome which has high collinearity with the wheat A genome donor *Triticum urartu* (2n = 2x = 14, A^uA^u genome). This makes T. monococcum a unique resource for novel gene discovery given its diploid and domesticated nature. This study aims to use a previously developed Triticum monococcum TA4420 mutant population to screen for dwarfing mutants which will then be selected based on their phenotypic strength and backcrossed with the wildtype to begin a MutMap approach to determine the causal SNP.

682

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ESTABLISHING THE ROLE OF HAIR CELLS IN COLEOCHAETE BREB.

Coleochaete is a multicellular streptophyte green alga that is closely related to embryophytes (land plants). Coleochaetophytes produce distinctive setae-bearing cells (sheathed hairs). These hairs are subcellular structures produced 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. We confirmed the presence of mitochondria within the hairs via mitotracker staining, found that the chloroplast continues to rotate at night, and noted the formation of what appear to be plugs within the hairs. We also determined that environmental factors such as nutrient concentration can cause variation in the production of hair cells within the thallus. This implies that the hairs of *Coleochaete* may be involved in nutrient uptake or sensing; this could have broader implications for the evolution of nutrient assimilation and uptake pathways in both green algae and plants.

686 **Phenotypic Assessment of Dollar Spot Response in Creeping Bentgrass under Controlled Conditions**

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Dollar spot, caused by *Clarireedia jacksonii*, is the most destructive foliar disease in cool-season turfgrass systems, significantly affecting creeping bentgrass (*Agrostis stolonifera*) and Bermudagrass (*Cynodon dactylon*). Managing this disease often requires more than ten fungicide applications per season, highlighting the need for sustainable alternatives such as host-plant resistance. This study aimed to develop a reliable phenotyping framework to evaluate dollar spot resistance across a panel of 210 creeping bentgrass accessions sourced from diverse geographic regions. Greenhouse trials were conducted under uniform, disease-conducive conditions without fungicide inputs. Disease severity was assessed across multiple time points using standardized visual ratings. Substantial phenotypic variation was observed among accessions, with the Area under the disease progress curve (AUDPC) values ranging from 45 to 155. Based on AUDPC thresholds, 28% of accessions were classified as resistant, 52% as moderately susceptible, and 20% as highly susceptible. Replication of the greenhouse phenotyping trial has been completed. These findings highlight progress toward achieving the study objectives and will provide valuable insights into the genetic mechanisms governing resistance to dollar spot. Understanding the genetic basis of resistance may ultimately lead to the development of improved turfgrass cultivars, offering a sustainable solution to mitigate the economic impact of this devastating disease.

Keywords

Creeping bentgrass, dollar spot, resistance, turfgrass

688 **Development and Characterization of Radiation Deletion Panel in Jagger Wheat for Trait Dissection and Gene Discovery**

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Forward genetic approaches remain critical for understanding the molecular basis of agronomic traits, particularly in polyploid crops like wheat. Here, we present a radiation-induced deletion panel derived from the hard red winter wheat cultivar *Jagger*, consisting of 834 mutant lines that have been genotyped via skim sequencing. This high-throughput genotyping has enabled precise detection of genomic deletions varying from less than 1 Mb deletions to large chromosomal deletions across the panel. To complete the genomic data, we phenotyped the entire deletion panel for multiple traits of agronomic and pathological relevance in the field and greenhouse conditions. The specific traits targeted include Fusarium head blight (FHB) resistance, powdery mildew susceptibility, plant height, spike length, number of fertile tillers, and spikelet numbers per spike. Useful diversity was recorded for all the traits of interest. This comprehensive deletion panel in wheat provides a powerful platform for associating genotype with phenotype in a high-resolution and cost-effective manner. The combination of deletion mapping with the trait data allows for rapid identification of candidate regions and genes underlying key traits, particularly those with recessive or dosage-sensitive effects that are difficult to detect using conventional mutant populations or natural diversity panels. Our ongoing analyses aim to integrate phenotypic and genotypic data to identify novel susceptible and resistant loci, as well as regulators of plant architecture. Ultimately, this panel will serve as a resource to accelerate gene discovery and functional validation in wheat.

