Abstract book

North East American Society of Plant Biologists

87th Annual Meeting 2024

April 20th -21st, 2024

VENUE:
148 Stocking Hall / Conference Center
Cornell University, 411 Tower Rd
Ithaca, NY 14853

Arranged by

Section Officers

Miguel Pineros (co-chair) (map25@cornell.edu)

Georg Jander (co-chair) (gj32@cornell.edu)

Subhash Minocha (secretary/treasurer) sminocha@unh.edu

Executive Committee Members

Joshua Gendron, Yale University joshua.gendron@yale.edu

Carolyn Lee-Parsons, Northeastern University ca.lee@neu.edu

Peter Melcher, Ithaca College pmelcher@ithaca.edu

Azam Noori nooria@merrimack.edu

Om Parkash parkash@umass.edu

ASPB Ambassadors

Erin Rehrig, Fitchburg State University erehrig@fitchburgstate.edu

Janeen Braynen, Cold Spring Harbor Laboratory braynen@cshl.edu

Audrey Fahey, Cold Spring Harbor Laboratory fahey@cshl.edu

Table of Contents

Program Outline

Saturday, April 20, 2024

10:30am **Registration opens at 148 Stocking Hall / Conference Center**

12:00pm Welcome and Introductions (Miguel Pineros and Georg Jander)

12:10pm **Doreen Ware**
Keynote Speaker: Plant Genomes: understanding their past and managing their future

1:00pm **Shuyao Kong**
Tradeoff Between Speed and Robustness in Primordium Initiation Mediated by Auxin-CUC1 Interaction

1:15pm **Maria Babar**
Exploring Graft Incompatibility Markers in Tomato (*Solanum lycopersicum* L.)

1:30pm **Lauren Cole-Osborn**
The CaMV 35S Promoter Can Activate Heterologous Promoters Even on Separate Co-Expressed Plasmids, Introducing Potential Artifacts into Reporter Assays in Plants

1:45pm **Erin Rehnig**
ASPB Ambassador presentation (Erin Rehnig, erehrig@fitchburgstate.edu)

2:00 – 2:30 pm Coffee break - BINGO – Networking
(Audrey Fahey, fahey@cshl.edu and Janeen Braynen, braynen@cshl.edu)

2:30pm **Yogita Singh**
CRISPR-Cas9 Editing of Silicon/Arsenic Transporter Genes to Reduce Arsenic Contents in Rice to Ensure Food Safety

2:45pm **Yang Liu**
Deciphering the Role of Redox-Responsive and Calcium-Binding Proteins BAP on Plant Immunity and Chilling Tolerance

3:00pm **Serena Fan**
Unravelling the Interplay within ASAP complex's Role in RNA Regulations in *Arabidopsis thaliana*

3:15pm **Sombir Rao**
The moonlighting function of a Nudix domain-containing protein in carotenoid biosynthesis and metabolon assembly

3:30pm **Seren Villwock**
Carotenoid-carbohydrate crosstalk: evidence for genetic and physiological interactions in storage tissues across crop species

3:45pm **Harper Lowrey**
CFH1 Conservation Sheds Light on Functionally Essential Amino Acids

4:00 pm **Josephine LoRicco**
Electron Tomography of Streptophyte Algae: Insights into the Subcellular Components that were Critical for Terrestrialization and Land Plant Evolution

4:15pm **Klaas van Wijk**
Organization and functions of the intra-chloroplast proteolysis network and discovery of the chloroplast N-degron pathway

POSTER SESSION

4:30pm – 5:30pm ODD NUMBERS
5:30pm – 6:30pm EVEN NUMBERS

DINNER
6:30pm – 8:30pm

Sunday, April 21, 2024

9:00am **Ed Buckler**
Keynote Speaker: From Climate Change to AI: Improving Agriculture by Learning from Global Biological Diversity

9:50am **Leeann Thornton (**ASPB, President)

10:00am **Ju-Chen Chia**
The role of Arabidopsis Oligopeptide Transporter 3 (AtOPT3) in shoot-to-root copper signaling and copper-iron crosstalk

10:15am **Simon Malcomber**
Update from NSF: Helping plant science bloom!

10:30 – 11:00 am Coffee Break

11:00am **Boaz Negin**
Catechol acetylglucose: A novel benzoxazinoid-regulated defensive metabolite in maize

11:15am **Gabriella Ballestas**
Development of Molecular Markers for Discrimination Between *Cynanchum wilfordii* and Its Close Relatives

11:30am **Greg Gregory**
Determining the Diurnal Cues Regulating CELLULOSE SYNTHASE A8 Expression in *Brachypodium distachyon*

11:45am **Bahman Khahani**
A Gene Regulatory Network for Secondary Wall Biosynthesis in the Leaf Nodal Roots of *Brachypodium distachyon*

12:00pm **Shiqi Zhang**
Quantitative imaging of Pi using FRET sensors in *B. distachyon* roots reveals diverse Pi distribution and fluctuation during AM symbiosis

12:15pm **Subhash Minocha**
NE-ASBP secretary/treasurer report, awards

12:30pm Tours of Cornell facilities

Cornell High Energy Synchrotron Source (CHESS), Boyce Thompson Institute automated phenotyping facility or Botanic Garden

Keynote speakers:

Plant Genomes: understanding their past and managing their future.

Doreen Ware

[1]Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724
[2]USDA-ARS-NAA, Ithaca, NY 14853

Doreen Ware is a Computational Biologist with the USDA Agriculture Research Service and an adjunct professor at Cold Spring Harbor Laboratory. Doreen's work focuses on understanding the evolution of plant genome sequences over time and its implications for agricultural improvement. By integrating Genetics and Evolutionary biology, her work seeks to answer questions on how are genes conserved and lost over time. What are the fates of duplicated genes? What is the impact of structural variation on phenotypic variation? Her work also focuses on understanding how genes are regulated in plants, by examining regulatory networks, targeting transcription factors and microRNA genes, with the goal of understanding how these parts of the plant genome work together in determining spatial and temporal expression of genes.

From Climate Change to AI: Improving Agriculture by Learning from Global Biological Diversity

Edward Buckler

[1]US Department of Agriculture-Agricultural Research Service, Plant, Soil and Nutrition Laboratory, Robert W. Holley Center for Agriculture and Health, 538 Tower Road Ithaca, NY 14853
[2]School of Integrative Plant Science, Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY 14853

Edward Buckler is a plant geneticist with the USDA Agricultural Research Service (ASDA-ARS) and an adjunct professor in the Section of Plant Breeding and Genetics at Cornell University. His work focuses on both quantitative and statistical genetics in maize (as well as other crops) to understand the genetic basis of trait variation and use this natural variation to improve crops. This research has provided insights into the genetic diversity of species, the genetic architecture of complex traits, hybrid vigor, and the genes controlling numerous traits related to plant flowering, development, starch, and pro-Vitamin A. His group is now exploring ways to re-engineer global agricultural production systems to reduce greenhouse gas emissions, ensure food security, improve nutrition, and respond to climate change. In 2012 he was elected an American Association for the Advancement of Science Fellow. In 2014, he was elected to the National Academy of Sciences, and in 2017 received the inaugural NAS Prize in Food and Agricultural Science.

Short Talks:

Tradeoff Between Speed and Robustness in Primordium Initiation Mediated by Auxin-CUC1 Interaction

Shuyao Kong[1,2], Mingyuan Zhu[1,2,3], David Pan[1,2], Brendan Lane[4], Richard S. Smith[4], Adrienne H. K. Roeder[1,2]

[1] Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY 14853, USA
[2] Section of Plant Biology, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, USA
[3] Present address: Department of Biology, Duke University, Durham, NC 27708, USA
[4] Department of Computational and Systems Biology, John Innes Centre, Norwich NR4 7UH, UK

Robustness is the reproducible development of a phenotype despite stochastic noise. It often involves tradeoffs with other performance metrics, but the mechanisms underlying such tradeoffs were largely unknown. An Arabidopsis flower robustly develops four sepals from four precisely positioned auxin maxima. The development related myb-like 1 (drmy1) mutant generates stochastic noise in auxin signaling that disrupts both the robust position and number of sepal primordia. Here, we found that increased expression of CUP-SHAPED COTYLEDON1 (CUC1), a boundary specification transcription factor, in the drmy1 mutant underlies this loss of robustness. CUC1 surrounds and amplifies stochastic auxin patches in drmy1 to form variably positioned auxin maxima and sepal primordia. Removing CUC1 from drmy1 provides time for the noise in auxin signaling to resolve into four precisely positioned auxin maxima, restoring robust sepal initiation. However, removing CUC1 decreases auxin maxima intensity and slows down sepal initiation. Thus, CUC1 increases morphogenesis speed but impairs robustness against auxin noise. Further, using a computational model, we found that the observed phenotype can be explained by the effect of CUC1 in repolarizing PIN FORMED1 (PIN1), a polar auxin transporter. Our model also predicted that decreasing growth rate restores developmental robustness in the drmy1 mutant, which we validated experimentally. Thus, our study highlights a tradeoff between speed and robustness during morphogenesis.

Exploring Graft Incompatibility Markers in Tomato (Solanum lycopersicum L.)

Maria Babar[1,2], Gwo Rong Wong[3], Purabi Mazumdar[3], Jenniffer Ann Harikrishna[3], Saddia Galani[2], Om Parkash Dhankher[1]
[1]Stockbridge School of Agriculture, University of Massachusetts, Amherst, USA
[2]Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering, University of Karachi, Pakistan 2.
[3]Centre for Research in Biotechnology for Agriculture, Universiti Malaya, 50603 Kuala Lumpur, Malaysia 3.

Grafting is an innovative technique commonly practiced in tomato to provide resistance against biotic and abiotic stresses. However, incompatibility limits the far-reaching grafting approaches in sustainable food production worldwide. An early and precise graft incompatibility detection will have great significance since graft compatibility between rootstock and scion is a pre-requisite for successful grafting. Recent studies have manifested the important role of microRNAs in graft union development in grafted plants. MicroRNA160a is a highly conserved miRNA that modulates the auxin transduction pathways by inhibiting auxin response factor (ARFs) genes, which are important for revascularization at the graft union. In the current study, sly-miR160a and its targeted genes (ARF10, ARF16 and ARF17) expression were examined in tomato self-grafts, intra-specific and inter-specific grafts at 4, 8 and 16 days after grafting (DAG) in addition to various biochemical and metabolic analyses. The results revealed that sly-miR160a was significantly downregulated and ARF10, ARF16 and ARF17 expression were upregulated in tomato self-grafts and intra-specific grafts, particularly at 4 and 8 DAG as compared to inter-specific grafts. In addition, intra-specific grafts depicted a vigorous resistance against oxidative damage by accumulating lower hydrogen peroxide, minimum membrane permeability and lipid peroxidation coupled with high antioxidants activity; while inter-specific grafts with low survival rates showed high accumulation of total phenolic and flavonoid content at 4, 8 and 16 DAG. During histological examinations of graft junctions, an apparent long adherence region at the graft junction of self-grafts and intra-specific grafts were observed. These variations at the molecular, biochemical and metabolic level may regulate graft incompatibility and may serve as markers to assess compatible germplasm in rootstock developmental research programs.

The CaMV 35S Promoter Can Activate Heterologous Promoters Even on Separate Co-Expressed Plasmids, Introducing Potential Artifacts into Reporter Assays in Plants

Lauren Cole-Osborn, Emma Meehan, Carolyn MA Lee-Parsons
Northeastern University, Boston, MA

Improvements in molecular and synthetic biology methods have accelerated in recent years, allowing efficient and effective exploration of gene and protein function in plants. Advancements in cloning allow high-throughput assembly of complex plasmids consisting of multiple transcriptional units, and advancements in transient transformation techniques have allowed rapid evaluation of gene function in many different plant species. However, co-expressed transcriptional units can have unexpected effects on each other's activity. It is important that we understand these interactions to ensure that our experiments are measuring the true regulatory relationship that we are trying to study rather than confounding artifacts. In this talk, I show that the Cauliflower mosaic virus 35S promoter/enhancer, widely used for transgene expression in plants, can activate heterologous promoters on separate co-expressed plasmids, interfering with quantitative dual-luciferase assays that investigate transcription factor function. The magnitude of this enhancing effect is not consistent between expressed coding sequences, indicating that the 35S promoter could interfere with quantitative results even when proper negative controls are used. Interestingly, this enhancer activity was orientation-dependent; when the 35S promoter was immediately adjacent to the transfer DNA (T-DNA) right border, activation was very strong, but this activation was weaker when the 35S promoter was immediately adjacent to the left border. Switching the 35S promoter to the Arabidopsis thaliana Ubiquitin 10 promoter, which does not have enhancer activity, significantly reduced this confounding activation. When designing experiments utilizing complex plasmids with multiple transcriptional units, it is imperative that researchers consider the interactions of these introduced transcriptional units and how they may influence interpretation of results.

CRISPR-Cas9 Editing of Silicon/Arsenic Transporter Genes to Reduce Arsenic Contents in Rice to Ensure Food Safety

Yogita Singh[1], Om Parkash Dhankher[1], Upendra Kumar[2]
[1]Stockbridge School of Agriculture, University of Massachusetts Amherst, MA, 01003, USA
[2]Department of Plant Sciences, MJP Rohilkhand University, Bareilly-243006, Uttar Pradesh, India

More than three billion people worldwide consume rice daily, which accounts for 20% of the world's dietary energy. Rice is a dominant source of inorganic arsenic exposure for populations consuming rice as a staple food. Arsenic (As) is a carcinogenic metalloid and it decreases the nutritious content of rice grains. Reducing arsenic in rice is critical for enhancing food safety. Also, rice straw takes longer to naturally degrade because of its high silicon (Si) (up to 10-12% of its dry weight) content. Rice straw burning contributes significantly to the global warming and decreases crop yield. Reducing the silica content of rice plants up to an ideal level could be a logical way to address the issue of excessive silica in rice straw, allowing the straw to still have its good benefits as animal feed while also being ploughed into the ground for quick decomposition. OsLsi1 and OsLsi2, the two main transporters for silicon uptake in rice roots, take up silicon and arsenite. Here, we investigated whether editing OsLsi1 and OsLsi2 can decrease As and Si accumulation in rice without compromising the plant growth and grain yield. We used the CRISPR/Cas9 technology to edit the promoter and exon region of OsLsi1 and OsLsi2, and we generated a total of four homozygous transgene free edited lines. Relative expression analysis showed that CRISPR-Cas9 induced mutations suppressed the expression of OsLsi1 and OsLsi2 transcripts in rice roots. Edited line generated by targeting the coding region of OsLsi2 showed no yield penalty. Mutant and wild type lines were treated with silicic acid and sodium arsenite in hydroponic experiments in order to evaluate the uptake of As and Si in their roots and shoots. A significant decrease in these element's concentration was found relative to wild type plants. Our study indicates that editing OsLsi2 can effectively reduce arsenic accumulation in rice, thereby enhancing food safety and grain yield without compromising the quality of the grain.

