88th Northeastern Section
American Society of Plant Biologist symposium

University of New Hampshire, NH

April 26-27, 2025

University of New Hampshire

American Society of Plant Biologists

Cultivating a better future through plant biology research.

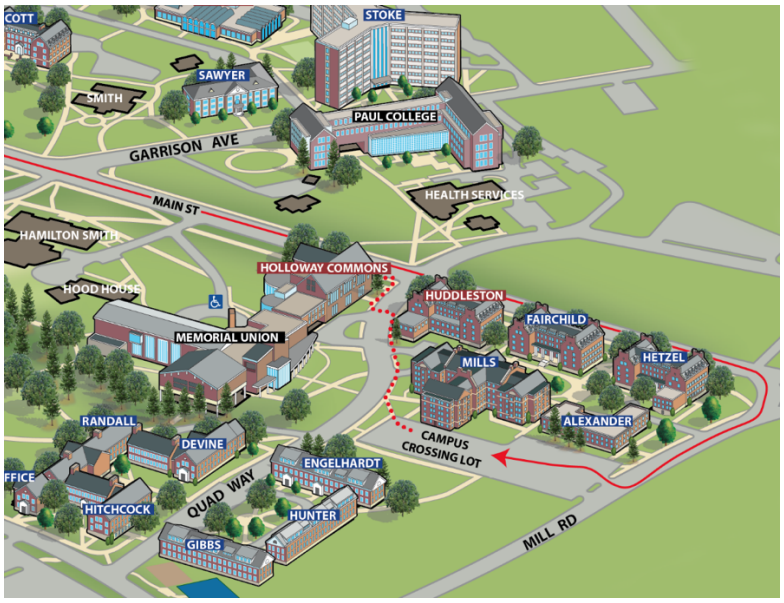
Driving Instructions

University of New Hampshire

Directions to Campus Crossing Visitor Lot

7 Mill Road, Durham, NH 03824

Piscataqua Room-Holloway Commons



From Boston, Massachusetts and Points South: Take I-95 North to Exit 4 (NH Lakes & Mountains/Spaulding Turnpike-Routes 4 & 16). Continue North about 5 miles to Exit 6 for Route 4 West to Durham and Concord. Follow Route 4 West to exit for Route 155A. Turn left at end of exit ramp. This turns into Main Street at UNH. Go through one set of lights and down a small hill. Holloway Commons (a brick building on the corner) will be on your right as you proceed down the hill.

From Portsmouth, New Hampshire and Points East: Take Spaulding Turnpike-Routes 4 & 16. Take the exit onto US-4 West. Turn left onto US-4 West. At the roundabout, take the second exit onto Piscataqua Road. Take the right exit/turn onto NH-108. Turn left onto Dover Road toward Durham/Newmarket. Turn left onto Newmarket Road. Turn right onto Mill Pond Road. Turn right onto Faculty Road. Turn right onto Mill Road. Campus Crossing Visitor Lot will be on the left side of Mill Road (if you pass the Hannaford plaza on the right side of the road, you have gone too far and will need to turn around).

From Portland, Maine and Points North: Take I-95 South, over the Maine-NH bridge and take Exit 5, which turns into Spaulding Turnpike Route 16 North. Continue North about 5 miles to Exit 6 for Route 4 West to Durham and Concord. Follow Route 4 West to exit for Route 155A. Turn left at end of exit ramp. This turns into Main Street at UNH. Go through one set of lights and down a small hill. Holloway Commons (a brick building on the corner) will be on your right as you proceed down the hill.

From Concord, NH and Points West: Follow Route 4 East to exit for Route 155A. Turn right at end of exit ramp. This turns into Main Street at UNH. Go through one set of lights and down a small hill. Holloway Commons (a brick building on the corner) will be on your right as you proceed down the hill.

From Manchester, NH and Points Southwest: Take Route 101 East to Exit 7 for Route 125 North. Pick up Route 4 East at the Lee, NH traffic circle. Take Route 155A exit and turn right at the end of the exit ramp. This turns into Main Street at UNH. Go through one set of lights and down a small hill. Holloway Commons (a brick building on the corner) will be on your right as you proceed down the hill.

CAMPUS CROSSING VISITOR LOT: Proceed past Holloway Commons and take the 2nd right onto Mill Road. Take the 2nd right into the entrance for the Campus Crossing Visitor Lot. After parking, printing your permit from one of the available kiosks in the lot, and putting the permit on your dashboard, walk to the back of the parking lot, cross the street (which is Quad Way), and proceed up the sidewalk toward the Holloway Commons building. The entrance to the building is at the corner of Quad Way and Main Street.

Parking Instructions

Conference Venue:
Piscataqua Room, Holloway Commons,
University of New Hampshire
75 Main St., Durham, NH 03824

Here are the instructions for parking at UNH. UNH Faculty parking is free over the weekend. Many of the faculty parking lots are within a couple of hundred yards from the Holloway Commons that we are having the meeting. There are a couple places that are not free until 6PM; others one has to pay.

Please don't park in the shopping center across the street from the Memorial Union Building and the Holloway Commons.

Program Schedule

Day 1- Saturday April 26, 2025

Session 1- Opening Remarks and Keynote Speakers

12:30- 12:35 Opening remarks – Prof. Subhash Minocha, University of New Hampshire

12:35-12:50 ASPB Presidential address- Leeann Thornton, ASPB President

12:50- 1:25 *Strategies for Limiting Arsenic Accumulation in Food Crops*
Om Parkash Dhankher, University of Massachusetts Amherst, MA

1:25- 2:00 *An Alternate Route for Cellulose Microfibril Biosynthesis*
Alison Roberts, University of Rhode Island, Kingston, RI

2:00-2:15 **Ambassadors program**

2:15-2:40 Coffee Break

Session 2- Abiotic and Biotic Stresses

2:40-2:55 *Exploring SUMO's role in heat-stress protection in the moss Physcomitrium patens*
Robert Augustine, Colby College, Waterville, ME

2:55-3:10 *Purification and Identification of SUMO Conjugates from Moss Exposed to Prolonged Moderate Heat- Stress Conditions*
Caitlin Haller, Colby College, Waterville, ME

3:10-3:25 *CYP72A9 Reduces Growth of Arabidopsis Plants in Response to Abiotic Stress*
Ellie Kreider, The College of New Jersey, Ewing, NJ

3:25-3:40 *AtOXP1 Maintains Glutamate Homeostasis, Promotes Arsenite and Mercury Tolerance via Reducing their Accumulation in Arabidopsis*
Ovais Sayed Aftab, University of Massachusetts Amherst, MA

3:40-3:55 *Developing Abiotic Stress-Tolerant Tobacco Through Overexpression of miR164*
Devyn Kelly, State University of New York, Cobleskill, NY

3:55-4:10 *Editing metacaspase (StMC7) gene enhances late blight resistance in Russet Burbank potato*
Bikram Poudel, McGill University, QC, Canada

4:10-4:25 *Genetic tools for characterizing and speed breeding blight tolerant transgenic American chestnut*
E. Han Tan, University of Maine, Orono, ME

4:25-4:40 *Protist-bacterial symbioses in the rhizosphere: unveiling novel auxin-mediated interactions*
Ravikumar Patel, The Connecticut Agricultural Experiment Station, New Haven, CT

4:40-6:40 Poster and reception

6:40- 8:15 Dinner

Day 2- Sunday April 27, 2025

Session 3 Specialized metabolites and Bioenergy crops

8:30- 9:05 **Keynote Speaker**
Development and Regulation of Secondary Cell Walls in Grass Nodal Roots: Uncovering Hidden Growth Patterns That Keep Plants Upright
Samuel Hazen, University of Massachusetts Amherst, MA

9:05-9:20 *Temperature signals drive grass secondary cell wall thickening*
Greg A. Gregory, University of Massachusetts Amherst, MA

9:15-9:35 *Genetic Engineering Male-Sterile and Drought-Tolerant Switchgrass by Agrobacterium -Mediated Transformation (in progress...)*
Xinyi Liu, State University of New York, Cobleskill, NY

9:30-9:50 *Genetic Engineering of Camelina sativa for Hyperaccumulation of Nickel (CaSH-Ni)*
Suresh Kumar Gupta, University of Massachusetts Amherst, MA

9:45-11:05 *Identification of UDP-dependent glycosyltransferases in the wallflower cardenolide biosynthesis pathway*
Patrick Owen, Williams College, Williamstown, MA

10:00-11:20 *Quantification of Caffeine in Simulated Kopi Luwak Coffee Beans via UV-Visible Spectroscopy & Reverse-Phase High-Performance Liquid Chromatography*
Matthew S. Bravo, State University of New York, Cobleskill, NY

10:20-10:45 Coffee Break

Session 4 **Genetics and Gene regulation**

11:00-11:15 *Integrating Computer Vision and Genomics to Dissect Iron Chlorosis in Direct-Seeded Rice*
Uzezi Okinedo, University of Massachusetts Boston, MA

11:15-11:30 *Evolution of Insertions Mitochondrial and Plastid DNA in the Nuclear Genome Across Arabidopsis thaliana Ecotypes*
Solomon T. Scheiner, State University of New York, Old Westbury, NY

11:30-11:45 *Functional analysis of a novel protein controlling apple fruit quality*
Alexandre Miaule, Cornell University, Ithaca, NY

11:45-12:00 *Decoding gene regulation in grasses: A DAP-seq atlas of Brachypodium distachyon*
Bahman Khahani, University of Massachusetts Amherst, MA

12:00-12:15 Talks and Posters awards

12:15-12:35 NEASPB Report and Elections

12:35 Adjourn

Keynote Speakers

KS-1

Strategies for Limiting Arsenic Accumulation in Food Crops

Om Parkash Dhankher
Stockbridge School of Agriculture, University of Massachusetts-Amherst, Amherst, MA

Arsenic (As) contamination is widespread and affects the health of millions of people worldwide. Arsenic contaminated soil and water are leading source of higher levels of As accumulation in food crops including Rice (*Oryza sativa*) and thus poses significant health risk to human health. We utilized several strategies for mitigating arsaenic threat in the environment and reducing arsenic accumulation in food crops. For developing strategies for phytoremediation of As contaminated soils, we engineered Arabidopsis plants co-expressing the *E. coli* arsC gene (arsenate reductase) in leaves and the γ-ECS (γ-glutamylcysteine synthetase) genes, constitutively. Transgenic lines showed significantly greater arsenic tolerance and accumulation in shoot than control plants. This As phytoremediation strategy was transferred to a high biomass non-food oilseed crop *Crambe abyssinica*, a member of Brassicaceae, for remediation of contaminated soil and water. Engineered Crambe plants exhibited phenotypes and As accumulation similar to those achieved in Arabidopsis.