690

Chromatin-remodeling factor PICKLE (PKL) contributing to nonhost resistance against powdery mildew in *Arabidopsis*

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Plant disease is a major biotic stress that significantly limits plant growth and causes substantial annual losses in global crop production. Understanding the genetic basis of disease tolerance in plants is therefore crucial. Nonhost resistance (NHR) refers to the effective immunity of an entire plant species against non-adapted, potentially pathogenic microbes. However, the molecular mechanisms underlying NHR are often poorly understood, largely due to limited intraspecific genetic variation. Here, we report the isolation of mutants defective in NHR using a genetically tailored pathosystem. The *Arabidopsis eds1pad4sid2* (*eps*) triple mutant is susceptible to the non-adapted sow thistle powdery mildew (PM) but remains resistant to strawberry PM, suggesting that it retains effective penetration resistance against the latter. Based on this, we screened EMS-mutagenized *eps* plants challenged with strawberry PM to identify "*snap*" mutants (susceptible to non-adapted PM). We obtained >100 snap mutants and identified 12 independent causal mutations, of which 5 are in *PEN2*, one is in *PEN3*, and one is in *PEN4*. Among the new genes identified to be required for penetration resistance, *SNAP2/3* encodes a CHD3 chromatin-remodeling factor PICKLE (PKL) determining H3K27me3 homeostasis.

Our study reveals chromatin-remodeling factor PICKLE (PKL) enhances disease tolerance in *Arabidopsis* by contributing to both host and nonhost resistance to powdery mildew (PM). We initially found that PKL mediates nonhost resistance (NHR) against non-adapted PM species (*UMSG4*, *UMSG1*, *UMSG3)*. In addition, PKL plays a role in host resistance to the adapted *Arabidopsis* PM isolate *UCSC1*. Genetic and phenotypic analyses further suggest that another chromatin remodeler homolog, CHR4, also contributes to both host and nonhost PM resistance, while CHR5 and CHR7 appear to be dispensable. Further infection assays with *Pseudomonas syringae* and *Xanthomonas* demonstrate PKL's involvement in both pattern-triggered immunity (PTI) and effector-triggered immunity (ETI). Transcriptomic and RT-qPCR analyses show that PKL modulates the expression of defense-related genes. Notably, PKL also regulates genes involved in glucosinolate (GS) biosynthesis, thereby enhancing penetration resistance. Collectively, our findings reveal that PKL as a key immunity regulator, acting through pre- and post-penetration resistance mechanisms against PM. This work highlights the potential of chromatin remodelers as promising targets for improving crop disease tolerance.

Key words: Chromatin remodeler; Plant immunity; Non-host resistance; Host-resistance; PTI; ETI

696 **Abstract withdrawn**

698 **Investigating a possible master regulator of endosperm and fruit development in a wild strawberry**

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Many domesticated crops are more susceptible to pathogens, pests and drought than their wild progenitors. On the other hand, domesticated crops have many desirable traits such as larger fruit and endosperm. Breeding techniques crossing wild plants with domesticated crops to introduce plant immunity genes can be time-consuming and can bring in unwanted traits. Additionally, transferring plant immunity genes between species can lead to GMO classification, posing regulatory and public acceptance challenges. Therefore, there are great advantages to precisely editing native genes, conferring beneficial traits to resilient wild crop species. In order to specifically target fruit and endosperm size, we must find and understand the genes that control these traits. Since strawberry is particularly vulnerable to many biotic and abiotic stresses, I aim to find a master regulator of endosperm and fruit size in wild diploid strawberry, *Fragaria vesca*. Strawberry fruit development requires auxin biosynthesis in the endosperm and auxin transport to the stem tip. The master regulators of auxin biosynthesis are largely unknown. We identified MYB119, a transcription factor highly and specifically expressed in the endosperm during fruit development, as a potential master regulator. Evidence suggests that it turns on auxin biosynthesis in the endosperm. Additionally, without a functional copy of this gene, plants are unable to make fruit or viable seeds. In the future, I aim to edit this gene to enhance its activity to increase fruit size in wild strawberry.