Deciphering the Role of Redox-Responsive and Calcium-Binding Proteins BAP on Plant Immunity and Chilling Tolerance

Yang Liu, Jian Hua
Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, USA

Redox and calcium signaling mechanisms play crucial roles in orchestrating plant responses to various biotic and abiotic stresses. Previous reports found redox-responsive and calcium-binding proteins BAP are negative regulators of immune responses in Arabidopsis thaliana and positive regulators of temperature stress responses in Vitis vinifera, yet the underlying regulatory mechanisms remain elusive. Our research identifies enhanced tolerance to chilling temperatures in Arabidopsis with overexpression of BAP1, as evidenced by two independent transgenic lines. Through bimolecular fluorescence complementation (BiFC) and reverse genetic analyses, we find the interaction between Arabidopsis BAP1 and BAP2 with all three catalases within the nuclei. Interestingly, overexpression of CAT2 may alleviate the autoimmune phenotype observed in bap1 mutants, hinting at a regulatory pathway where BAP recruits catalases to suppress redox-associated immune and stress responses, such as reactive oxygen species (ROS) burst and programmed cell death (PCD). In addition, utilizing calcium reporters GCamp.6f and YC3.6, we detect an elevated basal calcium level in bap1 loss-of-function mutants, potentially contributing to its autoimmune phenotype. Mutant forms of BAP with defective redox-sensing or calcium-binding are introduced into the bap mutants. Further studies on their downstream effect would collectively shed new light on the intricate interplay between BAP proteins, redox signaling, and calcium dynamics in modulating immune and temperature stress responses in plants.

Unravelling the Interplay within ASAP complex's Role in RNA Regulations in Arabidopsis thaliana.

Serena Fan, Christopher Chin, Xiao-Ning Zhang
Biochemistry Program, Department of Biology, St. Bonaventure University 3261 W. State Road, St. Bonaventure, NY 14778

The evolutionarily conserved apoptosis and splicing associated protein (ASAP) complex regulates transcription, splicing and nonsense-mediated decay in RNA metabolism. In Arabidopsis thaliana, the ASAP complex is composed of Serine/Arginine-rich (SR45), ACINUS, and Sin3-associated protein 18 (SAP18). Recent studies suggest that an ACINUS-SR45 dimer is required for SAP18 association, SAP18 recruits Histone Deacetylase 19 to silence a flowering suppressor gene (FLC) and promote flowering. In addition, the sr45-1 null mutant exhibits delayed root growth, late flowering, lower levels of the ASAP component proteins and a decreased nucleus:cytoplasic ratio for SAP18. This led us to hypothesize that the ASAP complex components regulate each other to achieve a homeostasis in transcriptional regulation. To test this, we generated all the ASAP single, double, and triple mutants. Phenotypic analyses showed that SR45 and ACINUS promote root growth and flowering, while the inhibitory role of SAP18 is dependent on SR45 and ACINUS. These initial findings prompted us to investigate their regulatory functions at the transcriptome level in Col-0, sr45-1, acin-2, sr45-1 acin-2, and sr45-1 acin-2 sap18-2. From 3D RNA-seq analysis, we observed a substantial overlap in ACINUS-activated and SR45-activated gene expressions, while SAP18 silences a subset of these overlapping genes. A nucleolus histone methyl transferase related protein coding gene (NuHMTRP) showed notable differential expression and an alternatively spliced isoform switch event in the sr45-1 acin-2 double mutant compared to other genotypes. The results were validated with qPCR and RT-PCR. Taken together, our findings suggest that there is a possible synergy between SR45 and ACINUS within the ASAP complex that SAP18 seems to partially antagonize. Future studies with all ASAP mutants would help reveal the mechanisms for cross-regulations within the ASAP complex and for their functions in RNA metabolism in plants.

The moonlighting function of a Nudix domain-containing protein in carotenoid biosynthesis and metabolon assembly.

Sombir Rao[1,2], Hongbo Cao[2], Li Li[1,2]
[1]Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, New York 14853, USA
[2]Robert W. Holley Center for Agriculture and Health, USDA-ARS, Cornell University, Ithaca, New York 14853, USA

Carotenoids are a group of pigments essential for plants. Carotenoid biosynthesis is highly regulated in response to diverse developmental and environmental signals. Carotenogenic enzymes are hypothesized to form metabolons to facilitate substrate channelling. However, the regulators conserved among plant species remain elusive and the facilitators required for metabolon formation are unknown. Phytoene synthase (PSY) is the major rate-limiting enzyme that catalyzes the highly regulated step of carotenogenesis. Geranylgeranyl diphosphate synthase (GGPPS) acts as a hub to interact with specific GGPP-utilizing enzymes for the synthesis of downstream isoprenoids. In this study, we discovered NUDX23, a Nudix domain-containing protein conserved among all plant species, as a novel posttranslational regulator of PSY and GGPPS, and an only known regulator required for carotenogenic enzyme metabolon assembly. Overexpression of NUDX23 significantly increases PSY and GGPPS protein levels and carotenoid production, whereas knockout of NUDX23 dramatically reduces their abundances and carotenoid accumulation in Arabidopsis. NUDX23 regulates carotenoid biosynthesis via direct interactions with PSY and GGPPS in chloroplasts, which enhances PSY and GGPPS protein stability in a large PSY-GGPPS enzyme complex. NUDX23 was found to co-migrate with PSY and GGPPS proteins in a high molecular weight complex and to be required for the complex assembly to facilitate metabolite channelling. The function of NUDX23 for the PSY-GGPPS enzyme complex assembly is conserved in bacteria and plants. Our findings uncover a new regulatory mechanism underlying carotenoid biosynthesis in plants and suggests a potential of NUDX23 as an effective genetic tool for developing carotenoid-enriched food crops.

Carotenoid-carbohydrate crosstalk: evidence for genetic and physiological interactions in storage tissues across crop species

Seren Villwock[1], Li Li[2] , Jean-Luc Jannink[2]
[1] Cornell University College of Agriculture and Life Sciences, School of Integrative Plant Science, Section of Plant Breeding and Genetics, 222 Tower Rd Ithaca NY 14853
[2] US Department of Agriculture-Agricultural Research Service, Plant, Soil and Nutrition Laboratory, Robert W. Holley Center for Agriculture and Health, 538 Tower Road Ithaca, NY 14853

Carotenoids play essential roles in photosynthesis, photoprotection, and human health. Provitamin A carotenoid biofortification efforts in staple crops like rice and cassava have been successful. Interestingly, in some cases, manipulating carotenoid content through selection or genetic engineering has been shown to affect other aspects of plant metabolism, influencing traits like sugar content, dry matter percentage, fatty acid content, stress tolerance, and phytohormone concentrations. Negative relationships between carotenoids and carbohydrates across diverse crop species suggest there may be a metabolic interaction. Despite ongoing research on carotenoids in fruit and leaf tissues, their interactions particularly in non-photosynthetic storage tissues remain unclear. We synthesize these observations and the evidence for four hypothesized mechanisms underlying this relationship: 1) direct competition for precursors; 2) physical impacts of co-located carotenoid and starch synthesis in plastids; 3) sugar or apocarotenoid signaling; and 4) non-mechanistic population or statistical artifacts. Areas where further research is needed into this interplay between primary and secondary metabolism are highlighted.

CFH1 Conservation Sheds Light on Functionally Essential Amino Acids

Harper Lowrey, Wei Liu, Anxu Xu, Josh Gendron
Yale University, New Haven, CT 06511

The circadian clock is vital to imparting rhythmicity on downstream biological processes, making it both ecologically and agriculturally important. However, while circadian transcriptional regulation has been well studied, far less is understood about the clock's posttranslational mechanisms. Recent work has identified CLOCK-REGULATED F-BOX WITH A LONG HYPOCOTYL 1 (CFH1) as a novel circadian output tethering the clock to photomorphogenic growth via protein degradation, but much remains unknown about the protein structure and evolutionary conservation of gene function. By combining evolutionary and structure-function analyses, we determined that CFH1 is widely conserved across land plants—even as far away as P. patens which diverged from flowering plants over 400 million years ago—and that this conservation is concentrated in two highly conserved domains: the F-box domain and a previously uncharacterized C-terminal domain. Within this C-terminal domain, we found that particular regions contribute to CFH1's hypocotyl regulation and PIF3 interactions, further illuminating its post-translational role in propagating circadian regulation and providing insight into potential functional orthologs in other species. Studying CFH1 expands our understanding of how plants integrate various environmental and endogenous signals to converge on key transcription factors that regulate growth. CFH1's function as a clock output creates new opportunities to regulate growth without disrupting the clock, presenting a novel avenue for future growth manipulation and crop optimization.

Electron Tomography of Streptophyte Algae: Insights into the Subcellular Components that were Critical for Terrestrialization and Land Plant Evolution

Josephine LoRicco, Li Kozel, Benjamin Gibeau, Kaylee Bagdan, David Domozych
Department of Biology and Skidmore Microscopy Imaging Center Skidmore College 815 N Broadway, Saratoga Springs, NY, 12866, USA.

All land plants are thought to have arisen from a single streptophyte algal ancestor that successfully colonized land approximately 500-600+ million years ago. The extracellular matrix (ECM) of these was particularly critical for this transformative evolutionary event. The ECM of streptophyte algae and land plants is a product of the highly coordinated interactions of the endomembrane system, cytoskeleton, plasma membrane and ECM itself. In turn, these subcellular systems modulate in response to complex developmental programs and abiotic/biotic stress. At present, there is limited structural information concerning the architecture of the endomembrane system and membrane trafficking networks in streptophyte algae. In this project, we applied electron tomographic technologies to initiate development of endomembrane atlases of streptophyte algae. Previous cell biology-based studies and the recently published genome of the unicellular zygnematophyte, Penium magaritaceum, make it a good model organism for understanding the "tools" available in ancient streptophytes that led to successful terrestrialization. Tomography quite literally adds an additional dimension to our understanding subcellular structures, allowing for additional structural information not apparent by traditional EM methods. We have used two tomography methods to interrogate structural changes Penium: (1) TEM tomography which allows for high resolution 3D reconstructions, and (2) serial array tomography which has slightly lower resolution, but allows for reconstruction of large volumes – even entire cells! Using these methods, we have characterized structural changes to subcellular components of Penium margaritaceum using both chemical and abiotic stress agents.

Organization and functions of the intra-chloroplast proteolysis network and discovery of the chloroplast N-degron pathway

Pratyush Routray, Marissa Annis, Claire Ravenburg, Klaas J.Van Wijk
Section of Plant Biology, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, USA

Different proteases and peptidases are present in chloroplasts to process precursor proteins, to remove cleaved chloroplast transit peptides, damaged, miss-folded proteins, or otherwise unwanted proteins. Collectively, these peptidases must form a proteostasis network, with build-in redundancies, hierarchies and complementary activities. The challenge is to determine the contributions of each peptidase (system) to this chloroplast network; this will require understanding of substrate recognition mechanisms for each protease system, substrate and product size limitation and protease capacity. Protein amino (N) termini are major determinants of protein stability in the cytosol of eukaryotes and prokaryotes, conceptualized in the N-end rule pathway, lately referred to as N-degron pathways. There are several independent observations that argue for the existence of N-degron pathways in plastids (chloroplasts) in higher plants. Structural and in vitro interaction analysis shows that the plastid ClpS homolog(s) has many features of bacterial ClpS recognin but with somewhat modified N-degron affinities. N-degron-bearing substrates are likely degraded by the Clp chaperone-protease system consisting of the ClpC1, ClpC2 and ClpD chaperones, the ClpPR protease and associated ClpT homologs, and possible one or more adaptors such as ClpF. I will discuss features and new unpublished findings of chloroplast degradation pathways, including novel methodologies to discover protease substrates and a novel chloroplasts N-degron reporter system. This talk will also illustrate the role of proteolysis in regulating chloroplast function throughout development and environmental challenges.

The role of Arabidopsis Oligopeptide Transporter 3 (AtOPT3) in shoot-to-root copper signaling and copper-iron crosstalk

Ju-Chen Chia[1], Arthur Woll[2], Louisa Smieska[2], Rong Huang[2], Ryan Tappero[3], Andrew Kiss[3], Jiapei Yan[1], Marta Faulkner[1], Eli Simons[1], Chen Jiao[4], Zhangjun Fei[4], Miguel Piñeros[5], Olena K. Vatamaniuk[1]
[1] Section of Plant Biology, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853
[2] Cornell High Energy Synchrotron Source (CHESS), Cornell University, Ithaca, NY14850
[3]National Light Source II, Brookhaven National Laboratory
[4] Boyce Thompson Institute for Plant Research, Ithaca, NY 14553, USA
[5] Robert W. Holley Center for Agriculture and Health, USDA-ARS

Micronutrients copper (Cu) and iron (Fe) are essential for plant growth but can be toxic when over-accumulated in cells. Thus, plants tightly regulate their root uptake systems to prevent deficiency while avoiding toxicity. This includes balancing Cu/Fe accumulation via the Cu-Fe crosstalk and systemic shoot-to-root signaling through the phloem to report the shoots' demand. However, only systemic Fe deficiency response has been documented. In Arabidopsis thaliana, it involves the oligopeptide transporter 3 (AtOPT3) that transports Fe into the phloem companion cells to reflect the real-time Fe status of the shoots to the roots. Consistently, shoot-to-root Fe status signaling is disrupted in the opt3 mutant. Using a combination of functional genomic approaches, including 2D Confocal Synchrotron X-ray Fluorescence Microscopy (C-SXRF) and 2D-SXRF, RNA-seq, phloem-feeding in the shoot followed by gene expression studies in the root and in vitro uptake studies in Xenopus oocytes, we showed that AtOPT3 also plays an important role in Cu delivery into the phloem in leaves, and this function is essential for the systemic shoot-to-root signaling of Cu deficiency. Specifically, we showed that the opt3 mutant contained less Cu in the phloem, was sensitive to Cu deficiency and mounted a transcriptional Cu deficiency response in roots and young leaves. Notably, direct phloem feeding of the opt3 mutant with Cu in leaves rescued the expression of not only components of Cu but also the Fe uptake system in roots. Direct phloem feeding of the opt3 mutant with Fe in the shoot also downregulated the expression of both Fe and Cu deficiency marker genes in the opt3 root. These data suggest the existence of shoot-to-root Cu signaling, highlight the complexity of Cu/Fe interactions, and the role of AtOPT3 in fine-tuning root transcriptional responses to reflect the plants' need for Cu and Fe.