To increase tolerance and reduced As accumulation in rice, we utilized the gene editing as well as novel nanomaterials applications. We studied the effect of nanoscale sulfur (NS) on counteracting the toxicity and accumulation of arsenic in rice. NS application showed fertilization effect and caused a 40% increase in seedling biomass and a 26% increase in seed yield, compared to untreated control plants. Arsenite (AsIII) exposure caused severe toxicity to rice; however, co-exposure of plants to AsIII and NS alleviated As toxicity. NS application significantly decreased arsenic accumulation (50-75%) in rice shoots, roots, and grains. Further, RNAi suppression of the members of Plasma Membrane Intrinsic Proteins (PIPs) subfamily of aquaporins in rice resulted in 19-26% reduction in AsIII accumulation in shoots and 16% in grains. The CRISPR/Cas9 editing of the AsIII transporters Lsi1 and Lsi2 (members of NIP subfamily), caused decrease As concentration in roots (21-32%) and shoots (62-74%), compared to wild-type plants.

All these approaches showed promising results for phytoremediation of As contaminated soils and in blocking the uptake and transport of arsenic in rice. Therefore, the deployment of these technologies could reduce the As and other toxic metals for improving food safety and human health.

KS-2

An Alternate Route for Cellulose Microfibril Biosynthesis

Alison Roberts
Department of Biological Sciences, University of Rhode Island, Kingston, RI 02881

The Cellulose Synthase (CESA) enzymes of land plants form hexagonal plasma membrane structures called Cellulose Synthesis Complexes (CSCs or rosettes) that move in the plasma membrane as they synthesize β-1,4-glucan chains that interact to form cellulose microfibrils. Cellulose synthase-like D (CSLD) proteins, which also synthesize β-1,4-glucan, are important for cell wall deposition during tip growth and cytokinesis in vascular plants, but the structural nature of their product was unknown. To investigate CSLD function in the absence of interfering CESA activity, we inactivated all eight CESA genes in *Physcomitrium patens* to create viable CESA-deficient moss lines. We discovered that they have plasma membrane rosettes that are morphologically indistinguishable from CESA-containing CSCs and their cell walls contain microfibrillar cellulose. Live-cell imaging showed that CSLDs move in the plasma membrane and that this movement requires catalytic activity. Compared to CESAs, CSLD movements were faster, shorter, less linear, and insensitive to the cellulose synthesis inhibitor isoxaben, and they also failed to track along microtubules. Thus CSLDs, like CESAs, assemble as distinct rosette CSCs that are propelled in the plasma membrane as they produce glucan chains that assemble into microfibrils and that the specialized roles of CSLDs in cytokinesis and tip growth are based on differential expression and different interactions with microtubules, rather than structural differences in the microfibrils they produce. Phylogenetic distribution of CESAs, CSLDs, and their CESA/CSLD-like common ancestor, characterized by a CSLD-like N-terminus and a CESA-like plant-conserved region, is consistent with an evolutionary history that includes gene loss in specific lineages. Contrary to earlier reports, we found abundant rosette CSCs in vegetative cells of Coloechaete orbicularis, which has CSLDs but not CESAs, and in *Klebsormidum nitens*, which has CESAs and CESA/CSLD-like proteins. This suggests that the rosette CSCs and the 18-chain cellulose microfibril evolved more than 830 mya.

KS-3

Development and Regulation of Secondary Cell Walls in Grass Nodal Roots: Uncovering Hidden Growth Patterns That Keep Plants Upright

Samuel Hazen
Department of Biology and Plant Biology Graduate Program, University of Massachusetts Amherst, MA 01003

Plants depend on the combined action of a shoot-root-soil system to maintain their anchorage to the soil. Mechanical failure of any component of this system results in lodging, a permanent and irreversible inability to maintain vertical orientation. Models of anchorage in grass crops identify the compressive strength of roots near the soil surface as key determinant of resistance to lodging. Indeed, studies of disparate grasses report a ring of thickened, sclerenchyma cells surrounding the root cortex, present only at the base of nodal roots. Here, in the investigation of the development and regulation of this agronomically important trait, we show that development of these cells is uncoupled from the maturation of other secondary cell wall-fortified cells, and that cortical sclerenchyma wall thickening is stimulated by mechanical forces transduced from the shoot to the root. We also show that exogenous application of gibberellic acid stimulates thickening of lignified cell types in the root, including cortical sclerenchyma, but is not sufficient to establish sclerenchyma identity in cortex cells. Leveraging the ability to manipulate cortex development via mechanical stimulus, we show that cortical sclerenchyma development alters root mechanical properties and improves resistance to lodging. We describe transcriptome changes associated with cortical sclerenchyma development under both ambient and mechanically stimulated conditions and identify SECONDARY WALL NAC7 as a putative regulator of mechanically responsive cortex cell wall development at the root base.

Oral Presentations

O-1

Exploring SUMO's role in heat-stress protection in the moss *Physcomitrium patens*

Augustine, Robert; Haller, Caitlin; Buetens, Celia; Stathis, Abigail
Colby College, 5730 Mayflower Hill Dr, Waterville, ME 04901

In order to survive and thrive, plants must quickly defend themselves from environmental challenges, which are becoming more prevalent due to the effects of climate change. Small ubiquitin-related modifier (SUMO) is rapidly attached to many nuclear proteins in response to various stress and imparts protection against stress, but exactly how it confers resilience is still an open question. To answer this, we are developing genetic and biochemical tools to better understand SUMOylation in plants using the moss *Physcomitrium patens* as a facile model. We characterized the moss SUMO system by identifying the suite of genes that mediate SUMO attachment and removal from target proteins, their expression levels, and show that they trigger SUMOylation in response to heat-stress. Using CRISPR/Cas9 and RNAi approaches, we are developing a collection of loss-of-function mutants to functionally test the importance of SUMOylation in stress and created His-tagged SUMO knock-in lines to purify SUMO-conjugated proteins for proteomic identification. Collectively, this work builds a foundation for dissecting the role of SUMOylation during heat- stress protection in moss.

O-2

Purification and Identification of SUMO Conjugates from Moss Exposed to Prolonged Moderate Heat- Stress Conditions

Haller, Caitlin; Augustine, Robert
Colby College 4000 Mayflower Hill Dr. Waterville, ME. 04901

As global temperatures rise, understanding how plants protect themselves from stress will be important for developing new strategies to engineer resilience into our food supply. Small Ubiquitin related Modifier (SUMO) is a protein post-translational modifier that is covalently attached to target proteins, especially in response to heat-stress, and is important for conferring thermotolerance. How SUMOylation protects plants from stress is still an open question. A deeper understanding is complicated by reliance on a complex 3-column purification scheme that is necessary to identify 6His-tagged SUMO-modified proteins. Here, we developed new genetic tools and protocols to purify and identify SUMOylated proteins in the moss, *Physcomitrium patens*. We hypothesized that a 10His-tagged SUMO would enable the use of higher imidazole washes to remove histidine-rich contaminant proteins, and thereby allow for a simplified 1-column purification scheme. We generated CRISPR/Cas9 and knock-in replacement constructs to integrate 10His tags into the endogenous moss Sumo1a and Sumo1b loci, and isolated 10His-Sumo1a replacement lines.

Using these lines, we stringently purified SUMO-modified proteins with nickel-affinity chromatography under strong denaturing conditions to remove non-covalently bound contaminant proteins. We optimized the purification to remove non-specific and histidine-rich contaminant proteins by adjusting buffer pH and imidazole concentrations. With these protocols in place, we are now primed to purify and identify SUMOylated proteins in moss by proteomics. The identity of SUMO targets subjected to heat stress should help to reveal the mechanism by which SUMO confers thermotolerance and provide new strategies for combatting the effects of climate change in crops.