700 **Identification and expression analysis of some dehydration-responsive element binding (DREB) transcription factors in *Musa* spp. under drought stress**

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Abstract:

Drought stress is a major abiotic factor that adversely affects the growth, development, and fruit yields of *Musa* spp. Several transcription factors (TFs), including dehydration-responsive element binding (DREB) TFs, play crucial roles in mediating responses to various abiotic stressors. In this study, in-silico analysis was used to identify 26 homologs of MaDREB TFs, each belonging to one of the A1–A6 categories, possessing the conserved AP2 domain motifs, which confirmed the uniqueness of these TFs. The drought stress experiment revealed noticeable phenotypic changes in the leaves and roots of *Musa* spp. compared to the control. Additionally, quantitative real-time PCR (qRT-PCR) was conducted to validate tissue-specific expression patterns of MaDREB transcription factors. The analysis revealed that the expression levels of *MaDREB2A*, *MaDREB2B*, MaDREB2C, *MaDREB1C*, *MaDREB1G*, and *MaDREB3* vary in a tissue-specific manner, each showing distinct patterns across the AAA, AAB, and ABB genomic groups of *Musa* spp. Heat maps showed that MaDREB2A and MaDREB2B are significantly upregulated in leaves and downregulated in the roots of the ABB group, suggesting that these genes could be promising candidate genes for enhancing crop resilience in banana plants. Therefore, an overexpression construct of *MaDREB2B* was developed with the intention to generate transgenic *Musa* spp. The study provides key insights into the role of *MaDREB*s genes under abiotic stress conditions, offering a pathway for improving crop resilience in *Musa* varieties.

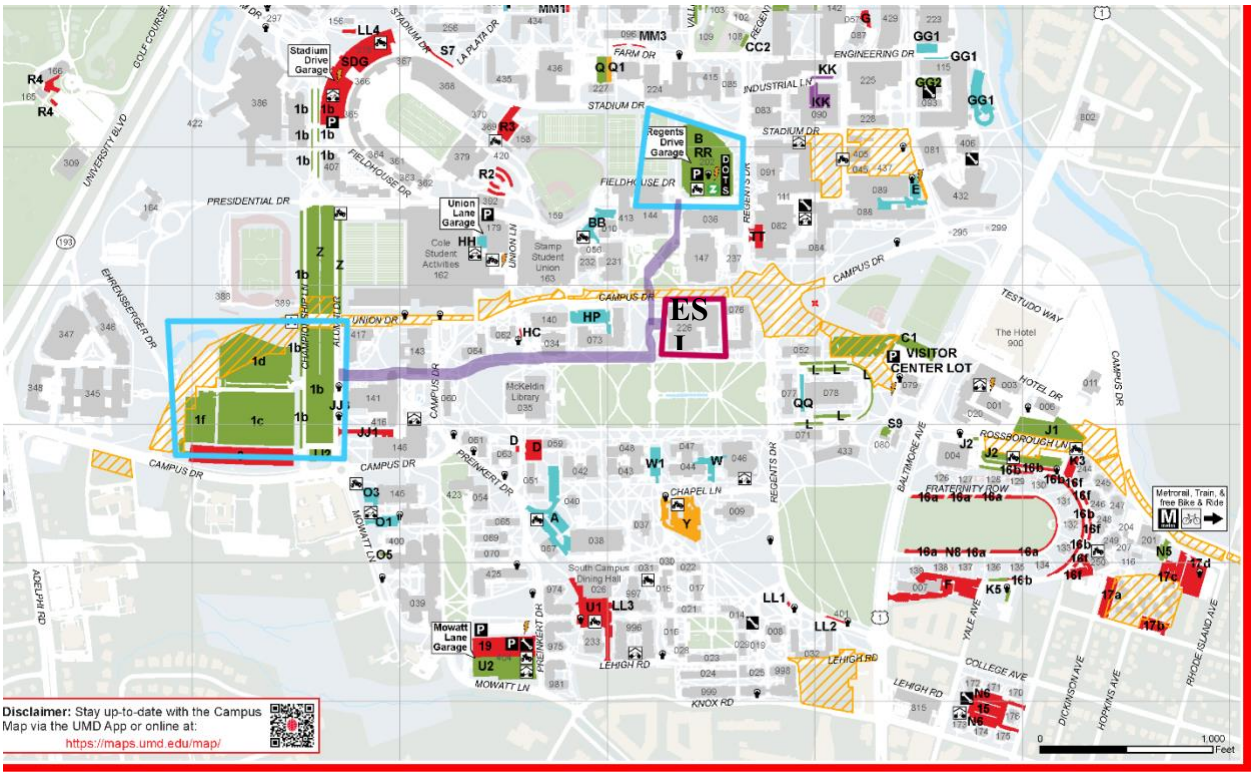
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Note: There may be several local high school graduations that will be hosted on campus this week so traffic to and from campus might be higher than usual. Please plan accordingly.



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