Catechol acetylglucose: A novel benzoxazinoid-regulated defensive metabolite in maize.

Richter Annett, Jander Georg, Negin Boaz
Boyce Thompson Institute, Ithaca, NY, 14853,USA

An enormous diversity of specialized metabolites is produced in the plant kingdom, with each individual plant synthesizing thousands of these compounds. Previous research showed that benzoxazinoids, the most abundant class of specialized metabolites in maize, also regulate the production callose as a defense response. In this study, we identified catechol acetylglucose (CAG) as a benzoxazinoid-regulated metabolite that is produced from salicylic acid via catechol and catechol-glucoside. Genome wide association studies of CAG abundance identified a gene encoding a predicted acetyltransferase. Knockout of this gene resulted in maize plants that lack CAG and over-accumulate catechol-glucoside. Upon tissue disruption, CAG breaks down to produce catechol, which inhibits Spodoptera frugiperda (fall armyworm) growth. Analysis of caterpillar frass showed that S. frugiperda detoxifies catechol by glycosylation, and the efficiency of catechol glycosylation was correlated with S. frugiperda growth on a catechol-containing diet. Thus, the success of S. frugiperda as an agricultural pest may depend partly on its ability to detoxify catechol, which is produced as a defensive metabolite by maize plants.

Development of Molecular Markers for Discrimination Between Cynanchum wilfordii and Its Close Relatives

Gabriella Ballestas, Michelle Yoo
Department of Biology, Clarkson University, Potsdam, NY 13699

The development of species-specific markers can aid in the identification and authentication of herbal products, ensuring their safety and efficacy in traditional medicine practices. Cynanchum wilfordii is an important medicinal plant in both Korean and Chinese traditional herbal medicine. In particular, the root of C. wilfordii has been used for a wide array of medicinal purposes, but the root tuber of C. auriculatum or C. boudieri is also sold under the same name due to their similar morphology and their relative ease of cultivation. To monitor and prevent adulteration, several molecular markers have been developed to differentiate these two species. However, most of them are derived from plastid DNA because of their high copy numbers as well as a single gene-like feature. While this method has proven to be useful, those markers may not be appropriate for differentiating C. wilfordii from other closely related Cynanchum species and identifying any hybrids between C. wilfordii and its close relatives, which may be sold in the market. Therefore, in this study, we aim to develop species-specific nuclear markers to differentiate C. wilfordii from its close relatives. We extracted DNA from multiple accessions of C. wilfordii and other Cynanchum species, such as C. boudieri, C. bungei, and C. auriculatum, and tested the effectiveness of two plastids (rps2, trnQ) and several nuclear markers designed to be specific to C. wilfordii or C. boudieri. Both plastid markers were able to differentiate the two species effectively, with different amplicon sizes for rps2 and trnQ. Also, nuclear markers can differentiate C. wilfordii from its close relatives based on amplicon sizes. This study highlights the importance of using proper molecular markers to prevent adulteration in the herbal medicine market and the need for further research in developing more effective markers.

Determining the Diurnal Cues Regulating CELLULOSE SYNTHASE A8 Expression in Brachypodium distachyon.

Greg Gregory[1,2], Joshua H. Coomey[1,2], Kira A. Gardner[1], David Follette[3], Samuel P. Hazen[1,2]
[1]Biology Department, [2]Plant Biology Graduate Program, [3]Institute for Applied Life Sciences, University of Massachusetts, Amherst, MA 01003, USA.

Cellulose is the predominant polymer in plant cells and is the most abundant biopolymer on earth. Cellulose is synthesized at the plasma membrane by multi-protein complexes that include CELLULOSE SYNTHASE A (CESA) proteins. In both the cereal model Brachypodium distachyon and Arabidopsis thaliana, three functionally similar enzymes, namely CESA4/7/8, operate within these complexes. Expression of these CESA genes is highly correlated with other secondary-wall-associated genes and is extremely high in developing stems as they thicken. In the developmental process, cells in stems undergo division, elongation, and subsequent thickening. In eudicots, elongation is circadian, but in B. distachyon elongation rate is controlled by temperature alone. Our understanding of CESA expression dynamics is limited to quantification of mRNA from destructively harvested tissues, which captures a single developmental stage and often lacks fine temporal and spatial resolution. To overcome this limitation, we developed a real-time bioluminescent reporter imaging system using the firefly Luciferase (LUC) gene to quantify transcription throughout the lifecycle of a plant. Through multi-day time-lapse imaging experiments, we visualized the expression of CESA8::LUC across diurnal light and temperature cycles. Additionally, we have performed analogous time-lapse experiments, manipulating thermocycles, photocycles, or both, to discern the cues regulating daily expression patterns of CESA8. The results suggest that temperature and not light or the circadian clock influences the expression of CESA8::LUC. We hypothesize that alterations in temperature impact the elongation rate of B. distachyon, thereby affecting secondary cell wall thickening and consequently, the expression of CESA8.

A Gene Regulatory Network for Secondary Wall Biosynthesis in the Leaf Nodal Roots of Brachypodium distachyon

Bahman Khahani[1], Ian W McCahill[1], Greg Gregory[1], Sophia Rinaldi[1], Kirk J-M Mackinnon[2], Baumgart, Leo[3], Ronan O'Malley[3], Samuel P Hazen[1]
[1]Biology Department, Plant Biology Graduate Program, University of Massachusetts, Amherst, MA 01003, USA
[2]Biology Department, Molecular and Cellular Biology Graduate Program, University of Massachusetts, Amherst, MA 01003, USA
[3]U.S. Department of Energy, Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

Grasses develop a root system originating from the nodes at the base of the plant, known as leaf nodal roots. This region serves a crucial role in anchoring the plant in the soil that is reinforced by the deposition of secondary cell walls. Additionally, leaf nodal roots undergo wall thickening in response to mechanical stimuli. The synthesis of secondary cell walls is governed by a complex regulatory network involving various transcription factors, including NAC, MYB, and TALE families. To gain deeper insights into the regulation of Brachypodium distachyon leaf nodal roots, we constructed a gene regulatory network by integrating protein-DNA interactions of four key transcription factors using DNA affinity purification sequencing. We investigated the expression of genes in both distal and proximal regions of the roots, as well as under mechanical stimulus. While we observed similarities in the binding site regions of the studied proteins compared to their orthologs in Arabidopsis thaliana, the target genes were mostly different. This indicates distinct regulatory mechanisms governing cell wall synthesis in these species. The transcription factor proteins activated the expression of genes primarily in the proximal zone and under mechanical stimulus, compared to the distal and proximal zones, respectively. The gene regulatory networks suggest that NAC proteins regulate secondary cell wall synthesis by directly regulating MYB transcription factors as well as wall biosynthesis genes. Our findings underscore the complexity of the regulatory network orchestrating cell wall synthesis in leaf nodal roots and highlight the intricate interactions between proteins and target genes, regulating pathways associated with lignin and polysaccharide synthesis.

Quantitative imaging of Pi using FRET sensors in B.distachyon roots reveals diverse Pi distribution and fluctuation during AM symbiosis

Shiqi Zhang[1,2], Lena M. Muller[1], Dierdra A. Daniels[1], Lucas Jurgensen[1], Sergey Ivanov[1], Wayne K. Versaw[3], Maria J. Harrison[1]
[1]Boyce Thompson Institute, Ithaca, NY, 14853,USA
[2]East Stoudsburg University, PA
[3]Department of Biology, Texas A&M University, College Station, TX 77843, USA

In many soils, limited plant-accessible phosphate (Pi) levels constrain plant growth. To overcome this, many plants establish symbiotic associations with arbuscular mycorrhizal (AM) fungi, thereby obtaining Pi from the fungal symbiont. This relationship alters root cell metabolism and physiology, particularly the pathways of Pi entry into the roots. The impact of AM symbiosis on the Pi content and response dynamics of root cells, however, remains to be fully understood. Utilizing fluorescence resonance energy transfer (FRET)-based Pi biosensors, we examined the cytosolic and plastidic Pi levels within intact mycorrhizal roots of Brachypodium distachyon. We discovered that colonized cells possess a higher cytosolic Pi content compared to non-colonized cortical and epidermal cells. Cells at the infection front demonstrated the highest plastidic Pi content. Application of Pi to the mycorrhizal root system resulted in transient cytosolic Pi alterations that varied based on cell type and arbuscule status. Further, applying Pi to extraradical hyphae induced measurable cytosolic Pi changes in colonized cells, noticeable 18 hours after application. These findings reveal how AM fungal colonization affects the intracellular Pi homeostasis of host root cells, emphasizing the diversity in Pi content and Pi response dynamics

Poster Abstracts:

P1

The Exogenous Application of Gibberellic Acid Influences Cell Wall Thickening in Brachypodium distachyon

Logayn T. Abushal[1,2]*, Ian W. McCahill[1,2], Cassandra F. Probert[1], Edward Li[1], Samuel P. Hazen[1,2]
[1]Biology Department, 2Plant Biology Graduate Program, University of Massachusetts, Amherst, MA 01003, USA

Gibberellic acid (GA) is a phytohormone known for its pivotal role in regulating plant growth and development. In addition to that, GA has emerged as a key factor in enhancing plant resilience to abiotic stresses which includes mechanical stimulation. Exposure to mechanical stress can lead to lodging, characterized by the destabilization of root anchorage. Plants respond to mechanical stress through sensory pathways, triggering significant cellular alterations and morphological changes aimed at bolstering structural integrity and stress resilience. These morphological changes are regulated by plant hormones and increase structural integrity and resilience to further mechanical perturbation. Unraveling the precise hormonal mechanisms governing these adaptations is essential for understanding plant reactions to mechanical stress. To explore the role of GA in mediating plant responses to mechanical stress, we investigated utilizing hydroponically grown Brachypodium distachyon plants as a model system. By subjecting plants to exogenous applications of GA, we aimed to elucidate the influence of the hormone on secondary cell wall development, specifically focusing on the proximal part of leaf nodal root sections. Our results revealed a notable increase in lignin deposition and cell wall thickness within the vascular cylinder and the first two cortex layers of leaf nodal root sections in GA-treated plants compared to control counterparts. These observations suggest a pivotal role for GA in promoting secondary cell wall development in grasses. Moreover, our study underscores the imperative for further examination to unravel the intricate regulatory networks governing GA-mediated responses to mechanical stress.

P2

Characterization of Rice OsNIP1;1 for its Role in Arsenic Transport in Indian Mustard (Brassica juncea)

Dylan Ashe, Sudesh Chhikara, Aditi Mankodi, Dr. Om Parkash Dhankher
Stockbridge School of Agriculture, University of Massachusetts Amherst, MA 01003

Arsenic (As) is a naturally occurring toxic element that is present throughout the Earth's crust. There are two general categories of arsenic: organic and inorganic. Inorganic As species, arsenite (AsIII) and arsenate (AsV), are the most prevalent forms of As in the environment. Long term exposure to inorganic As often results in various deleterious health effects including hyperkeratosis, diabetes, cardiovascular diseases, and cancers. Although As is not an essential nutrient for plant growth, arsenite and arsenate are chemically analogous to two essential plant nutrients, silicate and phosphate, respectively. Consequently, As is absorbed via corresponding silicon and phosphorus ion protein channels and accumulated within plant cells. In particular, the rice aquaporin protein OsNIP1;1, categorized in the Nodulin 26-like Intrinsic Protein (NIP) subfamily, has been observed to transport water as well as boron and silicon, and therefore arsenic. The purpose of this research is to determine the role of rice aquaporin protein OsNIP1;1 in As tolerance/sensitivity as well as the transport of As in Brassica juncea plants. Brassica juncea was chosen for the overexpression of OsNIP1;1 as B. juncea is widely implemented in the phytoremediation of arsenic due to fast growth, adequate mature biomass, accumulation of and tolerance to metalloids, and wide growth conditions. Overexpression of OsNIP1;1 in B. juncea resulted in As sensitivity within transgenic lines, demonstrating significant decreases in biomass and significant As translocation from roots to shoots, and thus significant increases in total As accumulation in the aboveground tissues compared to wild type (WT) plants. As such, our results indicate that OsNIP1;1 facilitates As uptake and transportation, indicating potential uses of this protein for the developing plants for the phytoremediation of As contaminated soils and water.

P3

Phenotypic Plasticity, Morphogenesis, and Cell Expansion: Experimental Manipulation of Penium margaritaceum

Kaylee Bagdan, Josie LoRicco, Stuart Malone, Abigail Becker,Nichole Xue,Anika Eastman, Gabriel Sgambettera, Aaron Winegrad, Benjamin Gibeau, Lindsay Bauer, Ruby Epstein, David Domozych
Department of Biology and the Skidmore Microscopy Imaging Center, Skidmore College, Saratoga Springs, NY, 12866, USA

Approximately 500 million years ago, an ancestor of a Charophycean (Streptophyta) green alga successfully invaded a terrestrial habitat and ultimately yielded modern day land plants. Through genomic analyses, Penium margaritaceum, a single celled green alga, is currently recognized as one of the closest living descendant to this divergence. This allows for Penium to be used as a model organism of great evolutionary significance and allows for comparison to both this common ancestor and higher land plants. One feature of this alga that correlates to more complex terrestrial plants include a cell wall primarily composed of pectin and cellulose. These cell wall materials are formed through complex coordination and transportation between organelles and systems including the Golgi apparatus, trans-Golgi network, vesicles, and the cytoskeletal network. Cell walls are areas of high interest due to their ability to respond to growth, maintenance, development, and abiotic and biotic stimuli through phenotypic plasticity. Zones of cell division and cell wall growth tend to be zones of phenotypic plasticity and hence morphogenesis. In higher plants, this has been visualized with root hairs and pollen tube tips. Within Penium, morphogenesis can be visualized at expansion zones, such as one located at its isthmus. At the isthmus within Penium, we see bands of microtubules analogous to the pre-prophase band in higher land plants. This research addresses the morphogenic responses to amiprophos methyl (APM), pectate lyase (PL), and the combination of these biochemical stimuli. Using confocal, scanning electron, and transmission electron microscopy to image Penium's phenotypic plasticity, this research addresses cell wall structural changes and related organelle alteration to better elucidate questions related to higher plant immunity, cell wall integrity, and evolutionary terrestrialization.