O-3

CYP72A9 Reduces Growth of Arabidopsis Plants in Response to Abiotic Stress

Kreider, Ellie; Thornton, Leeann E
Biology Department, The College of New Jersey, Ewing, NJ

Environmental stressors increase in severity every year. To acclimate to these harsh but transient stresses, plants reallocate resources from growth-related metabolism so that they can promote defense and acclimation metabolism. Although many genes are involved in these tradeoffs, we know much more about those involved in regulation of defensive molecules than those that catalyze stress-related growth modulation. Highly conserved enzymes, such as the Cytochrome P450 (CYP) enzyme family, are of particular interest as candidates for stress-induced metabolic changes. They are also significant because they are conserved across plant lineages and catalyze a diverse array of biochemical reactions. Enzymes in the CYP72A subfamily vary in enzymatic functions, some of which are species-specific and others that are conserved across flowering plants. For example, the CYP72A9 gene is a 13-hydroxylase that lowers the bioactivity of gibberellin (GA) growth hormones. Although CYP72A9 is involved in primary dormancy, it is also induced by abiotic stresses. Therefore, our work tests the hypothesis that CYP72A9 13-hydroxylase activity modulates GAs as part of the growth defense trade-off. Using a cyp72A9 knockout mutant and CYP72A9ox lines with constitutive CYP72A9 expression, we measured plant growth and the accumulation of several stress-related molecules in osmotic stress conditions. We found that knocking out CYP72A9 causes increased growth which is exacerbated in stress conditions, and that overexpression of CYP72A9 results in increased resilience to repeated stresses. These results support the hypothesis that CYP72A9 modulation activity is involved in the growth defense tradeoff for Arabidopsis abiotic stress acclimation.

O-4

AtOXP1 Maintains Glutamate Homeostasis, Promotes Arsenite and Mercury Tolerance via Reducing their Accumulation in Arabidopsis

Aftab, Syed Ovais; Singh, Gurpal; Dhankher, O.P.
Stockbridge School of Agriculture, University of Massachusetts, Amherst, MA, US

Glutathione (GSH) is a key antioxidant involved in maintaining redox balance and protecting plants against heavy metal stress. Its turnover is regulated through the γ-glutamyl cycle, but the genes responsible for GSH degradation and recycling are not fully characterized. In this study, we investigated the role of *Arabidopsis thaliana* Oxoprolinase 1 (AtOXP1) in mediating tolerance to arsenite (AsIII) and mercury (Hg) stress via GSH degradation and recycling of glutamate (Glu). T-DNA insertion mutants of AtOXP1 (atoxp1) exhibited hypersensitivity to Hg, with shoot and root biomass reduced by ~40% and ~86%, respectively, compared to wild-type (WT) plants. These mutants also accumulated ~45% more arsenic and mercury in the shoots.

Additionally, oxp1 mutants showed elevated levels of GSH (2.3–4.0-fold) and 5-oxoproline, a precursor of Glu by 96–265%, under metal stress conditions, while exhibiting significantly reduced Glu levels (15–78%) compared to WT. In contrast, AtOXP1 overexpression (OE) lines showed enhanced tolerance, with 18–43% greater biomass and significantly lower shoot accumulation of As (40%) and Hg (19–35%) than WT. OE lines also exhibited reduced GSH levels (44–57%) under toxic metals stress, suggesting GSH degradation and efficient Glu recycling. Isotope labeling further revealed a 29% increase in the 15N-Glu/Total Glu ratio in oxp1 mutants, indicating impaired Glu recycling, whereas OE lines were comparable to WT. Notably, under low nitrogen conditions, OE lines showed 15–22% higher shoot and ~77% higher root biomass compared to WT. Our findings highlight AtOXP1 as a key player in GSH degradation and Glu recycling. Enhancing AtOXP1 expression may offer a promising strategy to improve plant growth and reduce toxic metal(loid)s accumulation, with important implications for food safety and sustainable agriculture.

O-5

Developing Abiotic Stress-Tolerant Tobacco Through Overexpression of miR164 (in Progress)

Kelly, Devyn; Bateman, Alison; Zhao, Ning; Liu, Xingye; Wu, Wenxuan; Ballard Samantha; McMaster-Schuyler, Lynda; Zeng, Peiyu
SUNY Cobleskill, 106 Suffolk Circle, Cobleskill, NY

The miR164 is a highly conserved microRNA in plants, playing a crucial role in abiotic stress tolerance, particularly in response to drought and heat stress. It regulates the expression of target genes, including NAC transcription factors, which mediate various stress responses. Previous studies in Arabidopsis have shown that overexpression of miR164 enhances heat tolerance, whereas loss-of-function mutants exhibit increased heat sensitivity. In this study, we aim to develop abiotic stress-tolerant tobacco (*Nicotiana tabacum*) by

overexpressing miR164. Tobacco serves as an ideal model for Agrobacterium-mediated transformation due to its efficient callus formation and susceptibility to Agrobacterium tumefaciens infection. Our preliminary results indicate successful transformation; however, further genetic analyses are required to confirm the stable integration of miR164 into the tobacco genome. This research contributes to the development of stress- resilient crops and enhances our understanding of miR164's role in abiotic stress responses.

O-6

Editing metacaspase (StMC7) gene enhances late blight resistance in Russet Burbank potato

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McGill University, 21111 Lakeshore Road, Sainte-Anne-de-Bellevue, QC, Canada, H9X 3V9

Plants induce hypersensitive response programmed cell death (HR-PCD), upon biotrophic pathogen infection, to contain the pathogen to the point of infection. Apoptotic-like PCD (AL-PCD) has been reported upon prolonged hemibiotrophic and necrotrophic pathogen infection in potato, to feed on the dead cells for their growth. In potato, silencing of the gene StHRC lead to the suppression of AL-PCD, thus increasing resistance to blights in potato. This was also associated with a significant reduction in the expression of the metacaspase gene StMC7. Accordingly, the gene StMC7 was silenced in potato cultivar 'Russet Burbank' using CRISPR-Cas9 to improve disease resistance against late blight of potato caused by Phytophthora infestans. Following pathogen infection, the disease severity, pathogen biomass and StMC7 gene expression was lower in Stmc7 mutants as compared to wild type. Disease severity was also decreased in *Alternaria solani* inoculated Stmc7 mutants, compared to the wild type, suggesting possible multiple disease resistance in the Stmc7 knockdown mutants. This confirms that the silencing of StMC7 improves late blight disease resistance in potato.

O-7

Genetic tools for characterizing and speed breeding blight tolerant transgenic American chestnut

Tan, E. Han[1], Weigand, Isabella Weigand [1], May, Virginia [2], Walker, Aaliyah [2], Pilkey, Hannah [3], Oakes, Allison [4], Matthews, Dakota [3], Newhouse, Andrew [3], Klak, Thomas [2]
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[2]School of Marine and Environmental Programs, University of New England (UNE), Biddeford, ME 04005
[3]Department of Environmental Biology, SUNY College of Environmental Science and Forestry (ESF), Syracuse, NY 13210
[4]Department of Biology, Syracuse University, Syracuse NY 13210

The American chestnut, *Castanea dentata* (Marsh.) Borkh., is currently a IUCN Red List critically endangered tree species due to this species' complete susceptibility to the

imported fungal blight pathogen, *Cryphonectria parasitica*. Decades of scientific studies and multiple breeding efforts have been underway with the goal of repopulating blight-tolerant American chestnut to restore this foundational tree species in the Eastern United States. Transgenic pure American chestnut lines that constitutively express wheat oxalate oxidase (OxO) are promising candidates for restoration efforts and are currently under consideration for deregulation by USDA APHIS, EPA and FDA under the Coordinated Framework. As practitioners of the hypothesis-driven scientific method, we aim to assess blight tolerance of the Darling 54, a transgenic OxO-expressing American chestnut line, based on rigorous lab- and field-based testing. Therefore, we have developed a suite of molecular and breeding tools that facilitate rapid generation times for breeding and characterizing blight tolerant transgenic trees. One of these methods based on embryo rescue offers further benefits for breeding, conserving rare trees, and propagation. We will also highlight the development of and successful pollen production from a homozygous transgenic Darling 54, which also represents the first T-DNA mutant of American chestnut, as we work towards blight tolerant, restoration-level trees.

O-8

Temperature signals drive grass secondary cell wall thickening

Greg A. Gregory[1,2], Joshua H. Coomey[1,2], Bahman Khahani[1,2], Didier Gonze[3], Serene Omran[1], Kathryn A. McGillivray[1], Conrad E. Stewart[1], Kira A. Gardner[1], David Follette[4], Samuel P. Hazen[1,2]

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In grasses, stem elongation is driven by intercalary meristems at node-internode junctions, where cells divide, elongate, and in some cell types secondary wall maturation. Cellulose is the predominant polymer in plant cells and the most abundant biopolymer on Earth. It is synthesized at the plasma membrane by multi-protein complexes that include CELLULOSE SYNTHASE A (CESA) proteins. To investigate the spatiotemporal regulation of cellulose deposition during development, we developed a CESA8 luciferase gene expression reporter system in *Brachypodium distachyon*. High bioluminescence was observed in stem nodes, a specific region of elongating internodes, and the inflorescence, indicating sites of active secondary wall deposition.

Within internodes, luminescence followed a distinct pattern, with a "dark zone" directly above the node with minimal signal, followed by a "bright zone" approximately 5 mm above the node where bioluminescence peaked. Histological, biophysical, and transcript analysis confirmed that luminescence intensity correlates with thickened secondary cell walls, increased cellulose crystallinity, and elevated CESA8 transcript levels. Time-lapse imaging revealed that CESA8 expression follows a robust diurnal rhythm governed by thermocycles alone, with peak expression occurring in the early morning. Temperature pulse experiments

revealed an immediate but transient response of CESA8 to temperature shifts, which we modeled as an incoherent feed-forward loop. Finally, we found a strong correlation between CESA8 expression and stem elongation, highlighting the role of secondary cell wall thickening in supporting upright growth. These findings provide new insights into the regulation of secondary wall formation and its integration with environmental cues, advancing our understanding of grass stem development.

O-9

Genetic Engineering Male-Sterile and Drought-Tolerant Switchgrass by Agrobacterium -Mediated Transformation (in progress...)

Xinyi Liu, Siyuan Yang, Hongyu You, Qingyun Zhang, Ning Zhao, Siyi Shao, Xingye Liu, Ningzhang Pan, Ming Liu, Wenxuan Wu, Samantha Ballard, Lynda McMaster-Schuyler, Peiyu Zeng
Department of Animal and Natural Sciences, State University of New York, Cobleskill, NY 12043

Switchgrass (*Panicum virgatum* L.), a hardy, warm-season (C4) perennial grass native to North America, is a promising biofuel crop due to its high biomass yield and adaptability to suboptimal soils. However, salinity and drought significantly limit crop productivity, necessitating the development of stress-resistant cultivars. Arabidopsis vacuolar H^+-pyrophosphatase (AVP1) enhances drought and salt tolerance by increasing vacuolar solute accumulation and water retention. Overexpression of AVP1 in Arabidopsis and tomato has demonstrated improved resilience under abiotic stress (Gaxiola et al., 2001; Park et al., 2005), suggesting its potential for enhancing drought tolerance in switchgrass. In this study, we aim to develop male-sterile and drought-tolerant switchgrass by overexpressing AVP1 and silencing FLO/LFY via RNAi. Using Agrobacterium-mediated transformation, we generated transgenic switchgrass lines containing pHL1052 (PhiC31 recombinase) and pHL1060 (PhiC31 recognition sites & Cre/LoxP recombination system). Hybrid plants enabled site-specific recombination, removing unnecessary selectable markers and Cre recombinase, leaving only the genes of interest. Our approach ensures the stable expression of RNAi(FLO/LFY) for male sterility and AVP1 for drought tolerance, facilitating the development of improved switchgrass cultivars for biofuel production in drought-prone regions.

O-10

Genetic Engineering of *Camelina sativa* for Hyperaccumulation of Nickel (CaSH-Ni)

Suresh Kumar Gupta, Yogita Singh, Baoshan Xing, Om Parkash Dhankher
Stockbridge School of Agriculture, University of Massachusetts Amherst, MA 01003

The transition to clean energy, particularly for electric vehicles battreries, is set to increase nickel (Ni) demand by 40% by 2040, and it is imperative that we adopt sustainable approaches to meet this demand. Traditional mining and refining of Ni is highly energy

intensive and causes severe environmental degradation and leaves behind large quantities of mining tailings. Phytomining is an emerging technology for sustainable Ni production. While over 500 plant species are recognized as Ni hyperaccumulators, their global distribution is restricted due to factors like invasiveness, slow growth rates, and limited biomass. It is crucial that we advance our understanding of the molecular, biochemical, and physiological mechanisms that govern Ni uptake, transport, and tolerance in these hyperaccumulating plants. This project is at the beginning stage and will address three major limitations to sustainable and viable Ni phytomining crop production: (i) Decipher mechanistic understanding of Ni homeostasis and its accumulation in hyperaccumulating plants. (ii) Develop a non-food biofuel crop, *Camelina sativa*, as a Ni hyperaccumulator for cultivation on serpentine soils for Ni extraction as well as biofuels production. (iii) Establish phytomining as a feasible technology for extracting Ni and other valuable metals, produce biofuels from seed oils, and phytoremediate ultramafic and contaminated soils contributing to sustainable agriculture and economic development in the future. Additionally, soil amendment will be combined with advanced characterization of soil chemistry to increase Ni bioavailability. Our major aim to make Camelina, non-food crop, as a Ni hyperaccumulator that can achieve over 30 mg/g of dry biomass Ni (250 kg Ni/ hectare per year) while simultaneously produce biofuels on serpentine and ultramafic soils.

O-11

Identification of UDP-dependent glycosyltransferases in the wallflower cardenolide biosynthesis pathway

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Cardenolides are potent plant defensive metabolites that have been studied for decades for their significance in plant-insect interactions and their use in treating heart failure in humans. With recent advancements in genome and transcriptome sequencing, genes in the cardenolide biosynthetic pathway have begun to be identified. Here we employed gene co-expression network analysis using published data from the cardenolide-producing plant *Erysimum cheiranthoides* (wormseed wallflower) to identify two UDP- dependent glycosyltransferases, UGT73C44 and UGT73C45, that are capable of glucosylating the aglycone cardenolide digitoxigenin, as well as other predicted cardenolide pathway intermediates. In vitro and in planta assays revealed that UGT73C44 is specific for cardenolide pathway intermediates with a low Km value of 7 μM for digitoxigenin, while UGT73C45 displayed broader substrate promiscuity in vitro and could glucosylate diverse steroid and flavonoid substrates. A phylogeny and comparisons of structural models of UGT73C44 and UGT73C45 suggest that the enzymes have divergent active site architectures, which may account for their different substrate specificities. These data report the first plant-derived UGT specific to cardenolides, advancing our understanding of cardenolide biosynthesis and the enzymes that drive specialized metabolite diversity. These findings lay the foundation for future efforts to reconstitute the cardenolide pathway

in heterologous systems and design cardenolide analogs with the potential for improved therapeutic properties.

O-12

Quantification of Caffeine in Simulated Kopi Luwak Coffee Beans via UV-Visible Spectroscopy & Reverse-Phase High-Performance Liquid Chromatography

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There are some compounds present in coffee beans that contribute to the overall flavor profile in a cup of coffee. Among these compounds are some that contribute to that bitter flavor you taste while enjoying a cup of coffee. This bitter flavor is shown to be reduced in natural kopi Luwak coffee, and although every step of the process towards this final flavor is unknown, researchers attempt to recreate parts of the enzymatic process to simulate the gastric processing that occurs in the civet cat gut while digesting a coffee berry. In this study, we established six experimental groups (see Table 1). Through taste tests, group 6 was the most desirable, having a fruity aroma and taste while being considerably less bitter than other groups. As a continuation of this research, the chemical analysis of these groups was conducted, focusing on a compound shown to contribute to bitterness in coffee - caffeine. This compound was quantified using analytical techniques such as UV-visible spectroscopy and Reverse-Phase High-Performance Liquid Chromatography. The focus of this quantification was to observe levels of caffeine when compared to the control group, to empirically determine if treatment was successful in lowering levels of bitterness in coffee beans, or if differences in groups are statistically significant.

O-13

Protist-bacterial symbioses in the rhizosphere: unveiling novel auxin-mediated interactions

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Protists are key regulators of rhizosphere microbial communities, significantly influencing microbial composition and ecosystem function. Beyond simple predator and prey relationships, protists can form symbiotic associations with bacteria in the rhizosphere. Understanding these protist-bacterial interactions is crucial for harnessing their potential to enhance plant health and productivity. In the present study, we investigated bacterial associates of heterotrophic protists from the maize rhizosphere using metagenome sequencing and a culture-based approach. This combined approach identified 61 unique bacterial genomes across the ten protists. An analysis of bacterial genomes for plant beneficial traits revealed an abundance of genes for phytohormone, phosphate solubilization, siderophore production, and antibiosis. Further, we validated the gene annotation predictions by lab testing and found IAA is the most prevalent trait; 68% of the

bacteria produce it. This suggests that auxin production is highly conserved and functionally redundant among protist-associated bacterial populations. Having shown that most bacteria associated with protists could produce auxin, we next asked if protists would respond to exogenously supplemented auxin. Auxin treatment significantly increased the growth of all ten protists, ranging from 130 to 300% at 10 µM concentration. We performed a transcriptomic analysis to identify the genes and pathways associated with auxin signaling in the protists using *Colpoda sp*. Over 1,700 genes were differentially expressed in response to exogenous IAA, including genes with homology to those identified in auxin transport, metabolism, and cell cycle regulation in other auxin-responsive organisms. Our findings reveal diverse heterotrophic rhizosphere protists associated with auxin-producing bacteria, exhibiting auxin-dependent growth phenotypes and gene expression. This is the first evidence of auxin-dependent growth in heterotrophic single eukaryotes.

O-14

Integrating Computer Vision and Genomics to Dissect Iron Chlorosis in Direct-Seeded Rice

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Direct-seeded rice (DSR) presents a sustainable alternative to flooded rice systems by minimizing water use and methane emissions. Iron (Fe) deficiency in the aerobic soils typical of DSR frequently leads to chlorosis and yield loss. However, the genetic basis for tolerance to this stress in DSR cultivation remains underexplored. To address this, we combined AI-driven phenotyping and genomics to investigate Fe deficiency tolerance in the 3K mini-core rice panel under field conditions. We developed a scalable computer vision (CV) pipeline that analyzed ~1,500 canopy images to quantify chlorosis severity. This standardized approach showed a significant correlation with expert visual ratings (ρ = 0.514, $p < 2e{-}16$). Despite challenges in differentiating intermediate symptoms, the model achieved strong diagnostic accuracy for healthy (AUC = 0.83) and severely chlorotic plants (AUC = 0.85). A Random Forest classifier further improved interpretability, correctly classifying 64% of severely stressed plants with 85% specificity.

Using CV-derived traits, GWAS identified nine significant SNPs, some overlapping with loci detected through traditional phenotyping. Candidate genes in these regions include OsGRXC2, involved in iron homeostasis, and OsGLDH, linked to ascorbate metabolism—both crucial for regulating iron uptake. These genes exhibited stress-responsive expression, with OsGRXC2 especially elevated in young leaves, consistent with early-stage chlorosis. Our study highlights how scalable phenotyping integrated with genomic analysis can accelerate the discovery of stress-resilient traits and support the breeding of Fe-efficient rice for future-ready agriculture.