P4

Molecular Mechanisms of Cell Type Specification in Grafted Tomato

Elise Boisvert
Cornell University 236 Tower rd. Ithaca, NY 14852

I am working to uncover non-cell autonomous signals that are involved in vascular patterning during graft healing and vascular reconnection, and to determine the role that plasmodesmata plays in signaling across the graft junction. I will use single nucleus sequencing to look for transcriptional profiles at the single-cell level to help reveal putative signals in various cell types. Because I am particularly interested in transitional cell-types, I will sample at different timepoints prior to vascular reconnection to temporally anchor my cell-lineage analysis, and I will use a constitutive fluorescent marker in the rootstock to spatially anchor my analysis in rootstock and scion. Simultaneously, I will use fluorescent imaging to assess the timing and placement of new plasmodesmata between rootstock and scion and look for correlation in timing with the transcription of small peptides and other signaling molecules.

P5

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

Shelby Boulanger *,Valeria Lacouture,Ian W McCahill, Shira Milo,Ludmila Tyler, Ana Caicedo, Li-Jun Ma, Samuel Hazen
University of Massachusetts Amherst, 181 Presidents Dr. Amherst, MA 01003

Interactions between plants and microbes can lead to the mutual benefit of both organisms. 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. By exploiting accessible openings, it is able to infiltrate roots of plants such as Arabidopsis thaliana and Solanum lycopersicum. While capable of colonizing the root interior, F. oxysporum Fo47 does not penetrate the vascular system, therefore having a neutral – or beneficial – effect on its hosts. 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 the interactions between F. oxysporum Fo47 and Brachypodium distachyon roots and found that the presence of Fo47 caused an increase in above ground plant area, dry mass, and seed mass. The degree of response differed among B. distachyon accessions, indicating that genetic variation within this grass species affects its interactions with 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.

P6

Exploring Antifungal Activity of Steroidal Glycoalkaloids in Wild Tomato Species from Roots to Leaves

Trey Bourassa[1]*, Maryam Rashidzade[2], Ana Caicedo[3]
[1]Plant & Soil Science, [2]Graduate Program Plant Biology, [3]Plant Biology Department
University of Massachusetts Amherst 240 Thatcher Rd Amherst, MA

Because plants are stationary, they have evolved multiple mechanisms to defend themselves from pathogens and herbivores. Tomatoes and other species in the Solanaceae family produce a class of secondary metabolites known as Steroidal Glycoalkaloids (SGAs), which are believed to act as defense compounds against fungal pathogens. However, effects of wild plant derived SGAs on pathogenic fungi have not been well documented, and the precise role that SGA variation plays in defense efficacy is unknown. Furthering our knowledge of wild plant defenses could provide insight into potential applications of these traits in agricultural systems. In a previous study, we extracted SGAs from the leaves of 13 wild tomato species, and documented a range of antifungal activity against the specialist pathogen Fusarium oxysporum f.sp. lycopersici MN25 (FOL) using in-vitro assays. A targeted metabolomics analysis revealed that alpha-tomatine and dehydrotomatine exhibited the highest inhibitory effects on FOL, and their amounts varied across tomato species. However, as a soil pathogen, initial interactions between FOL and tomatoes occur in roots and stems. We thus examined whether stem and root extracts of wild tomatoes contain sufficient SGAs to impede FOL growth. We selected three species (Solanum lycopersicum, S. habrochaites, and S. galapagense) to extract SGAs from stem and root tissues, and conducted in vitro inhibition assays. Our results so far reveal no significant differences in mycelium growth between the stem and root treatments compared to our negative control, suggesting an absence of effective defense in these tissues. For future research, we aim to quantify the SGA content in stem and root extracts through targeted metabolomics.

P7

Cellulose Synthase-Like D Participation in Cell Plate Formation During Cytokinesis in Physcomitrium patens

Lia Bozza, Ryan Hennessey, Alison Roberts
University of Rhode Island, 45 Upper College Road, Kingston, RI 02881

Plant cell walls are made of cellulose and other polysaccharides that give cells structure and diverse shapes. Cellulose Synthase-like D (CSLD) is a cellulose synthase known to participate in cell plate formation. In the moss model, Physcomitrium patens, 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, cell separations, and incomplete midrib formation. When these mutants were grown on ammonium-free medium to promote gametophore development, the severity of the phenotype was more extreme, including incompletely formed cell plates and multinucleated cells. The P. patens genome encodes eight CSLDs. If any of the other six CSLDs are involved in cytokinesis in gametophores, we expect that knocking out the corresponding genes will increase the severity of the phenotype on both media. To test this, we transformed a csld2/6KO line with a CRISPR-Cas9 vector targeting two sites within CSLD1 to create a deletion. Colonies selected for transient antibiotic resistance were PCR genotyped to identify lines with deletions. The PCR products were sequenced to confirm that the deletions resulted in frameshifts. Four confirmed csld2/6/1KO lines were grown with and without ammonium to compare the phenotypes with the csld2/6KO lines. The cslsd2/6/1KO mutants had no phenotypic differences compared to csld2/6KO on standard or ammonium-free media. Additional P. patens csldKOs will be created and tested for phenotype severity to determine which CSLD genes are involved in gametophore cytokinesis. We are also investigating the role of ammonium in moderating the csld2/6KO cytokinesis phenotype.

P8

Evaluating Nitrate Tolerance in Male Sterile Sorghum Lines for implications for safe forage Production

Janeen Braynen[1], Sunita Kumari[1], Michael Regulski[1], Andrew Olson[1], William L. Rooney[2], Robert Klein[3], Nicholas Boerman[4], Doreen Ware[1,5]
[1]Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724
[2]Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas, USA
[3]2881 F&B Road College Station, Texas 77845
[4]USDA-ARS, Livestock, Forage, and Pasture Management Research Unit, Woodward, OK 73801
[5]USDA-ARS-NAA, Ithaca, NY 14850

Sorghum serves as a vital forage crop within varied farming systems, where nitrogen's role is key to both crop yield and plant vitality. To enhance crop yields and avert nitrogen shortages, farming methods often lean on heavy nitrogen fertilizer application. This practice, however, leads to notable environmental concerns. Excess nitrogen, surpassing plant uptake capabilities, can infiltrate water bodies, instigating eutrophication and harming aquatic ecosystems. Moreover, residual nitrogen in soil can lead to nitrate accumulation in plants, raising concerns over nitrate poisoning, which can be deadly for ruminant animals consuming this forage. In light of this, a study by Ralston, et al., 2023, revealed varying nitrate levels in 20 sorghum inbred lines, exploring genotypic differences in nitrate storage among these lines. Our study progresses Ralston, et al. 's research by investigating four specific male sterile sorghum A-lines, chosen for their diverse nitrate content profiles, alongside BTx623 as the benchmark genome. The research methodology included growing more than 50 plants from the four A-lines (ATx645, ATx3408, A.11022, and A.07258bst) and BTx623, both in hydroponic and sand-based mediums in a greenhouse, under both high (20mM) and standard (1mM) ammonium nitrate levels, to mimic varied nitrate conditions. Through sequential transcriptome analysis of hydroponically grown samples, a notable variance was observed in gene expression under high nitrate conditions, especially in genes related to nitrate reduction and photosynthesis, exhibiting distinct and consistent trends among lines with different historical nitrate accumulation rates. These results confirm significant genetic diversity among the studied sorghum lines for breeding purposes, aiming to reduce leaf nitrate levels below the toxicity threshold of 10,000 μg g−1, while also improving overall crop productivity.

P9

Genome-wide Response to Iron Availability and Identification of Leaf-level Metal Acclimation Factors in Four Sorghum Carbon Partitioning NAM Populations.

Janeen Braynen[1], Sunita Kumari[1], Michael Regulski[1], Aditi Bhat[2], Dimitru Tedesse[2], Tim Paape[3,4], Meng Xie[2], Doreen Ware[1,5]
1 Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724
2 Brookhaven National Laboratory, Upton, NY
3 Institute For Advancing Health Through Agriculture, Texas A&M, College Station, TX
4 USDA-ARS Children's Nutrition Research Center, Huston, TX
5 USDA-ARS-NEA, Ithaca, NY 14850.

Iron (Fe) is essential for photosynthesis, respiration, and chlorophyll production in plants. Its uptake involves a network of transporters and chelators, optimizing transport from soil to tissue and preventing toxic buildup. This study explores the impact of Fe limitation and excess on growth and resilience in four Sorghum Carbon Partitioning Nested Association Mapping (CP-NAM) populations (Grassl, Leoti, Pink Kafir, 1S13633) and the reference genome (BTx623) in controlled hydroponic chambers. We used RNA-seq to identify gene expression patterns and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to measure metal concentrations, providing insights into micronutrient interactions and carbon allocation. Chlorosis was noted under Fe stress at 7 and 14 days in all lines except Leoti, which remained green. ICP-MS revealed variability in elements like Ca44, S34, Na2, and B11, with significant differences in Fe54 concentrations between the observed time points under low Fe conditions. Time-series RNA-seq shows that specific gene clusters are critical for adapting to Fe variations, effectively directing carbon to essential functions under stress. These findings inform the genetic basis of micronutrient utilization and have implications for developing crop varieties with better growth and yield in Fe-deficient soils. Targeted breeding could enhance iron-use efficiency and carbon distribution, addressing challenges in nutrient-depleted environments. This project was funded by the USDA-CRIS, award number 8062-21000-044-000D.

P10

Generating an Epitope-Tagged RAP2.6L in Arabidopsis thaliana to Investigate Its Role in Wounding and Defense Pathways

Lauren Cardarelli, Patricia Vequetini, Alison Palasek, Heather Marella
Bridgewater State University, 131 Summer St, Bridgewater, MA 02325

Parasitic root-knot nematodes (Meloidogyne incognita; RKN) damage the vascular tissue of plant roots causing ~100 billion dollars of crop loss across the world every year. The disruption in the root system can cause increased susceptibility to disease and damage the delivery system of water and nutrients to the rest of the plant. To further understand how RKN manipulate their plant hosts, I am studying the gene RAP2.6L in Arabidopsis thaliana, which encodes a transcription factor known for wound healing and defense responses in plants. RAP2.6L, a member of the AP2/ERF protein family, is encoded by At5g13330 and localizes in the nucleus. It is expressed in the presence of stressors like salt, drought, and ethylene and jasmonic acid treatments. RAP2.6L expression is induced by the RKN. In fact, the expression of RAP2.6L was found to be in the giant cells created by the RKN as determined by microarray analysis of laser-captured RNA from giant cells. The rap2.6l-1 mutant, a knock-out mutant of the gene, supports fewer adult female nematodes and produces more adult male nematodes. However, the genes regulated by RAP2.6L have not yet been identified and so we lack a clear picture of the gene expression network regulated through RAP2.6L. Lacking expedient and inexpensive options to study the pathway, I have been working to incorporate epitope tagged RAP2.6L into the genome of Arabidopsis by creating a transgenic plant line which will be an important tool that will allow us to determine which genes RAP2.6L regulates. The resulting transgenic line can be further used for Chromatin Immunoprecipitation (ChIP) to determine gene targets of RAP2.6L.

P11

Enhancing RuBisCO Kinetics and Thermal Tolerance in Solanaceae Crops: Use of a Novel Genotype to Study Unique RuBisCO Isotypes in planta.

Vishal Chaudhari, Maureen Hanson
Dept. of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

RuBisCO plays a pivotal role in the productivity of terrestrial plants by catalyzing the fixation of carbon dioxide. RuBisCO's slow reaction rate and its propensity to inadvertently fix oxygen instead, significantly impede carbon assimilation, particularly in C3 plants. For years, scientists have been striving to enhance RuBisCO's selectivity and efficiency. However, progress has been slow due to the complex nature of its structural determinants, its dependence on a suite of chaperone proteins, and the challenges associated with in-vivo testing of genetic alterations. Using recent advancements in assembling RuBisCO in E. coli, we evaluated hypothetical ancestral rubiscos for enhanced kinetics and identified promising candidate pairs of large and small subunits for Solanaceae. These candidates showed better kinetic profiles and improved thermal tolerance. To evaluate these candidates in planta, we developed a tobacco plant platform NOR (no other rubiscos) devoid of native large and 11 small rubisco subunits, through a series of CRISPR knockouts and chloroplast transformations. This platform offers a unique opportunity to study RuBisCO's kinetics in-planta, one isotype at a time, without the influence of the wild-type background. Subsequently, we generated several plants expressing single isotypes including ancestral forms. These plants showed robust photosynthesis and expression of RuBisCO. Our research provides tools to significantly improve the efficiency and productivity of crops through optimizing RuBisCOs.

P12

The role of the Golden2-like (GLK) transcription factor in regulating terpenoid indole alkaloid biosynthesis in Catharanthus roseus

Lauren Cole-Osborn*, Shannon McCallan, Olga Prifti, Rafay Abu, Virginie Sjoelund, Carolyn Lee-Parsons
Northeastern University, Boston, MA

Catharanthus roseus is the sole source of the chemotherapeutic terpenoid indole alkaloids (TIAs) vinblastine and vincristine. TIA pathway genes, particularly genes in the vindoline pathway, are expressed at higher levels in immature versus mature leaves, but the molecular mechanisms responsible for this developmental regulation are unknown. We investigated the role of GOLDEN2-LIKE (GLK) transcription factors in contributing to this ontogenetic regulation since GLKs are active in seedlings upon light exposure and in the leaf's early development, but their activity is repressed as leaves age and senesce. We identified a GLK homologue in C. roseus and functionally characterized its role in regulating TIA biosynthesis, with a focus on the vindoline pathway, by transiently reducing its expression through two separate methods: virus-induced gene silencing (VIGS) and application of chloroplast retrograde signaling inducers, norflurazon and lincomycin. Reducing CrGLK levels with each method reduced chlorophyll accumulation and the expression of the light harvesting complex subunit (LHCB2.2), confirming its functional homology with GLKs in other plant species. In contrast, reducing CrGLK via VIGS or lincomycin increased TIA accumulation and TIA pathway gene expression, suggesting that CrGLK may repress TIA biosynthesis. However, norflurazon had no effect on TIA gene expression, indicating that reducing CrGLK alone is not sufficient to induce TIA biosynthesis. Future work is needed to clarify the specific molecular mechanisms leading to increased TIA biosynthesis with CrGLK silencing. This is the first identification and characterization of GLK in C. roseus and the first investigation of how chloroplast retrograde signaling might regulate TIA biosynthesis.