O-15

Evolution of Insertions Mitochondrial and Plastid DNA in the Nuclear Genome Across Arabidopsis thaliana Ecotypes

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Utilizing bioinformatic techniques, we were able to identify insertions of mitochondrial and plastid DNA within the nuclear genome of 80 plant species. The organellar insertions discovered have since adapted to the nuclear environment and are now known as norgDNA. NorgDNA has been identified in protein coding regions, pseudogenes, long non-coding RNA, and microRNA. Past studies have found that genes fully composed of norgDNA have a high likelihood of being pseudogenes; however, our results show that genes partially composed of norgDNA have a higher potential of being protein coding genes. In genes partially composed of norgDNA, it has been found that norgDNA is not limited to specific regions, but rather has the potential to be inserted into introns, exons and/or UTRs. Given this, we took a closer look at *Arabidopsis thaliana* genes partially composed of norgDNA. We utilized a recently published A. thaliana pangenome containing 69 ecotypes from around the world and bioinformatic techniques to examine the evolution of norgDNA across these ecotypes. We have found norgDNA to be ecotype specific and their evolutionary trajectories post insertion to differ. This study further contributes to our understanding of genome evolution and endosymbiotic theory.

O-16

Functional analysis of a novel protein controlling apple fruit quality

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The fresh apple industry will reach approximately $101 billion in 2024. However, a significant financial challenge facing this industry is post-harvest losses, which account for over 25% of apple volume loss due to inadequate storage and infections. Apple fruit acidity influences multiple fruit properties, and while it is a primary target for growers aiming to meet consumers' taste preferences, fruit acidity also plays a significant modulator role in maintaining fruit quality during storage. Apple fruit acidity, an important modulation target in apple breeding, is predominantly stored as malic acid content in the vacuoles of apple pulp cells. Ma1 and Ma3 are two major quantitative trait loci (QTL) that account for up to 66% of the variation in apple fruit acidity. Ma1 contains MdALMT9, a gene encoding an anion channel protein belonging to the Aluminum- activated malate transporter (ALMT) family. In apples, up to 42% of the natural variation in apple fruit acidity can be attributed to the allelic differences in the MdALMT9. Ma3, the second major QTL, contains a gene encoding a protein belonging to a scaffolding protein family, which, for clarity, is hereafter

referred to as Ma3. Two variants, Ma3-H and Ma3-L, are associated with high and low fruit acidity, respectively. The Ma1 and Ma3 alleles have been shown to have dominant and incomplete dominance effects on fruit acidity, respectively. Notably, there is an epistatic interaction between the Ma1 and Ma3 QTLs, such as high acidity levels requiring the presence of both wild-type Ma1 and Ma3 alleles. In this project, I explore the hypothesis that the physical interaction between the Ma3 and ALMT9 proteins and the Ma3 and Ma1 gene products accounts for most of the apple fruit's acidity variation, with Ma3 being a significant determinant of apple fruit quality.

O-17

Decoding gene regulation in grasses: A DAP-seq atlas of *Brachypodium distachyon*

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Cis-regulatory elements (CREs) are crucial for controlling gene expression by serving as binding sites for transcription factor proteins. While cis-regulatory elements have been extensively characterized in Arabidopsis thaliana, their identity, diversity, and regulatory functions remain less understood in grasses. To address this gap, we applied DNA affinity purification sequencing (DAP-seq) to identify genome-wide transcription factor binding sites in *Brachypodium distachyon*, a model grass species. We profiled 1,120 proteins across 49 families, with over 38% meeting quality thresholds. Several families, including bZIP, ERF, and NAC, exhibited identifiable motifs and corresponding target genes. Most binding sites were enriched in promoter regions (defined as 2 kb upstream of transcription start sites), although Trihelix and ERFs also showed unique and substantial binding in 5'-UTR regions. Notably, motif analysis revealed strong conservation within protein families, with members often targeting highly similar sequences. Integrating DNA-binding sites identified by sequencing with conserved non-coding sequences revealed that overlapping regions were more enriched, suggesting they are functionally important. To further explore regulatory functions, we combined DAP-seq data with transcriptomic profiles across developmental stages, enabling predictions of transcription factor function as activators or repressors. For example, ERF binding in promoter regions correlated with increased gene expression, consistent with a role in transcriptional activation.

Together, this work provides an atlas of transcription factor binding and regulatory motifs in *B. distachyon*, enhancing our understanding of gene regulation in grasses. These insights have broad implications for transferring regulatory knowledge to related cereal crops like wheat and barley.

Posters

P-1

Investigating the Role of Arabidopsis Ferroportin3

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Iron (Fe) is an indispensable micronutrient essential for plant growth and development. However, maintaining cellular iron levels within physiological bounds is crucial to prevent cytotoxicity. To achieve this balance, plants have evolved intricate mechanisms to regulate iron at inter and intracellular levels, including various iron transporters. The adaptation of plant roots to changing nutrient levels involves alterations in endodermis suberization, which is nutrient-dependent. Recently, Arabidopsis Ferroportin 3 (FPN3) was characterized as an iron exporter targeted to mitochondria and chloroplasts. Analysis of fpn3 T-DNA insertion mutants revealed distinct endodermal suberization patterns in response to iron-sufficient and iron-deficient conditions compared to wild-type seedlings. Transmission electron microscopy studies showed abnormal mitochondrial ultrastructure in fpn3 mutants, resembling vesicular-tubular structures associated with suberin deposition in endodermal cells discovered by another research group. Currently, we are investigating why fpn3 mutants show different suberization patterns. Experiments are underway to elucidate the potential role of FPN3 in the suberization process.

P-2

Elucidating the grape malate tonoplast transporter: A targeted approach for controlling fruit and wine acidity, and improving grapevine tolerance to biotic and abiotic stresses

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Malate is a predominant organic acid that accumulates in the vacuoles of fleshy fruit cells, influencing pH, acidity, and overall fruit quality. In commercial grape cultivars (*Vitis vinifera*), malate levels are inversely related to ambient temperature, resulting in suboptimal acidity in fruit grown in warm climates. In contrast, wild Vitis grapes often exhibit excessive malate levels, resulting in overly tart and unpalatable fruit. This complicates efforts to introgress valuable traits such as disease resistance and cold hardiness from wild species into commercial varieties. Thus, modulating malate levels is crucial for the economic and environmental sustainability of viticulture. Evidence from tomato and apple indicates malate levels in these fruits are controlled by the activity of members of the aluminum-activated malate transporter (ALMT) family. These anion channels mediate malate flux into the vacuole. Recently, VvALMT6, the grape homolog of

the apple ALMT9, was found to be expressed in the fruit. This gene contains conserved, non-synonymous polymorphisms between Vitis species with contrasting levels of malate in the berries. We hypothesize that these polymorphisms underline structural and functional differences between VvALMT6 alleles that lead to differential accumulation of malate between species.

To better understand the role of these transporters, this project aims to characterize the function of VvALMT9 and investigates potential differences between VvALMT alleles. Current work includes determining the subcellular localization of VvALMT9 and two VvALMT6 alleles via transient expression in Nicotiana benthamiana. Additionally, I am characterizing the functional properties of VvALMT9 heterologously expressed in Xenopus oocytes via two-electrode voltage clamp (TEVC) electrophysiology. These studies aim to provide insights into how ALMT transporters regulate malate homeostasis in grapes, contributing to advancements in grape breeding and cultivation.

P-3

Decoding MATEs: Domain Analysis of Citrate Binding Plant Membrane Transporters

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A unique sub-group of membrane transport proteins belonging to the Multidrug and Toxic-Compound Extrusion (MATE) family mediates citrate transport in plants. Citrate chelates and detoxifies phytotoxic aluminum ions (Al3+) in acid soils, thereby providing resistance and preventing it from damaging the growing root. The properties underlying substrate recognition (i.e., citrate binding) in MATEs are poorly understood. Protein sequence alignments of functionally characterized plant MATEs exhibit significant sequence differences (i.e., domains) between citrate-transporting and non-citrate-transporting plant MATEs. We hypothesize that conserved protein domains underlie their distinctive substrate recognition and transport ability. I am utilizing chimeric proteins constructed by swapping these domains among various MATEs to identify domains of citrate transport essential for recognition. I utilize heterologous systems (i.e., *X. laevis* oocytes and *S. cerevisiae*) and transgenic Arabidopsis to analyze the transport and structural properties of these chimeras that enable a select group of plant MATEs to mediate the beneficial exudation of citrate.

P-4

Generating SUMO Knockdown Lines in the Moss *Physcomitrium patens*

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Climate change is increasing the frequency and intensity of extreme weather conditions, and understanding how plants protect themselves from abiotic stress will be critical for

engineering more resilient crops. Small ubiquitin-related modifier (SUMO) is a post-translational modification necessary for plant stress defense.
SUMO is essential for viability and thus it is not possible to generate knockout lines to study this modification's function. Instead, we cloned an RNA interference (RNAi) construct that should reduce, but not eliminate SUMO expression in the moss *Physcomitrium patens*. We designed our inverted-repeats RNAi construct to stably incorporate into the genome by homologous recombination, and facilitated this incorporation by engineering CRISPR/Cas9 constructs that would introduce double-stranded breaks at the insertion site. Our RNAi construct simultaneously targets both Sumo1 genes and adenine phosphoribosyltransferase (Apt) for knockdown. The latter serves as a way to select for plants actively undergoing RNAi silencing because APT converts 2-fluoroadenine (2-FA) into a toxic product, whereas plants with reduced APT levels survive on 2-FA media. Stable RNAi transformants have decreased Sumo1a and stable or increased Sumo1b mRNA levels compared to the wild type, suggesting that a threshold of Sumo1 expression is necessary for plant survival. We continue to screen for stable Sumo1-RNAi lines and are now also attempting transient RNAi assays to test gene function. Sumo-RNAi plants will be useful for testing how SUMO protects plants from stress.

P-5

Investigating lignin biosynthesis in grasses with a bioluminescent reporter of p-Coumaroyl-CoA Monolignol Transferase gene expression

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The plant secondary cell wall provides the rigidity necessary for vertical growth, protection against biotic and abiotic challenges, and structure and hydrophobicity to facilitate water transport. It is also the largest reservoir of atmospheric carbon on Earth. We previously demonstrated that the rate of grass leaf and stem elongation is temperature-dependent, with warmer temperatures stimulating growth. However, it remains unknown whether secondary wall synthesis is similarly regulated by temperature. Given rising temperatures due to climate change, we expect plant growth and carbon sequestration to be altered. Using the grass model *Brachypodium distachyon*, we are investigating how photo- and thermocycles influence growth and secondary cell wall synthesis. Lignin, a key structural component of the secondary wall, provides rigidity and strength. We monitored growth using time-lapse imaging of plants expressing a bioluminescent gene expression reporter where the p-Coumaroyl-CoA Monolignol Transferase (PMT) cis-regulatory region was fused to the firefly Luciferase gene (PMT::LUC). Under thermocycle, with warm days and cool nights, PMT::LUC expression exhibited a daily rhythm with an approximately 24-hour period, peaking in the morning. To test if removing photocycles or thermocycles will impact secondary wall synthesis, we grew plants under normal, warm night, and continuous light conditions. Comparable time-lapse experiments with either thermocycles or photocycles revealed that PMT::LUC expression exhibited a daily rhythm in thermocycles but not

photocycles. This study aims to provide insight into how environmental factors influenced by climate change may affect carbon sequestration and cereal crop physiology

P-6

Optimizing Nickel Bioavailability for Phytomining using Camelina sativa

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Nickel (Ni) is among the critical minerals in renewable energy and various industrial applications. As a key component in batteries for electric vehicles (EVs) and renewable energy storage systems, demand for Ni is projected to grow over 200% by 2050 (IFC, 2023). However, conventional Ni mining practices pose significant environmental risks, such as habitat destruction, soil and water contamination, and high carbon emissions. This urges a need for sustainable resource retrieval alternatives such as phytomining. Ni and other rare elements enriched ultramafic ('serpentine') soils remain an underutilized resource due to challenges in plant growth and low metal bioavailability. Developing effective soil amendment strategies is critical to enhancing Ni bioavailability and plant uptake, ultimately making phytomining scalable.

In this study, we aim to improve the Ni uptake in *Camelina sativa*, a non-nickel accumulator, under different soil amendments to create standardized conditions that maximize Ni bioavailability and accumulation that can be transferred to ultramafic soil conditions for phytomining applications. Camelina is a high-value bioenergy crop, ideal for phytomining due to its fast growth rate, short lifecycle, high biomass, adaptability to marginal soils, and ease of genetic engineering for desired traits. We assessed Ni uptake and accumulation in Camelina across varying pH levels (4.5 to 6.5). Our preliminary results indicate a positive correlation between acidic conditions and Ni uptake. A notable increase in metal concentration in both roots and shoots was observed at lower soil pH. However, plant biomass reciprocally declined with increasing acidic conditions. These results provide a basis upon which further amendment strategies can be developed, balancing optimal Ni uptake without penalties on plant biomass. This study will provide insight into improving the effectiveness and economic feasibility of phytomining in response to growing global demand for Ni.

P-7

Defining Roles of ATAD3 Proteins in *Arabidopsis thaliana* Through Partial Complementation

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Plants depend on the function of a critical cellular organelle, the mitochondrion, to produce the energy necessary for growth and development. My project focuses on the conserved mitochondrial ATPase family AAA domain-containing 3 (ATAD3) proteins, which

uniquely span the mitochondrial membranes from the matrix to the cytosol and are crucial for plant viability. ATAD3 proteins are important for mitochondrial DNA maintenance and cellular respiration, and their disruption in plants has been shown to disrupt nucleoid structure and accumulation of complex I. The model plant Arabidopsis thaliana has four ATAD3 proteins divided into two evolutionary clades: ATAD3A1 and A2, and ATAD3B1 and B2. Single ATAD3 mutants are viable and show no obvious phenotype due to gene duplication, while double mutants of clade A or B are lethal. In order to explore ATAD3 function, I am working to identify a homozygous clade B (b1b2) double mutant transformed with B1 or B2 C-terminally fused to the fluorescent tag GFP. These fusion proteins rescue mutant lethality, but only partially restore function, allowing for phenotypic analysis. I am genotyping progeny of ATAD3 b1b1/b2+ mutant plant lines transformed with the ATAD3-GFP fusions to identify homozygous b1b1b2b2 complemented lines. After the expression of the GFP-tagged ATAD3 genes is confirmed, phenotypic analysis will be performed on these plants to gain insights regarding ATAD3 mutant growth and development.

P-8

Root to Shoot Conversion in *Cannabis sativa* Root Explants After *Agrobacterium*-Mediated Transformation.

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Cannabis sativa is known for its recalcitrance to in vitro regeneration, posing significant challenges in producing transgenic plantlets following genetic transformation. This limitation hinders the development of improved Cannabis varieties to support the budding industry. In this investigation, we evaluate a regeneration strategy in which lateral root primordia are induced and converted into shoots following Agrobacterium- mediated transformation. Root explants are cultured on an auxin-rich medium for three days to stimulate the development of early lateral roots, which are competent for shoot conversion. Explants are then transferred to a cytokinin-rich medium for seven days to promote the conversion of root meristems to shoot pro meristems, leading to shoot meristem development. After both hormone treatments, lateral protuberances from responding explants are assessed to determine whether they developed into roots or shoots and if they are GFP-UV positive. This method presents an alternative strategy for regenerating transgenic shoots in a short period. Additionally, this approach could produce large quantities of micropropagates for commercial purposes.

P-9

Resilience Amid Rising Temperatures: Functionally Examining the Machinery Behind SUMO Attachment in *Physcomitrium Patens*

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As climate change worsens, heat stress threatens to reduce agricultural yield. Thus, a better understanding of how plants protect themselves from stress could open new avenues for improving crop resilience. Small ubiquitin-related modifier (SUMO) is a post-translational modification that allows plants to maintain resilience under rising temperatures. An E1-E2-E3 enzyme cascade covalently attaches SUMO onto target proteins to alter their function and promote stress protection. Precisely how SUMOylation does this remains unknown. To investigate how SUMOylation protects plants, I am inactivating the genes that encode E2 and E3 enzymes via CRISPR-Cas9 gene editing in *Physcomitrium patens*, a moss that is highly amenable to genetic analyses. To achieve this, I have cloned protospacers that target both E2 genes into a CRISPR-Cas9 plasmid using Gibson Assembly Cloning. To examine the function of the E3 ligase, I am using PCR and Sanger sequencing to screen moss lines that have been transformed with CRISPR-Cas9 plasmids targeting one or more of the four Siz1 E3 ligase genes. Thus far, I have identified single, double, and triple siz1 knockouts with frameshift mutations at each locus, and continue to screen for quadruple siz1 knockout plants. I will measure plant phenotypes under normal and heat stress conditions to determine whether these E3s are critical for stress protection and, if so, determine what temperature conditions are lethal to moss SUMO system mutants. In a warming environment, understanding precisely how SUMO helps to withstand heat stress will aid in engineering more resilient crops to feed the growing population.

P-10

Investigation into the Roles of Protein S-Acyltransferases in the Roots of *Arabidopsis thaliana*

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Palmitoylation is the post-translational modification of proteins by covalent attachment of a 16-carbon fatty acid to a cysteine residue. This reaction is catalyzed by protein-S-acyltransferases (PATs). The majority of the 24 Arabidopsis thaliana PAT genes have no characterized mutant phenotype, although several pat genes are known to affect elongation of tip growing cells (pollen tubes or root hairs). Using our collection of single and double mutants, we investigated whether mutations in PAT2, PAT4, or PAT8 affected root hair length. pat2 mutants did not exhibit a reduction in root hair length compared to wildtype, but root hairs of pat4 or pat8 mutants were significantly shorter than wildtype. The root hairs of pat4;pat8 double mutants were significantly shorter than any single mutant. This research indicates that multiple PAT genes are involved in regulating root hair elongation in Arabidopsis.

P-11

Expression of a hyperthermophilic endoglucanase results in alteration in carbon allocation and improved saccharification in poplar

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Poplar is a biomass feedstock of significant economic importance, but efficient production and processing of biomass requires costly pretreatments and enzyme additives. Improving biomass quality and processing requires further investigation into poplar secondary cell wall composition and development in the context of both abiotic stressors and transgenic alteration. Previously, we developed and characterized a transgenic line of poplar expressing a bacterial hyperthermophilic endoglucanase (TnCelB) in hybrid poplar (*Populus alba* × *grandidentata*) that used post-harvest heat activation to improve saccharification efficiency and reduce the need for chemical pretreatment.

Here, we investigate the effects on the TnCelB transgenic poplar line when under a reduced nutrient regime. Biomass allocation and photosynthetic limitations of wild type and TnCelB plants under 100% and 15% strength nutrient treatments were characterized at three timepoints over three months. Overall, genotypic differences were minimal, and most of the significant changes observed were between nutrient treatments. One exception is that the transgenic plants dedicated less biomass to roots and had decreased instantaneous water use efficiency relative to the wild type, but had slightly less predicted xylem vulnerability to cavitation and similar net assimilation values. These results may be explained by localization of the TnCelB enzyme to the plant apoplast. The TnCelB line presents a viable option as a poplar biofeedstock genotype, offering biomass comparable to wild type poplar and more efficient processing, even under limiting nutrient conditions.