P13

Reducing Energy Requirements in Controlled Environment Agriculture through Pulsed Lighting Sequences

Lily Donaldson, Robert Karlicek, Elsebeth Kolmos,
Rensselaer Polytechnic Institute, 110 8th St, Troy, NY 12180

Controlled Environment Agriculture (CEA) research aims to make growing plants in the built environment more efficient, hardier, and more appealing to consumers than traditional open-field agriculture, but vertical farming has a much larger carbon footprint than open-field agriculture, with electric lighting accounting for two-thirds of energy use. There are many use-cases, however, where CEA may be necessary to feed the local population, including in remote locations and in areas where climate-related plant stressors inhibit outdoor crop growth. In addition, CEA use-cases often have strict energy availability. With electric lighting being the highest energy user in CEA, there exists the largest opportunity for energy use reduction. Horticultural lighting quantity and quality is incredibly important to a plant's ability to thrive, but delivery of too little or too much light and degraded spectral quality can cause plant stress responses and morphological changes. The goal of a CEA environment's lighting system then is to use the least amount of light (and therefore energy) possible to achieve the desired growth outcomes. Light emitting diodes (LEDs) can be spectrally tuned, allowing for finer control of spectrum-based photomorphogenesis and increased spectral efficacy of fixtures, and they allow for easier regulation of light intensity. However, spectral tuning, especially short-term time-based spectral changes, and pulsed lighting (on the order of kilohertz) have been demonstrated to be opportunities for further LED grow-light optimization but are understudied. My thesis research focuses on these two optimization opportunities using modified commercially available horticultural lights, which are color, intensity, and pulsing tunable, and allow for a wide variety of lighting recipes to be rendered to the experiment plant.

P14

Characterization of Transcription Factor Family's Functional Role in Arabidopsis thaliana Primary Root Growth

Kate Dooling*[1], Kai Wang[1], Lifang Zhang[1], Vivek Kumar[1], Audrey Fahey[1], Fangle Hu[1], Kapeel Chougule[1], Doreen Ware[1,2]
[1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
[2]USDA-ARS Robert Holley Center, Ithaca, NY"

Agricultural yield is a multifaceted trait encompassing various traits, including root formation and growth, as well as plant responses to abiotic and biotic stress factors. Such processes generally involve transcription factor-mediated regulation of a wide array of genes. In this preliminary study, 167 Arabidopsis thaliana germplasms with mutated genes in various transcription factor (TF) families were screened to determine their role in primary root growth. It was hypothesized that loss of function mutant genes expressed in the roots might exhibit distinct root phenotypes. Amplified seeds from each of the 167 germplasms obtained from the Salk Institute's T-DNA Insertional Mutant Collection were plated on standard media and grown for 7 days. Plates were then scanned using a high-resolution flatbed scanner and roots were traced to measure length based on a semi-automated approach using SmartRoot, a tool in ImageJ. All germplasms were also screened for phenotypic consistency based on primary root length and assumed homozygosity of given TF alleles. Out of all phenotypically consistent germplasms, 5 mutated genes produced short root phenotypes and 14 produced long root phenotypes. The 19 genes that produced extreme root length phenotypes may potentially be valuable in crop species. To investigate this, we identified which of the 19 genes in Arabidopsis had orthologs present in Sorghum. These orthologs were then reviewed in SorghumBase and curated to identify conservation among predicted protein sequence models. The presence of conservation between predicted protein models can potentially contribute to future investigation of the significance of TF families in important agricultural crops. Funding: USDA-CRIS award number 8062-21000-044-000D.

P15

Binding Site Preferences of Sorghum GRAS Transcription Factors Across Monocots

Audrey Fahey[1], Sunita Kumari[1], Michael Regulski[1], Doreen Ware[1,2], Nicholas Gladman[1,2]
[1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
[2]USDA-ARS Robert Holley Center, Ithaca, NY

The GRAS transcription factor (TF) family is a plant-specific TF family that is heavily involved in plant inflorescence development and root architecture. The effect of these TFs has been characterized more in Arabidopsis, and less so in Sorghum bicolor and other monocots. In order to address this, three representative sorghum GRAS TFs (SHORT ROOT, SCARECROW-LIKE 3, and SCARECROW-LIKE 23) were characterized using DNA affinity purification and sequencing (DAP-seq). To do this, each TF precipitated genomic DNA fragments from Sorghum bicolor, Zea mays, and Oryza sativa. The precipitated fragments were sequenced, then data processed to determine the TF binding sites in each background. This has generated the first-ever DAP-seq profile of these TFs in sorghum and produced data for cross-species comparison. Promoters with high GRAS TF binding hits were often associated with genes involved in amino acid processing, stress response, and potential root architecture development. Motif analysis of novel and unique DNA motifs showed a unique recognition motif signature for each TF. We identified putative GRAS-specific DNA binding motifs that were over-represented in promoter sequences of protein coding genes. Then, we confirmed them as real motifs through frequency evaluation across all promoter regions in sorghum, maize, and rice. They were unique compared to other well-known TF family recognition motifs like WRKY and NAC. We combined these GRAS binding profiles with epigenetic data to further curate potential regulatory non-coding target sequences that could be candidates for CRISPR editing for downstream functional characterization and agronomic trait generation. This project was funded by the USDA-ARS award number 8062-21000-044-000D.

P16

An aphid salivary protein interacts with the plant autophagy pathway.

Leila Feiz, Navid Movahed, Georg Jander
Boyce Thompson Institute, Ithaca NY

Various phytopathogens have evolved specialized effector proteins to manipulate plant autophagy by targeting its core proteins and diverting their functions to serve the pathogen. Aphids are sap-sucking pests whose interactions with plants involve a wide array of mostly unknown small molecules, proteins, and signaling pathways, constituting a dynamic co-evolutionary battle between these insect pests and their host plants. A proteome profiling of the aphid saliva led to the identification of an effector protein, Sp11, which elicits cell death reminiscent of plant responses that bacterial effectors induce. Subcellular localization investigations revealed that Sp11 exhibits colocalization with plant autophagosome and pre-autophagosomal structure (PAS) marker proteins, ATG8 and ATG9, respectively, within PAS punctate structures. Co-immuno precipitation of Sp11 with its putative plant partners, followed by identifying candidates by mass spectrometry, revealed its binding affinity towards small GTP-binding proteins, RabD1 and RabD2a. In Arabidopsis, RabD2a plays a regulatory role in both vesicle-mediated transport between the endoplasmic reticulum and Golgi apparatus and autophagosome biogenesis—subsequent reciprocal co-immunoprecipitations and immunoblotting validated these interactions. Additional investigations demonstrated that the C-terminal RAB-binding domain of Sp11 is crucial for its association with Rabs and its induction of cell death in plant cells. We demonstrate that Sp11, through its binding to Rab, facilitates lipid recruitment to PAS and initiates autophagy. Sp11 represents the first aphid effector discovered to influence plant autophagy, thereby enhancing aphid survival and reproduction.

P17

Monitoring the Effect of Arabidopsis Growth on Aerial CO2 Concentration Using an Aranet4 CO2 Monitor

Samuel Griffin, Xiao-Ning Zhang
St. Bonaventure University 3261 W State St

From 1979 to 2022, the average CO2 concentration has increased from 339 ppm to 417 ppm, or 20%, largely due to human activities. The increased CO2 concentration is responsible for trapping heat in the atmosphere and increasing global temperatures. Plants play an important role in regulating the concentration of CO2. During the day when light is available, plants undergo photosynthesis and cellular respiration, taking in CO2 at a greater rate than they release it. However, during the night when light is absent, photosynthesis turns off and plants only undergo cellular respiration, releasing CO2. The purpose of this experiment is to set a baseline for how the aerial day and night concentration of CO2 at parts per million (ppm) in a Percival AR41L2 growth chamber is affected as Arabidopsis plants grow. We placed 4 flats of Arabidopsis plants in a Percival AR41L2 growth chamber and recorded the concentration of CO2 every 5 minutes for 5 weeks using an Aranet4 CO2 monitor. The growth chamber was set at a 12-hour day/night cycle and kept at 22°C and 60% humidity. For each day, we found that the aerial CO2 concentration decreased during the day and increased during the night, while the overall daily difference between the aerial CO2 concentration increased as the plants grew from juveniles to adults. The Aranet4 CO2 monitor was sensitive enough to detect the CO2 fluctuations due to chamber access and rhythmic shift when Day Light Saving occurred. This experiment provides a proof of concept for an economic experimental setup to study how different genotypes and environmental conditions may affect plant's ability to alleviate the increasing CO2 level in the air.

P18

Serine/Arginine-rich 45 Promotes Arabidopsis thaliana Reproduction by Upregulating DAZ1 and Alternative Splicing of RAD4

Iesh Gujral, Arden Bui, Xiao-Ning Zhang
St. Bonaventure University 3261 W. State Rd., St. Bonaventure, NY USA 14778

In flowering plants, pollen grains are the male gametophyte that produces sperms. Arabidopsis Serine/Arginine-rich 45 (SR45) is an evolutionarily conserved RNA-binding protein that regulates transcription and pre-mRNA splicing. It is highly expressed during pollen development. The sr45-1 null mutant exhibits mild sterility and a reduced seed set. To investigate SR45's regulatory role in reproduction, sperm cells were isolated from wild-type (Col-0) and sr45-1 mutant mature pollen using FACS. The bulk sperm transcriptome was sequenced and compared between the genotypes. RNA-seq data analyses suggest that SR45 promotes gene expression in pollen tube development. Comparisons of sperm markers in Col-0 and sr45-1 background showed significantly lower protein abundance of sperm-specific transcription factor DUOI-Activated Zinc Finger 1 (DAZ1-mCherry) in sr45-1. Since DAZ1 promotes G2/M transition, SR45 likely upregulates DAZ1 to promote cell cycle progression in pollen. 3D RNA-seq analysis also identified a list of differential alternatively spliced isoform switch events. Among genes exhibiting significant isoform switches, a DNA repair gene RAD4 was selected to explore isoform-specific expression and functional differences. Predicted protein structures indicate that the isoform that is significantly highly expressed in sr45-1 (RAD4.6) has a longer RAD4 domain. It's C-terminal coiled-coil domain is missing in the other isoform (RAD4.2). Preliminary comparisons of RAD4.6-GFP in Col-0 and sr45-1 background suggest visible differences in expression. Future studies should investigate possible functional differences between the two RAD4 isoforms.

P19

Long-distance intercellular communication: deciphering signals from the noise using interspecies grafting.

Michelle Heeney[1,2], Muhammad Rizwan Riaz[3], Elise Boisvert[2], Amy Marshall-Colon[3], Margaret Frank[2], Adrienne Roeder[1,2]

[1]Weill Institute for Cell and Molecular Biology
[2] Section of Plant Biology, School of Integrative Plant Sciences, Cornell University, Ithaca, NY 14853, USA
[3]Department of Plant Biology, University of Illinois Urbana-Champaign, Urbana, Illinois, 61801 USA

Grafting, with its ability to increase yield and stress resilience, benefits many crops, including Cucurbitaceous and Solanaceous crops. Mobile mRNA in grafted plants has been identified in multiple studies and has been shown to move between root and shoot systems over long distances in a bidirectional manner. The identity of these mobile transcripts in addition to the quantity, function, and functional significance, have garnered much debate. We developed a stringent bioinformatic pipeline for mobile mRNA detection in tomato (Solanum lycopersicum) and eggplant (Solanum melongena) heterografts to gain insight into the true quantity and identity of mobile signals. Using exclusive alignment to a false-concatenated eggplant-tomato genome, we identified regions of the genome that, under less stringent conditions, falsely inform our understanding of mobility. Applying this understanding, we observed patterns of genome-wide mRNA exchange in heterografts over diurnal time. From the application of this pipeline, we identified mobile mRNA transcripts— enriched in RNA, DNA, and transport-related functions with no impact on mobility due to diurnal time. Our analysis pipeline provides a unique tool for mobile signal detection that can be used in systems with divergent genomes.

P20

Convergent evolution of one-step methyl anthranilate biosynthesis in sweet orange, grapes, and maize

Michael Fallon[1], Hisham Tadfie[1], Aracely Watson[1], Madeline Dyke[1], Christopher Flores[1],Nathan Cook[1], Zhangjun Fei[2], Cynthia Holland[1]

[1]Williams College, Department of Biology, Williamstown, MA 01267, USA.
[2]Boyce Thompson Institute, Ithaca, NY 14850 USA

Plants synthesize an array of volatile compounds, many of which serve ecological roles in attracting pollinators, deterring herbivores, and communicating with their surroundings. Methyl anthranilate is a volatile derived from an intermediate in tryptophan biosynthesis and is responsible for grape aroma. Its biosynthesis has convergently evolved in several agriculturally relevant plants, including citrus, grapes, and maize. Many angiosperms methylate the plant hormone salicylic acid to produce methyl salicylate, which acts as a plant-to-plant communication molecule, and anthranilate methyltransferases have evolved from salicylic acid methyltransferases. Unlike maize, which uses a one-step anthranilate methyltransferase, grapes have been thought to use a two-step pathway for methyl anthranilate biosynthesis. After identifying additional one-step anthranilate methyltransferases in Citrus sinensis (sweet orange), we hypothesized that grapes may also use a similar strategy. By mining available 'omics' data, we identified two anthranilate methyltransferases in Vitis vinifera (wine grape), as well as one ortholog in 'Concord' grape. Because the citrus enzyme indiscriminately methylated both anthranilate and salicylic acid, we used this enzyme to examine the evolution of anthranilate activity by introducing rational mutations, which identified two residues that increase anthranilate activity. Reversing this approach, we traced the evolution of anthranilate activity in the maize anthranilate methyltransferase by introducing mutations that imparted ancestral activity with salicylic acid, which uncovered different active site residues from those in the citrus enzyme. These data demonstrate the molecular mechanisms underpinning the convergent evolution of anthranilate activity in three plant orders and reveals an additional pathway by which grapes synthesize methyl anthranilate.