P-12

Overexpressing a Bottleneck Gene in *Catharanthus Roseus* with CRISPR Activation

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The medicinal plant Catharanthus roseus is the sole source of chemotherapy drugs vinblastine (VBL) and vincristine (VCR). Shortages of these drugs made national headlines in 2019, highlighting the need for improved processes for VBL and VCR production. One major driver of drug shortages is the low concentration of the compounds in the leaves; since VBL and VCR are secondary metabolites, many genes in the ~30-step biosynthetic pathway are lowly expressed. One approach to improve VBL and VCR concentrations in C. roseus is by overexpressing important pathway genes that act as bottlenecks to VBL and VCR accumulation.

In this project, we use CRISPR activation (CRISPRa) to target catharanthine synthase (HL1), a lowly expressed gene in the pathway. Using a promoter-transactivation assay, we present insights on the optimal CRISPRa design; factors such as the number of

guide RNAs (gRNAs), guide location, and percent GC content are studied to determine the optimal design for overexpression of important promoters. Notably, we found that having 4 gRNAs significantly improves promoter activation compared to 3 gRNAs or single gRNAs. Elucidating the optimal gRNA parameters will allow researchers to streamline the CRISPRa design when targeting lowly expressed genes in plants.

P-13

Maize CYP72A124 Enhances Cold Stress Tolerance

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Climate change impacts crop productivity due to worsening environmental conditions, such as chilling, salinity, or caterpillar feeding. To combat these stresses, plants evolved a variety of defense mechanisms that include physical or chemical defenses and growth modulation. Induction of gene expression at the transcriptional level and alternative splicing are both mechanisms for regulating the abundance or function of proteins related to stress responses. We are focusing on the Cytochrome P450 (CYP) superfamily of enzymes, which are involved in the biosynthesis of many protective secondary metabolites that are induced by environmental stresses. Specifically, the CYP72A subfamily has been shown to possess functional diversity among flowering plants that correlates to their diverse metabolomes. It appears that intron retention in CYP72A124 in maize could create a protein isoform that affects the protein's ability to function and interact with the reductase partner. CYP72A124 is also upregulated under cold stress in maize, suggesting that it enhances cold stress tolerance; however, we did not have genetic resources to test this directly in maize. To test the role of CYP72A124 in cold tolerance, we generated Arabidopsis mutants overexpressing CYP72A124. The CYP72A124ox plants were subjected to cold stress to test the expression of cold stress- related genes and measure other cold tolerance phenotypes relative to wild type Arabidopsis plants. CYP72A124 confers cold stress resistance in Arabidopsis overexpression mutants. This data improves our understanding of the contributions of the CYP72A124 in plant stress acclimation.

P-14

Endophytic *Fusarium oxysporum* Fo47 Increases Growth in Different Genotypes of *Brachypodium distachyon*

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Interactions between plants and microbes can lead to the mutual benefit of both organisms, and understanding such interactions provides key insight into plant growth and development. *Fusarium oxysporum* Fo47 is a non-virulent endophytic fungus commonly found within the rhizosphere. Although well-characterized in *A. thaliana*, *S. lycopersicum*,

and other eudicot species, neither the capability to colonize grass root systems nor its effects on this potential host have been reported. To that end, we investigated potential interactions between *F. oxysporum* Fo47 and *Brachypodium distachyon*. Using fluorescent microscopy we found evidence of Fo47 growing both externally and endophytically on inoculated *B. distachyon* roots. We also found that inoculating with Fo47 resulted in an increase in plant area, dry mass, and seed mass. The degree of response differed between *B. distachyon* accessions, indicating that genetic variation within this species affects its interactions with Fo47. Three potential QTLs have been identified as potential influences on the response of *B. distachyon* to the presence of Fo47. These findings suggest that *B. distachyon* can be colonized by *F. oxysporum,* and the two together function as a tractable system for studying beneficial plant- microbe interactions.

P-15

Molecular fates of anthranilates in plants

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Plants synthesize an array of small molecules used for chemical defense against pathogenic microorganisms and herbivores. Many of these compounds are derived from aromatic amino acids or intermediates in aromatic amino acid biosynthesis. Anthranilate is generated in the first step of tryptophan biosynthesis, where it is then conjugated to a phosphoribosyl sugar by phosphoribosyl anthranilate transferase (PAT1). As a single-copy gene in plants, all fixed carbon flux to tryptophan for protein synthesis, specialized metabolism, and auxin growth hormone biosynthesis proceeds through PAT1. Using a structure-informed biochemical examination, we identified variations in activity, efficiency, specificity, and enzyme-level regulation across PAT1s from evolutionarily diverse plants. In agricultural crops such as citrus and oats, anthranilate is siphoned away from tryptophan biosynthesis by methylating it on the amine. The resulting N-methyl anthranilate can be glycosylated in black oat (*Avena strigosa*) as an intermediate in the biosynthesis of avenacins, which are anti-microbial glycosylated specialized metabolites released from roots to protect against pathogens in the soil. Recently, we have begun probing the substrate specificity of the oat UGT74H5 for activity with various plant hormones and structurally similar plant metabolites. By shedding light on the function and regulation of these anthranilate-using enzymes, our findings may aid in our understanding of how plants balance anthranilate flux to tryptophan with specialized metabolism.

P-16

Contributions of Cellulose Synthase-Like D and Pectin Methylesterase to Cell Plate Formation and Cell Expansion in *Physcomitrium patens*

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Plant cell walls are made of cellulose and other polysaccharides that give cells structure and diverse shapes. Cellulose Synthase-like D (CSLD) is a family of cellulose synthases known to participate in cell plate formation. The moss model species *Physcomitrium patens* has eight CSLD genes. Double csld2/6 knockout (KO) mutants were previously shown to have gametophore leaf phenotypes that are consistent with defects in cytokinesis, including irregular cell shape and cell separations. When these mutants were grown on ammonium-free medium, the severity of the phenotype was more extreme, including excessive cell expansion, incompletely formed cell plates, and multinucleated cells. It was also reported that expression of several genes that encode enzymes promoting cell wall expansion, including three Pectin Methylesterase (PME) genes, are downregulated by ammonium in P. patens, possibly explaining this observation. To test this, we transformed csld2/6KO mutants with three plasmids, each expressing Cas9 and an sgRNA targeting one of the three PME genes along with homology-directed repair oligonucleotides designed to create frame shift mutations by inserting two nucleotides downstream of the start codons of the target PME genes. Three independent triple pme1/2/3KO mutants were selected and verified by PCR genotyping and sequencing.

Mutants are growing for documentation of phenotypic differences, and they will be compared to csld2/6KO and wild type lines on standard and ammonium-free media. If the PME mutants show reduced severity of the csld2/6KO mutant phenotype when grown on ammonium-free medium, we can conclude that CSLDs in P. patens work together with PME proteins to balance cell division and expansion. We have also knocked out four additional CSLD genes in the csld2/6KO line to test whether they contribute to cell plate formation in the gametophores. If so, we expect increased phenotype severity in the csld2/6/1/3/5/8KO mutants grown in medium containing ammonium.

P-17

Knock Out Lines of Secret Agent (SEC) a N-acetylglucosamine Transferase Gene in *Physcometirum patens*

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The Secret Agent proteins (SEC) participate in post translational modification of other proteins, including members of the DELLA family, which act as primary repressors of gibberellin-activated growth in Arabidopsis. SEC acts as an N-acetylglucosamine (O-glcNAc) transferase to release DELLA from its interacting proteins, which can then regulate transcription. SEC is the main O-glcNAc transferase in Arabidopsis and has nearly 500 targets. The moss *Physcomitrium patens* has two SEC genes, but DELLA is not regulated by

gibberellin. The goal of this project was to produce a double knock out of the SEC genes in *P. patens* for investigating evolution of the role of O-glcNAcylation in plant signaling. Two pMH-Cas9- sgRNA vectors were prepared through HiFi reactions, which incorporated protospacers that target either the SEC1 or SEC2 gene. Protoplasts from wild type P. patens were transformed with plasmids targeting SEC1, SEC2 or both genes and homology-directed repair oligonucleotides designed to create frame-shift mutations. For genotyping, tissue samples were collected and a Shorty prep procedure was done to extract the DNA. The extracted DNA was amplified by PCR with primers designed to specifically-amplify wild-type or mutated sequences. The products were run on agarose gels to select lines that amplified preferentially with primers that targeted the mutated sequence. Sequencing tested whether selected lines included the correct frameshift mutation. We have identified three confirmed sec1 knockouts from a transformation with the SEC1-targeted plasmid and at least one double knockout line from a transformation with both plasmids. Identification and testing of additional double knockouts is in progress. The viable sec1/2 double knockout lines will be used for proteome-scale analysis of O-glcNAcylation to test for O-glcNAc transferase activity and identify the SEC targets.

P-18

Investigating Hormonal Influence on Secondary Cell Wall Thickening in Leaf Nodal Roots of *Brachypodium distachyon* Using a Hydroponic Cultivation System

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Grass root systems, particularly leaf nodal roots are essential conduits for water and nutrient transport that play a pivotal role in plant vigor and adaptability. To explore these root structures, we employed a hydroponic system which offers a soil free, highly controlled environment ideal for investigating root responses to external cues like phytohormone application. Our study focused on understanding the effects of exogenous phytohormone application on secondary cell walls in the leaf nodal roots of *Brachypodium distachyon*, a model grass species. To address this, we established a reproducible hydroponic system, enabling precise administration of four pairs of phytohormones and their respective inhibitors, enabling us to assess their impact on root secondary wall deposition across key root tissues, including apoplastic barrier layers, cortex, xylem, and pith. Among the treatments, gibberellic acid showed significant lignin accumulation throughout the mentioned tissues, whereas p-Chlorophenoxyisobutyric acid, an inhibitor of IAA signaling, demonstrated elevated lignin deposition in the stele. In contrast, brassinosteroid and methyl jasmonate did not significantly influence lignin levels, indicating hormone-specific roles in secondary wall thickening. These findings highlight not only the functional specificity of plant hormone regulation in leaf nodal root development but also show the value of our established hydroponic system as an accessible research platform. By enabling unimpeded access to roots, this established system stands as a

powerful tool for researchers aiming to advance their understanding of root growth, development, and structural resilience in plants under diverse abiotic conditions such as salinity, nutrient limitations and toxicity.