P21

Elucidating the Biosynthesis and Structural Diversity of Resin Glycosides – Defense Compounds Specific to Morning Glory (Convolvulaceae) Family

Mohammad Irfan, Lars Kruse, Alexandra Bennett, Gaurav Moghe
Plant Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, USA

Resin glycosides (RGs), a subclass of acylsugars are plant defense compounds specific to Convolvulaceae family. These RGs exhibit cytotoxic properties against herbivores, nematodes, plant and human pathogens. The use of Convolvulaceae plants containing RGs in traditional herbal medicine also suggests their potential in ethnomedicinal practices and natural product-based drug discovery. RGs possess diverse structures, consisting of oligosaccharide core, hydroxyacyl chains, and acyl groups. Phylogenetic assessment of structural diversity of RGs in leaves and roots using LCMS revealed dozens of RGs per species and substantial diversification at organ, species and genus levels. Structural variations include the length and types of sugars, esterified acyl groups, long-chain fatty acids (C14-C18), and site of macrolactone formation. Nevertheless, it is still unclear how RG structural diversity in family Convolvulaceae is formed. In our study, we conducted de novo RNA sequencing of leaf and root samples from two Convolvulaceae species; Ipomoea tricolor, producing RGs in both leaf and root, and Dichondra argentea, lacking RGs in leaves. Transcriptomic analysis revealed hundreds of differentially expressed transcripts between root and leaf of each species. 12 potential BAHD acyltransferases were selected based on differential or high expression in I. tricolor to study their role in acylating the RG core. Through heterologous expression, purification, and assay, ItBAHD5 was identified as enzyme acylating the RG core with linear and branch chain C4 and C5 CoAs specifically butyryl CoA, isobutyryl CoA, 2-methylbutyryl CoA and isovaleryl CoA. In vivo functional validation of ItBAHD5 using VIGS is currently underway to elucidate its role in RG structural diversification. Our study provides molecular insights into biosynthesis of RGs in Convolvulaceae, shedding light on their structural diversity and their significance in agriculture and pharmacological applications.

P22

Characterization of a Putative Biosynthetic Gene Cluster Involved in Pathogen Stress Response in Medicago truncatula

Monirul Islam, Jessie Dorff, Sibongile Mafu
Department of Biochemistry and Molecular Biology, University of Massachusetts-Amherst, Amherst MA 01003, USA.

Plants produce a wide range of distinct metabolites to protect themselves from pests and pathogens, attract pollinators, and communicate with other organisms. MtTPS10 which produces himachalol, was recently shown to improve resistance against a root rot pathogen. Biosynthetic genes for many specialized metabolites are arranged in biosynthetic gene clusters (BGCs), which facilitate their co-inheritance and regulation. Here, we report the identification and functional characterization of a putative sesquiterpenoid cluster consisting of two known terpene synthases MtTPS10 and MtTPS11, which produce himachalol and longicyclene respectively and three full- length cytochromes P450 colocalized within a fifty kilobase pair region. Combinatorial pathway reconstruction in a modular microbial engineering system revealed that the P450s specifically reacted with himachalol resulting in oxygenated downstream metabolites at distinct positions, C4-hydroxylation and an aldehyde at the C15 position. To assess a potential stress response role, we evaluated gene expression of these putative genes after treatment with chemical elicitors methyl jasmonate, salicylic acid, or copper as oxidative stress. The genes display inducible transcription in response elicitation with salicylic acid and copper sulfate suggesting a role in pathogen and abiotic stress.

P23

CRISPR-Cas9 Editing and Agrobacterium-Mediated in Planta Transformation of the Medicinal Plant Catharanthus roseus

Molly Johnson[1], Emma Meehan[2], Lauren Cole-Osborn[2], Natalie Soens[2], Carolyn Lee-Parsons[1,2,3]
[1]Department of Bioengineering, [2]Chemical Engineering, and [3]Chemistry & Chemical Biology
Northeastern University 360 Huntington Ave, Boston, MA 02115

The Madagascar periwinkle plant, scientifically known as Catharanthus roseus, produces the anti-cancer drugs vinblastine and vincristine through a complex biosynthetic pathway. These drugs are produced at a very low natural abundance, making their industrial production and availability challenging. Towards engineering their increased abundance, we are utilizing the Agrobacterium-mediated in planta transformation method to introduce CRISPR-Cas9 mutations and yield transgenic C. roseus plants. In contrast to commonly used methods for stable transformation followed by regeneration, the Agrobacterium-mediated in planta transformation method has a shorter timeline and higher regeneration success. We cloned a plasmid encoding CRISPR-Cas9 to mutate the ChLH gene in the chlorophyll biosynthetic pathway, which will result in leaves with a white phenotype if the transformation is successful. This visible reporter gene will be used to evaluate and improve the in planta transformation rate. With the addition of CRISPR-Cas 9 editing, the Agrobacterium-mediated in planta transformation method will enable researchers to create stable, comparatively faster, and targeted edits in the C. roseus genome towards increased production and availability of vinblastine and vincristine.

P24

ATHB2 is a novel component of transcriptional networks regulating copper homeostasis in reproductive organs in Arabidopsis thaliana.

Yana Kavulych, Yan Jiapei,Tetiana-Olena Zavodna, Ju-Chen Chia, Olena Vatamaniuk
Plant Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, 14853 USA

Copper (Cu) is a redox-active micronutrient required for nearly every aspect of the growth and development of plants. However, Cu is also toxic when it accumulates in excess in plant cells. Thus, plants tightly regulate Cu uptake and internal transport to avoid deficiency and preclude toxicity. In Arabidopsis thaliana, Cu homeostasis is controlled by a conserved transcription factor, SPL7 (SQUAMOSA PROMOTER BINDING PROTEIN LIKE7), a homolog of the algal Cu sensor, CRR1 (COPPER RESPONSE REGULATOR1). In our past studies, we identified another regulator of Cu homeostasis, a transcription factor CITF1 (Cu-DEFICIENCY INDUCED TRANSCRIPTION FACTOR1). We found that CITF1 responds transcriptionally to Cu availability and is essential for the growth and development of A. thaliana under Cu deficiency. CITF1 has been considered among the downstream targets of SPL7. However, our functional genetic studies pointed to the existence of a complex SPL7-CITF1-integrative pathway, the disruption of which leads to extreme sensitivity to even small fluctuations in Cu availability and results in seedling lethality and infertility. Further, our data suggested that other transcriptional regulators besides SPL7 mediate the transcriptional response of CITF1 to Cu availability in roots and flowers. Using yeast one-hybrid (Y1H) analyses and chromatin immunoprecipitation assays (ChIP), we identified ATHB2 (Arabidopsis Thaliana Homeobox protein 2), a light-responsive transcription factor, as one of the upstream regulators of Cu-responsive CITF1 expression in flowers. Functional genetic studies of the crosstalk between ATHB2 and the CITF1-SPL7-controlled pathway will be presented, and possible interactions between light and Cu-status signaling will be discussed.

P25

CYP72A9 Modulates Plant Growth in Response to Osmotic Stress

Ellie Kreider*, Luke Rogers, Leeann Thornton
Biology Department, The College of New Jersey, Ewing NJ

Plants regularly experience mild stresses for which they are equipped to adjust gene expression and biochemistry to maintain growth and reproduction. Metabolic tradeoffs between growth and defense allow plants to optimize resource use in response to adverse environmental conditions. Little is known about the subtle changes in secondary metabolites and growth promoting hormones during stress acclimation. Many cytochrome P450 enzymes (CYPs) are induced by environmental stresses to facilitate shifts in metabolism. Plant genome sequencing has revealed the presence of thousands of CYP genes with an average of about 300 genes per plant. The CYP72A subfamily appears to have members in all angiosperms and provides the potential for diverse biochemical functions in each plant species in response to external stresses. CYP72A9 from Arabidopsis acts as a GA hydroxylase, inactivating gibberellins as part of the dormancy process in immature seeds. This hydroxylase activity is also conserved in several other CYPs across the plant world. The gene encoding CYP72A9 is induced by various abiotic stresses, such as heat and osmotic stress. Our work tests the hypothesis that CYP72A9 modulates plant growth in response to environmental stress. We measured plant growth at various stages of development, as well as GA metabolism gene expression, when CYP72A9 mutant and wild type plants are exposed to abiotic stresses. Plants deficient in CYP72A9 are larger than wild type plants even when not exposed to stress due to upregulation of regulatory GA genes. However, this growth difference is exacerbated under stress conditions. These results suggest that under a variety of adverse environmental conditions, CYP72A9 can have an effect on the acclimatization to the stressor(s). Unraveling the stress-induced regulation of gibberellin homeostasis contributes to our understanding of the growth-defense tradeoffs in plant acclimation to adverse conditions.

P26

Plant Microbe Interactions Between Brachypodium distachyon and Fusarium oxysporum Across Seven Accessions

*Valeria Lacouture[1], Shelby E. Boulanger[1,2], Shira Milo[1,2], Ludmila Tyler[2,3], Ana L. Caicedo[1,2], Li-Jun Ma[2,3], Samuel P. Hazen[1,2]
[1]Department of Biology, [2]Plant Biology Graduate Program, [3]Department of Biochemistry and Molecular Biology, University of Massachusetts, Amherst, MA 01003, USA

Plant-microbe interactions form essential alliances crucial for agriculture and environmental sustainability. Particularly noteworthy is the symbiotic relationship with microorganisms like fungi, which fosters mutual benefits, thereby enhancing plant growth, health, and soil fertility. This study aimed to investigate the response of Brachypodium distachyon to Fusarium oxysporum Fo47. B. distachyon serves as a model system for functional genomics in grasses due to its small stature and genome size, rapid life cycle, and its close phylogenetic relation to cereal crops including wheat and barley. These characteristics render it ideal for exploring the intricate dynamics of plant-microbe interactions and their implications for sustainable agriculture. Similarly, Fo47 is nonpathogenic and known to have growth-promoting properties. Following two weeks of greenhouse cultivation, the roots of seven different B. distachyon accessions were inoculated with fungal cultures, while the other half received distilled water. Subsequently, after a four-week post-inoculation period, comprehensive imaging and analysis of the plants revealed a significant increase in above-ground biomass for two accessions, Bd21 and Spa-S6D. Conversely, the remaining accessions did not exhibit a response to the fungus. This study underscores the capacity of F. oxysporum to influence above-ground biomass in B. distachyon in an accession-specific manner. It sheds light on the nuanced interactions between plants and beneficial fungi, emphasizing the importance of understanding the specificity and variability in such relationships for optimizing agricultural practices and fostering sustainability.

P27

Root Exudates & Resistance to Toxic Heavy Metals: Functional Analysis of a Subset Group of Citrate Recognizing Plant MATE Transporters

Patricia Leyva[1] and Miguel Piñeros[1,2]
[1]Department of Plant Biology, Cornell University, Ithaca, NY. 14853
[2]Robert W. Holley Center for Agriculture and Health, USDA Agricultural Research Service, Cornell University, Ithaca, NY. 14853

A unique sub-group of membrane transport proteins belonging to the Multidrug and Toxic - compound Extrusion (MATE) family has gained significant attention as they mediate the root exudation of citrate into the rhizosphere. Citrate chelates and detoxifies phytotoxic aluminum ions (Al^{3+}) in acid soils, thereby providing resistance and preventing it from damaging the growing root. The molecular and structural properties underlying citrate binding (i.e., substrate recognition) and subsequent transport remain unknown. Protein sequence alignments of functionally characterized plant MATEs exhibit significant sequence differences (i.e., domains) between citrate-transporting and non-citrate-transporting plant MATEs. Through functional screening analysis in *Xenopus laevis* (African claw frog) oocytes, our lab has shown that *Sorghum bicolor* MATE1 (SbMATE1) facilitates citrate transport, and *Zea mays* MATE2 (ZmMATE2) does not. We hypothesize that conserved protein domains underlie their distinctive substrate recognition and transport ability. I am utilizing chimeric proteins constructed of swapped citrate-transporting (SbMATE1) and non-transporting (ZmMATE2) protein domains to test this hypothesis. To identify protein domains of citrate transporting MATEs essential for functionality and substrate recognition, I will utilize two complementary heterologous systems: *X. laevis* oocytes, which will allow me to investigate the electrogenic nature of the transporter, and *S. cerevisiae* (yeast) to analyze substrate (i.e., citrate) recognition. In addition, I will address the physiological effects caused by the MATE structural changes by comparing transgenic plant lines expressing the chimeric variants to unmodified SbMATE1 and ZmMATE2 plants. The outcomes of this research will increase our understanding of the structural properties that enable a select group of plant MATEs to mediate the beneficial exudation of citrate.

P28

Understanding Silver Nanoparticle Translocation and Elemental Interactions in Lycopersicon esculentum

Erin Lincoln, Kajal Purohit, Azam Noori
Department of Biology, Merrimack College, North Andover, MA

Silver nanoparticles (AgNPs) are versatile molecules lauded for their unique physicochemical composition. They are used in a wide variety of fields, including the textile, cosmetic, food, agricultural, pharmaceutical, and biomedical industries. However, the impact of AgNPs once they are inevitably released into the environment has remained unclear. This study aims to analyze the mechanisms of AgNPs translocation in Lycopersicon esculentum and its interaction with essential elements. In this study, L. esculentum were exposed to 30 mg/L of AgNPs and silver nitrate (AgNO3) for 30 and 70 days. To better understand the role of membrane transporter aquaporin on the translocation of AgNPs, the mutant L. esculentum for aquaporin (AQ) in addition to the wild type (WT) were exposed to AgNPs and AgNO3. The concentration of silver and essential elements were analyzed in soil and different organs of L. esculentum to determine the translocation rate and accumulation of silver and its interaction with other elements. The findings of this study showed that silver was transferred to all organs. The highest concentration of silver (1.99 μg/g) was detected in the younger leaves of plants exposed to AgNPs and was significantly higher ($p < 0.05$) than silver concentrations in other parts of the plant shoot. Significantly higher ($p < 0.05$) levels of silver were also detected in younger roots exposed to AgNPs when compared to older roots. Exposure to silver in particulate or ionic forms impacted the concentration of essential elements. In upper leaves exposed to AgNPs, there was a significant negative correlation ($r = -0.70$) between silver and iron, while in upper leaves exposed to AgNO3, there was a positive correlation ($r = 0.74$) between these two elements. The results of this study are essential to further understanding the translocation of AgNPs in plants and their influence on essential elements. Keywords: silver nanoparticles, elemental analysis, Lycopersicon esculentum, aquaporins.

P29

Deciphering the Role of Redox-Responsive and Calcium-Binding Proteins BAP on Plant Immunity and Chilling Tolerance

Yang Liu, Jian Hua
Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, USA

Redox and calcium signaling mechanisms play crucial roles in orchestrating plant responses to various biotic and abiotic stresses. Previous reports found redox-responsive and calcium-binding proteins BAP are negative regulators of immune responses in Arabidopsis thaliana and positive regulators of temperature stress responses in Vitis vinifera, yet the underlying regulatory mechanisms remain elusive. Our research identifies enhanced tolerance to chilling temperatures in Arabidopsis with overexpression of BAP1, as evidenced by two independent transgenic lines. Through bimolecular fluorescence complementation (BiFC) and reverse genetic analyses, we find the interaction between Arabidopsis BAP1 and BAP2 with all three catalases within the nuclei. Interestingly, overexpression of CAT2 may alleviate the autoimmune phenotype observed in bap1 mutants, hinting at a regulatory pathway where BAP recruits catalases to suppress redox-associated immune and stress responses, such as reactive oxygen species (ROS) burst and programmed cell death (PCD). In addition, utilizing calcium reporters GCamp.6f and YC3.6, we detect an elevated basal calcium level in bap1 loss-of-function mutants, potentially contributing to its autoimmune phenotype. Mutant forms of BAP with defective redox-sensing or calcium-binding are introduced into the bap mutants. Further studies on their downstream effect would collectively shed new light on the intricate interplay between BAP proteins, redox signaling, and calcium dynamics in modulating immune and temperature stress responses in plants.

P30

Accessory proteins increase the efficiency of RNA editing by Arabidopsis chloroplast editosomes

Jose Lombana*, Maureen R. Hanson, and Stéphane Bentolila
Department of Molecular Biology and Genetics, Biotechnology Building, Cornell University, Ithaca, NY 14853

We investigated the intricate process of RNA editing in terrestrial plants, characterized by cytidine-to-uridine (C-to-U) conversions. This biochemical modification is predominantly orchestrated by a specialized class of proteins known as pentatricopeptide repeat (PPR) proteins. These proteins exhibit specificity for RNA sequences, binding to them to either directly facilitate deamination or to recruit catalytic partners, such as the DYW deaminase domain, which can be integral to the PPR protein (in cis) or function independently (in trans). Despite the identification of key molecular players and target RNA sites within this biological framework, the mechanistic intricacies governing plant organelle RNA editing remain largely elusive. A significant gap in our understanding pertains to the role of the accessory proteins, which are necessary in angiosperms but not required for RNA editing in non-vascular plant lineages.

To bridge this knowledge gap, we have developed an *Escherichia coli*-based heterologous expression system designed for rapid and effective screening of RNA editing components. By expressing and co-expressing essential editosome constituents, we scrutinized their contribution to RNA editing efficiency, using RT_PCR bulk sequencing or Illumina RNA-seq to measure RNA editing extent. The co-expression of RNA-editing Interacting Proteins (RIPs) and the Organelle RNA Recognition Motif (ORRM1) protein along with PPR proteins markedly elevates editing efficiency in *E. coli* to levels observed in planta. We observed that accessory proteins increased the affinity of the PPR protein for the target transcript. We have made novel insights into the functional dynamics of the editosome complex.

P31

Study of an Intrinsic vacuolar protein for modulating apple fruit acidity

Alexandre Miaule[1], Chunlong Li[2], Kenong Xu[3], Lailiang Cheng[2], Miguel Piñeros[1,4]
[1] Plant Biology Section, [2] Horticulture Section, [3] Horticulture Section – Geneva, Cornell University, Ithaca NY
[4] Robert W. Holley Center for Agriculture and Health, United States Department of Agriculture-Agricultural Research Service, Cornell University, Ithaca, New York 14853

Fruit acidity and sweetness in apples (*Malus domestica)* are largely conferred by the malate content of the vacuole. Previous studies have found that allelic variation in the tonoplastic membrane transport protein Ma1, an orthologue of *ALUMINUM-ACTIVATED MALATE TRANSPORTER9* (*ALMT9*) in Arabidopsis *(Arabidopsis thaliana*), results in large differences in fruit acidity [1]. The recessive allele, ma1, contains a single nucleotide substitution which results in a premature STOP, truncating the cytosolic C-terminal domain. When expressed in heterologous systems the transporter encoded by ma1 shows a decreased transport activity, as well as a reduced expression at the vacuolar membrane, which is associated with a reduced fruit acidity [2]. Here, we examined the regulatory role of the ALMT C-terminus. Using a fluorometric quantification approach, we were able to estimate protein longevity at the membrane [3]. Preliminary results indicated that truncation of the C-term in the ma1 protein does not result in increased degradation at the membrane, but rather may result from a reduction in trafficking to the tonoplast. A pharmaceutical approach is being implemented to elucidate trafficking differences between the two alleles and provide insight into the role that the C-terminus plays in trafficking of membrane-bound proteins to the tonoplast membrane. Furthermore, additional loci have been associated with variations in fruit acidity [4], among which is one locus whose causal phenotype has been narrowed to a protein homologous to a mammalian scaffolding protein family. Preliminary data suggests a potential interaction between this putative protein and the membrane transporter Ma1 indicating a possible additional regulatory mechanism for Ma1 transport activity. Our current aim is to elucidate if the putative scaffolding protein plays a stabilizing role on Ma1, and therefore its association with a given fruit acidity phenotype.

Literature Cited:

[1] Ban, S., Xu, K. Identification of two QTLs associated with high fruit acidity in apple using pooled genome sequencing analysis. *Hortic Res* **7**, 171 (2020). https://doi.org/10.1038/s41438-020-00393-y
[2] Chunlong Li, Laura Dougherty, Alison E. Coluccio, Dong Meng, Islam El-Sharkawy, Ewa Borejsza-Wysocka, Dong Liang, Miguel A. Piñeros, Kenong Xu, Lailiang Cheng, Apple ALMT9 Requires a Conserved C-Terminal Domain for Malate Transport Underlying Fruit Acidity , *Plant Physiology*, Volume 182, Issue 2, February 2020, Pages 992–1006
[3] Hongtao Zhang, Eric Linster, Lucy Gannon, Wiebke Leemhuis, Chelsea A. Rundle, Frederica L. Theodoulou, Markus Wirtz, Tandem Fluorescent Protein Timers for Noninvasive Relative Protein Lifetime Measurement in Plants, *Plant Physiology*, Volume 180, Issue 2, June 2019, Pages 718–731,
[4] Verma, S., Evans, K., Guan, Y. *et al.* Two large-effect QTLs, *Ma* and *Ma3*, determine genetic potential for acidity in apple fruit: breeding insights from a multi-family study. *Tree Genetics & Genomes* **15**, 18 (2019). https://doi.org/10.1007/s11295-019-1324-y

P32

Investigating the role of thermo- and photocycles on p-Coumaroyl-CoA Monolignol Transferase expression in the model grass Brachypodium distachyon

Serene Omran[1], Greg Gregory[2] ,Joshua Coomey[2], Emil Mah[1], Kira Gardner[1], David Follette[3], Sam Hazen[2]
[1]Biology Department, [2]Biology Department and Plant Biology Graduate Program, [3]Institute for Applied Life Sciences, University of Massachusetts, Amherst, MA 01003, USA

The plant secondary cell wall provides the rigidity necessary for vertical growth, protection from biotic and abiotic challenges, as well as structure and hydrophobicity to facilitate water transport. Previously we demonstrated in the model grass Brachypodium distachyon that rate of elongation is regulated by temperature with warmer temperatures increasing the elongation rate. Whether secondary wall synthesis is similarly temperature-driven is unknown. Lignin, one of the main structural components of the secondary wall, provides rigidity and strength. In grasses, there are three monolignol subunits for lignin polymers: p-hydroxyphenyl, guaiacyl, and syringyl units. Some monolignols may be acylated with p-coumaric acid before being incorporated into a growing lignin chain, which results in the partial acylation of the polymer, but the function of this acylation is unknown. In B. distachyon, the enzyme p-coumaryl-CoA-monolignol transferase (PMT) acylates monolignols. We seek to determine the location of PMT expression during stem elongation and the external or internal cues that may drive a daily expression rhythm. We developed a bioluminescent gene reporter in B. distachyon by fusing the PMT promoter region to the firefly Luciferase coding sequence (PMT::LUC). Comparable time-lapse experiments with either thermocycles or photocycles revealed that PMT::LUC expression exhibited a daily rhythm in thermocycles, but not photocycles. These results are consistent with our recent reports of temperate and not light or the circadian clock regulating both cell elongation and secondary wall thickening in grasses.

P33

Investigating the Role of Jasmonic Acid Carboxyl Methyltransferase in Meloidogyne incognita Infection of Arabidopsis thaliana

Lexi Papamechail, Tyler Gallagher, Destiny Sotomayor, Heather Marella
Bridgewater State University 131 Summer St Bridgewater MA 02325

Root-knot nematodes (Meloidogyne spp.) are obligate plant parasites which have a wide host range including many agriculturally important crops. They manipulate their hosts through complex signaling regimes that have not been fully elucidated but implicate several phytohormone pathways, including jasmonates. Jasmonates are a class of phytohormones with diverse roles in plant defense signaling pathways and plant development. The jasmonic acid carboxyl methyltransferase (JMT) is the enzyme that catalyzes the formation of methyl jasmonate (MeJA) in Arabidopsis. In order to determine if MeJA plays a role in root-knot nematode infection through the action of JMT, we generated a jmt mutant using CRISPR-Cas9. The resulting jmt mutant has a small deletion in exon 2. We verified that the mutation was impacting the expression of JMT by RT-qPCR which showed a significant decrease in expression compared to the Columbia wild type control. We next investigated whether the jmt mutant altered infection by root-knot nematodes. Plates of the jmt mutant or the Columbia wild type were inoculated with 1000 Meloidogyne incognita eggs and after 7 weeks the number of resulting female nematodes producing egg masses were counted. The jmt mutants displayed significantly more females than the Columbia wild type. These results point towards a role for JMT and MeJA in host defense against root-knot nematode infection.

P34

Mitochondrial Calcium Ion Import May Promote Cold Tolerance in Plants

Veronica Perez, Jian Hua
Cornell University School of Integrative Plant Science Plant Biology Section, 158 Emerson Hall

Cold tolerance is an agriculturally significant trait that has been examined at many biologically significant levels, yet the impact translational regulation has on cold response and tolerance is comparatively unknown. To identify genes with significantly altered translation upon cold in maize (Zea mays), RNA-Seq and DEG analyses were conducted on total RNA and translationally active, polysome-bound RNA from control and cold-treated plants. From these analyses, 'calcium ion homeostasis' was identified as a significantly enriched GO term of differentially expressed genes (DEGs) between cold and control plants for both total and polysomal RNA. Several DEGs upregulated by cold were further characterized, including genes homologous to Mitochondrial Calcium Uniporter (MCU) and Mitochondrial Calcium Uptake (MICU) which showed greater induction in polysomal RNA than total RNA in maize. Examination of Arabidopsis mutants lacking these genes showed that micu and mcu123 mutants are more susceptible to chilling stress and freezing stress respectively. Taken together, these results suggest that mitochondrial calcium homeostasis plays a role in plant cold tolerance.

P35

Phytotoxicological Impacts of Silver Nanoparticles on Lycopersicon esculentum

Kajal Purohit*, Erin Lincoln, Azam Noori
Department of Biology, School of Arts & Sciences, Merrimack College, North Andover, MA

Silver nanoparticles (AgNPs) have gained significant attention due to their unique properties and wide-ranging applications across various fields, including medicine, agriculture, and industry. This study investigates the effects of AgNPs and silver nitrate (AgNO3) on Lycopersicon esculentum (tomato) by focusing on the translocation mechanism of AgNPs and assessing their impact on the anatomy of the plant. Two genotypes of L. esculentum, wild type (WT) and aquaporin mutant (AQ), were used to determine the role of aquaporin channels in the transportation of Ag and AgNPs. To proceed with this study both WT and AQ L. esculentum were exposed to 30 mg/L of AgNPs or AgNO3 for 30 days. The half maximum inhibitory concentration (IC50), the effects of Ag on seeds germination, plant growth, the cellular structure, and the translocation of silver were studied in plants. The bioaccumulation factor (BF) and the translocation factor (TF) were determined in both WT and AQ genotypes exposed to AgNPs, and AgNO3 for 30 days. The IC-50 analysis of the WT group revealed that exposure to 30 mg/L of AgNPs had 50% inhibitory effects on seeds germination and growth. The germination rate in AQ was higher than the WT group at 30 mg/L exposure. However, root length was significantly less in AgNO3 and AgNP-exposed plants compared to controls of the WT group. Additionally, xylem cell size was significantly less in the AQ group exposed to AgNO3 and AgNP-exposed L. esculentum compared to controls. The bioaccumulation and translocation factors were calculated for the WT group. The results showed significantly higher translocation of silver in AgNO3 compared with the AgNPs group. This research provides information regarding the impact of AgNPs on plant growth from seeds germination to fruit development. Keywords: Silver nanoparticles, anatomical analysis, phytotoxicity, analytical analysis

P36

How Domestication Heightens Tomato Vulnerability to Fusarium Wilt Disease

Maryam Rashidzade, Brian Kubi, Ana Caicedo
University of Massachusetts, Amherst, Massachusetts, USA

Fusarium oxysporum f. sp. lycopersici (FOL) is a fungal pathogen ranked among the world's top ten fungal disease agents, causing vascular wilt in tomatoes, a threat to global tomato production resulting in up to 80% yield losses. Evidence suggests a shared ancestry between non-pathogenic Fusarium strains and FOL, potentially evolving in wild tomato species in the Andes, particularly in Peru. Genomic data has elucidated the domestication history of tomatoes, revealing multiple populations of wild (Solanum pimpinellifolium), semi-domesticated (S. lycopersicum L. var cerasiforme), and domesticated (S. lycopersicum L. var lycopersicum) groups across diverse habitats. FOL's growth and regeneration are influenced by environmental factors such as temperature and humidity, prompting us to take advantage of tomato natural diversity to assess how levels of resistance to FOL are related to the plant population environment and degree of domestication. We inoculated 130 accessions representing various populations of wild, semi-domesticated, and domesticated tomatoes with FOL race 3, and measured disease symptoms. Our findings indicate a gradual loss of resistance to FOL throughout tomato domestication, with the most resistant population found among wild tomatoes from North Ecuador. Subsequently, we aim to explore the correlation between historical environmental data and FOL resistance among the studied populations.