P-19

Investigating regulation of secondary cell wall thickening in the node of the model cereal grass *Brachypodium distachyon*

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In plants, secondary cell walls provide the mechanical strength and the structural integrity necessary for the transport of water and nutrients. These walls are primarily composed of cellulose, hemicellulose, and lignin. The forces generated by water transport impose significant mechanical demand on the plant vasculature, requiring cells with heavily thickened and lignified secondary walls. Grass stems are made up of repeating units called phytomers, which consist of internodes, leaves and sheaths, connected by nodes. These nodes serve as a crucial vascular junction for the transport of photosynthates and water. While the regulation of secondary cell wall thickening in the internode is well characterized, nodes remain understudied. Using histological techniques, we identified distinct regions within the stem and found that, in fully senesced plants, nodes exhibit more intense lignification and enlarged vascular bundles compared to internodes These features likely serve to slow transpirational flow and retain nutrients for longer periods. To investigate the regulation of secondary cell wall thickening in the node, we examined phenotypic differences in node architecture between wild-type and mutant KNOTTED OF BRACHYPODIUM 7 (knob7-1) plants. KNOB7 is a transcriptional repressor of secondary cell wall thickening. While its role is well documented in the internode, little is known about its function in the node. Our analysis shows that knob7-1 plants exhibit significantly thicker cell walls in both internode and node tissue, suggesting a shared regulatory mechanism throughout the stem. By elucidating node structure and regulatory mechanisms, our study aims to advance our understanding of cell wall thickening and its coordination across tissue types.

P-20

Exploring the mechanism of corn CYP72A28 in acclimation to cold and drought stress

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Plants rely on complex biochemical pathways to respond to various environmental stresses, such as drought, cold, or salinity stress. To effectively manage changes in their environment, plants manage a tradeoff between growth and stress tolerance, modulating growth to bolster the stress response, and vice versa. Cytochrome P450s are a diverse

superfamily of enzymes that catalyze a variety of chemical reactions in plants. The CYP72A subfamily has been shown to be involved in modulating species-specific secondary metabolism, but they exhibit high functional variation and remain widely uncharacterized. Previous studies have implicated some CYP72As in the inactivation of the growth hormone Gibberellin (GA). CYP72A2A is induced by abiotic stresses in maize, and it is homologous to a rice GA-inactivating CYP72A enzyme. We investigated the possible role of CYP72A28 in GA inactivation, and whether it confers tolerance to cold and drought stress as part of the growth-defense tradeoff. To characterize the possible role of CYP72A28 in hormone regulation and stress tolerance, we generated CYP72A28ox Arabidopsis mutants and exposed them to cold and drought stress. Overexpression of maize CYP72A28 in Arabidopsis alters responses to stress through the accumulation of anthocyanins and transcriptional changes in stress response genes and stress modulated hormone biosynthesis genes. This work broadens our understanding of how CYP72A enzymes regulate acclimation responses.

P-21

Effect of CSLD3 Complex Disruption on *Physcomitrium patens* Cell Wall Composition

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Every plant cell wall is largely composed of cellulose, which acts as the main structural component. Cellulose works alongside many other supporting polysaccharides and proteins that serve varying functions. In *Physcomitrium patens*, cellulose is synthesized by proteins encoded by two gene families, including the Cellulose Synthase-like Ds (CSLDs), which are localized at growing cell tips. It has been determined through previous research that CSLD3 is the highest expressed protein of the gene family. In this experiment, a CRISPR plasmid vector and a homology-directed repair oligonucleotide template for site-directed mutagenesis were used to implement a dominant negative L995F mutation in CSLD3 to investigate the effects on plant growth and cell wall composition. Mutant lines were selected by comparing amplification with PCR primers specific for the wild-type and mutated sequences. DNA sequencing confirmed four mutant lines. We observed that mutant colonies expressed a more compact phenotype due to shortened protonemal filaments and less caulonemal filaments compared to the wild type. Immunolabeling with antibodies LM2, LM6, and LM19 was carried out to compare the cell wall polysaccharide and protein composition to the wild- type plant. LM2 labels arabinogalactan proteins, which function in growth of apical tip growing cells, and was expressed in the protonema tip and cell junction between branching filaments and the main filament.

LM6 labels the 1,5-α-L-arabinan side chains of the rhamnogalacturonan I pectin domain, which maintains cell adhesion and promotes cell wall flexibility, and was expressed along the edges and cell junctions of the protonema, but not at the tip. LM19 labels the unesterified homogalacturonan domain of pectin, which increases cell wall

stiffness, and is expressed along the edges of the protonema, but not at the tip. There were no obvious differences between the labeling patterns of the wild type and L995F mutant.

P-22

Immunolabeling Analysis of Cell Wall Gene Knockouts in *Physcomitrium patens*

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The plant cell wall is composed of cellulose, various polysaccharides and structural proteins that contribute to its rigidity and integrity. Cellulose Synthase-Like D(CSLD) genes encode proteins that synthesize cellulose microfibrils. To better understand the synthesis of cellulose, *Physcomitrium patens* is an exceptional model organism to work with due to its predominantly haploid life cycle, high efficiency of homologous recombination and fully sequenced genome. In P. patens, CSLDs play key roles in protonemal tip growth, cytokinesis and primary cell wall formation during cell expansion. Previous studies have shown that csld2/6 knockout (KO) lines grown without ammonium exhibit an enhanced phenotype characterized by dramatic expansion of gametophore leaf cells after the formation of the first two normal leaves. A published transcriptome analysis showed that genes associated with cell expansion, Extensin1(EXT1) and Xyloglucan Endotransglucosylase/Endohydrolase1(XTH1), are downregulated by ammonium, possibly explaining this phenotype. CRISPR gene editing methods were used to introduce frameshift mutations into EXT1 and XTH1 in a csld2/6KO line. We hypothesized that knocking out EXT1 and XTH1 might restore the wild-type phenotype in csld2/6KO, however, this was not observed. To investigate potential alterations in cell wall composition, we employed immunolabeling, which utilizes monoclonal antibodies and a fluorescently tagged secondary antibody to detect and visualize specific polysaccharides. This enables direct comparison of cell wall components between the csld2/6KO and the newly generated csld2/6+ext1+xth1KO mutant lines.

Immunolabeling analysis revealed that arabinogalactan protein, the 1,5-α-arabinan epitope of rhamnogalacturonanI, and unesterified homogalacturonan show consistent localization patterns in both the csld2/6KO and the csld2/6+ext1+xth1KO mutant lines, suggesting these genes do not significantly affect the distribution of these cell wall components.

P-23

A dual recombination system for transgene control and elimination in switchgrass

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Switchgrass (*Panicum virgatum*) is a hardy perennial C4 grass native to North America, valued for its high biomass yield, and adaptability to various soils and climates. Genetic engineering of switchgrass using transgenic technology allows for plant improvement that is difficult or impossible to achieve through traditional breeding. However, the risk of transgene escape requires development of strategies for gene containment. We have developed an integrated approach that combines a dual site-specific recombination system and total sterility induction mechanisms to produce transgenic products self-contained for desirable transgene, but free of undesirable foreign DNAs. The system is universal and can be applied to different crop species to address transgene escape issue facilitating commercialization of transgenic switchgrass.

P24

Identifying the Significance of LAP1 and LAP2 Genes on Heavy Metals in *Arabidopsis thaliana*

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Toxic metals such as cadmium (Cd), mercury (Hg) and arsenic (As) induce oxidative damages and poses a significant threat to plant development and productivity. To mitigate such oxidative damage, plants rely on several defense mechanisms, one of the most crucial being the glutathione (GSH) homeostasis maintained by g-glutamyl cycle. This cycle involves the synthesis, degradation, and recycling of constituent amino acids- glutamate, cysteine, and glycine. GSH plays a dual role by directly scavenging ROS and by forming conjugates with toxic metals, thereby facilitating their detoxification and sequestration. We are investigating the roles of two genes, leucine aminopeptidase 1 (LAP1) and leucine aminopeptidase 2 (LAP2), in Arabidopsis thaliana, which are hypothesized to be involved in GSH-mediated detoxification pathways. To confirm the genetic identity of homozygous knockdown lines, PCR-based genotyping was performed using allele-specific primers. These mutant lines were subsequently screened for phenotypic responses under Cd, As, and Hg stress conditions. Preliminary results indicate that lap1 and lap2 T-DNA insertion lines exhibited heightened sensitivity to toxic metal stress compared to wild-type plants. This suggests that both genes may play important roles in regulating oxidative stress and contributing to detoxification processes. Based on their expression patterns and the phenotypic data, it is plausible that LAP1 and LAP2 are involved in maintaining glutathione homeostasis—either by influencing GSH biosynthesis, facilitating amino acid recycling, or

regulating downstream detoxification mechanisms. Ongoing experiments, including ROS quantification and qPCR analysis of GSH-related genes, aim to further elucidate the molecular relationship between LAP1, LAP2, and glutathione metabolism. These insights could enhance our understanding of plant tolerance to toxic metals and identify new targets for engineering stress-resilient crops.

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