P37

Identifying Insertions of Mitochondrial and Chloroplast DNA Within the Nuclear Genomes of 82 Plants

Solomon Scheiner, Christos Noutsos,
SUNY Old Westbury, Old Westbury, NY, 11568, USA

Through the use of bioinformatic analysis, insertions of mitochondrial and chloroplast DNA within the nuclear genome of plants were identified and their functions were studied. The organellar insertions discovered have since adapted to the nuclear environment and are now known as norgDNA. It was hypothesized that norgDNA could impact both protein coding regions as well as non-coding regulatory elements within the nuclear genome. norgDNA of various lengths were identified within the nuclear genomes of all 82 plants being studied. The number of base pairs made up of norgDNA within the plants being studied varied between 299 bp in the plant with the least amount of norgDNA and 24.08 mbp in the plant with the greatest amount of norgDNA. These results confirmed a previously discovered 641 kb segment of norgDNA of mitochondrial origin in chromosome 2 in the Arabidopsis thaliana nuclear genome. Using data from GFF files, 37,349 genes and 850 long non-coding segments either entirely or partially composed of the identified norgDNA were found. Additionally, using data from the GreeNC database 1002 long non-coding RNA regions and using data from the PmiREN database 124 microRNA regions also composed either entirely or partially of norgDNA were found as well. Overall, there were found to be a significantly high number of functional nuclear loci impacted by norgDNA.

P38

Nanosulfur application for minimizing Cadmium Accumulation in Wheat

Sudhir Sharma[1], Om Parkash Dhankher[1], Jason White[3]
[1]UMass Amherst, Stockbridge School of Agriculture, University of Massachusetts, Amherst, MA 01003, USA
[2]The Connecticut Agricultural Experiment Station, New Haven, CT 06504, USA.

The accumulation of Cadmium (Cd), a harmful heavy metal, in crops like wheat (Triticum aestivum) raises significant food safety concerns. Cadmium, a known carcinogen, can enter the human diet via crops like wheat and present considerable health risks. Thus, exploring effective strategies to limit Cd absorption and buildup in agricultural products is essential for maintaining food safety and security. Our research aimed to evaluate the impact of different sulfur-based soil treatments on reducing Cd absorption and concentration in wheat. We investigated four sulfur variants: elemental sulfur, nanoscale sulfur (uncoated), nanoscale sulfur (stearic acid-coated), and ionic sulfur (sodium sulfate), to identify sustainable solutions for alleviating Cd stress in wheat and curtailing its presence in the food supply. Initial findings from a hydroponic setup indicated that stearic acid-coated nanosulfur was the most effective, enhancing plant shoot biomass by ~60% in treatments combining sulfur and Cd, compared to Cd alone treatments. This result was linked with improved photosynthetic efficiency and diminished stress markers in the plants. Ongoing research seeks to uncover how sulfur helps mitigate Cd stress in wheat, focusing on the soil's sulfur-Cd interactions and the wheat plants' physiological and biochemical reactions to various sulfur and Cd treatments. Our examination includes looking at Cd absorption, distribution, and accumulation in plant parts, as well as the activity of antioxidant enzymes and the expression of genes involved in Cd detoxification/sequestering. The insights gained from this investigation will advance our understanding of how sulfur amendments in soil can effectively reduce Cd toxicity in wheat plants, thereby addressing the critical issue of food safety for Cd contamination in wheat globally.

P39

The Role of the Micronutrient Copper in the Development of Female and Male Reproductive Organs and Fertility in Arabidopsis thaliana

Shatokhina H.[1]*, Chia, J.[1], Rahmati Ishka, M.[1], Yan, J.[1], Yezerska I.[1], Zavodna, O.[1], Yang, Y.[2], Woll, A.[3], Smieska, L.[3], Vatamaniuk, O.K.[1]
[1]Plant Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, USA
[2] National Synchrotron Light Source II, Brookhaven National Laboratory, USA
[3]Cornell High Energy Synchrotron Source (CHESS), Cornell University, Ithaca, NY14850, USA

It has been known for decades that the micronutrient copper is essential for plant growth, development, fertility, and seed/grain yield. However, which plant reproductive organs require copper, how copper is delivered to these structures, and how it acts to ensure fertility is not entirely understood. I will report our recent studies deriving from the use of synchrotron x-ray fluorescence microscopy (2D-SXRF) at the nanoscale, 3D and 2D confocal synchrotron x-ray fluorescence microscopy (2D and 3D C-SXRF) and 3D-SXRF-Computed Tomography that allowed us to establish the spatial distribution of copper at different resolution scales in floral organs of a model plant Arabidopsis thaliana. I will also discuss the role of two transcription factors, CITF1 and SPL7, in controlling copper uptake, internal transport, and delivery to the specific sites in flowers and the impact of these transcription factors on the development of the male and female gametophytes. Importantly, we show that the role of copper and these transcription factors in reproductive processes is conserved in other plant species. Our new data not only significantly increase our understanding of the role of copper in plant reproduction but also invoke the possibility of its signalling role in reproductive organ development.

P40

Generation of Mutant Lines of Arabidopsis thaliana Chloroplast Small Heat Shock Proteins

Alina Shkurikhina*, Fabian Suri-Payer, Dr. Elizabeth Vierling
University of Massachusetts Amherst

When plants are subjected to increased temperatures, they undergo a conserved change in gene expression known as the heat shock response. One class of proteins that is upregulated during heat stress is small heat shock proteins (sHSPs), which are proposed to act as molecular chaperones to prevent irreversible denaturation and aggregation of heat-sensitive proteins. Though these proteins are vital for surviving high temperature and other stresses, much remains unclear about their mechanism of action. The focus of this project is the HSP25.3 protein, the only sHSP that is localized solely to the chloroplast. It is hypothesized that HSP25.3 assists in maintaining homeostasis in the chloroplast, but previous studies addressing its function have not shown a definitive phenotype and much is still not understood about its role and interaction. Phenotypic analysis of chloroplast sHSP mutants in Arabidopsis thaliana could shed light on the function of this highly conserved protein. To prepare for phenotypic analysis, it was required to characterize available chloroplast HSP25.3 null mutant lines and perform background research to determine potential phenotypic assays for future experiments. Two protein null mutant plant lines generated by CRISPR mutagenesis (hsp25.3-2 and hsp25.3-3) in A. thaliana were backcrossed to remove background mutations and the CRISPR machinery, reisolating the homozygous mutants in the F2 generation. A new mutant line was also developed in which the protein null line hsp25.3-1 is complemented with a wild type HSP25.3 gene containing a C-terminal affinity tag that can be used to recover HSP25.3-interacting proteins.

P41

Effects of Environmental Stress on the Gene Expression of CYP72A124 in Maize

Aliyah Siddiqui*, Leeann Thornton
Biology Department, The College of New Jersey, Ewing, NJ

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. Furthermore, plants have utilized regulatory mechanisms, such as alternative splicing, to adjust the abundance or function of certain genes and proteins related to stress responses. Previous studies have shown that intron retention is the predominant mechanism of alternative splicing in plants, creating transcripts that can protect the plant in various ways. However, there are few studies that have investigated the role of intron retention in maize, particularly in connection to acclimation to environmental changes. 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. We hypothesize that CYP72A124 in maize undergoes intron retention in response to several environmental conditions and that there is a difference in splicing between different maize recombinant inbred lines. To study the effects of environmental stresses on the intron retention of CYP72A124, we performed single and combined stress experiments and analyzed mRNA. We found that CYP72A124 is differentially expressed under different stress conditions and that intron retention varies between stresses, introns, and maize lines. This data improves our understanding of the contributions of the CYP72A124 in plant stress acclimation.

P42

X-rays for Life, Environmental, Agricultural, and Plant Sciences (XLEAP): A New Beamline Under Construction at the Cornell High Energy Synchrotron Source

Louisa Smieska[1], Arthur Woll[1], Ernest Fontes[1], Olena Vatamaniuk[2], Carlos Cabrera[3], Joel Brock[1]
[1]Cornell High Energy Synchrotron Source, Cornell University, Ithaca, NY 14853
[2]School of Integrative Plant Science, Plant Biology Section, Cornell University, Ithaca, NY 14853
[3]Chemistry and Biochemistry, The University of Texas at El Paso, El Paso, TX 79968

On February 13, 2024, the U.S. National Science Foundation announced an award that will support construction of a new x-ray beamline customized for research in plant and soil sciences at the Cornell High Energy Synchrotron Source (CHESS). The new beamline project, X-rays for Life, Environmental, Agricultural, and Plant sciences (XLEAP) will specialize in x-ray fluorescence microscopy, enabling quantitative imaging of micronutrients in biological systems at length scales ranging from whole tissues to cells. The science priorities for XLEAP include (1) fundamental mechanisms in plant sciences, such as micronutrient uptake, transport, and storage; (2) how plants respond to external stimuli such as nanoparticles, microplastics, bacteria, and fungi, as well as climate and environmental factors; (3) mechanisms of elemental transport and cycling at the root-soil interface and in soil degradation processes; and (4) mechanisms of elemental uptake and cycling in aquatic flora, seaweeds, and algae. We will discuss the planned x-ray capabilities of the XLEAP beamline as well as its complementary microscopy, plant growth, and sample preparation facilities, highlighting the potential for in-situ x-ray measurements with a custom plant growth environment directly on the beamline. The CHESS XLEAP beamline will be constructed over the next four years with user operations planned to begin in 2028. During construction, graduate students and faculty from the University of Texas at El Paso will collaborate with CHESS staff and Cornell faculty on pilot experiments that develop future user capabilities and workflows for XLEAP.

P43

FuncFetch: A GPT-4 Enabled Workflow for Rapidly Mining Enzyme-Substrate Interactions from PubMed-Indexed Manuscripts

Nathaniel Smith, Chesney Melissinos, Xinyu Yuan, Gaurav Moghe
Plant Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, USA 14853

Over the past decade, the surge in genome sequencing has significantly expanded the number of publicly available genomes. However, the pace of gene function annotation has not matched this growth, with only a small fraction of verified gene functions — mostly from model species — making it into key databases like UniProt, BRENDA, and KEGG. This issue isn't due to a lack of research output; rather, many functional insights remain within the confines of literature, not integrated into databases. The bottleneck for this information is biocuration, a critical yet labor-intensive task that transfers detailed scientific findings into database-friendly formats. Despite its importance, the scarcity of biocurators — only 5 focusing on plants across 40 databases — exacerbates the challenge, leaving the plant science community reliant on outdated information. To address this critical gap, we developed the FuncFetch pipeline for automated extraction of enzyme activities from manuscripts. Utilizing the power of OpenAI's GPT-4 model, FuncFetch can screen thousands of published papers and extract hundreds of activities in a few hours, with limited human assistance. In comparison to a high-quality manually curated dataset of 1112 BAHD acyltransferase enzyme activities, FuncFetch demonstrated remarkable accuracy, recovering 50% of the activities present in the dataset and outputting 90% correct activities. Of the correct activities, 21% were not identified by manual curation efforts, suggesting there are additional functional annotations present in previously reviewed papers which rarely get a second look from biocurators. A new data repository will make available compilations of thousands of activities from ~10 enzyme families for researchers worldwide. With simple adjustments to the prompts, the FuncFetch approach can be adapted to address other gaps in functional annotation and expand the set of information accessible to researchers across the plant sciences.

P44

Designing CRISPR Based Activation for Increasing Gene Expression in Catharanthus roseus

Natalie Soens*, Emma Meehan*, Krystyna Traverse, Carolyn Lee-Parsons
Department of Chemical Engineering, Northeastern University, Boston, Massachusetts 02115, United States

Catharanthus roseus (Madagascar periwinkle) is the only commercial source of several medicinally important terpenoid indole alkaloids (TIAs). The most notable TIAs from C. roseus are vinblastine and vincristine. These unique chemotherapeutics are limited in supply due to inconsistent production and low yield from plants. The ~30 genes which comprise the TIA biosynthetic pathway have been identified. To increase expression of the biosynthetic genes, we are using CRISPR activation tools. Here, we demonstrate the advantage of combining several guide RNAs (gRNAs) in a multiplexed CRISPR-activation tool towards increasing gene expression. We compared how the design of the CRISPR activation system affected gene activation, as it has been previously reported that increasing the number of guide RNAs improves CRISPR efficacy. Notably, we saw a 15-fold increase in gene activation using four gRNAs via promoter-transactivation studies. We also confirmed this trend via qPCR on endogenous gene expression. This work is translatable to other plant species and provides a more effective approach towards gene activation.

P45

A Genetic Test for Serpentine-Specific Natural Selection in the Lyre-Leaf Rockcress, Arabidopsis lyrata, an Important Element of the State Line Serpentine Barrens of Eastern North America.

Christopher Stieha, Christopher Hardy, Maja Klosinska
Department of Biology, Millersville University, Millersville, PA 17551

A short-lived perennial in the mustard family, Arabidopsis lyrata is often used in studies of plant ecology and evolution due to its high level of morphological and genetic polymorphism. Arabidopsis lyrata can grow in disturbed or disadvantaged habitats, such as on nutrient poor serpentine soils high in heavy metals. We aimed to provide insight into adaptation and evolution of this species through examining gene flows between A. lyrata populations growing in the same geographic area (Mid-Atlantic) but in drastically different habitats, specifically comparing more hospitable non-serpentine soils to nutrient poor and toxic serpentine soils of serpentine barrens. Gene flow between populations was investigated through comparison of alleles of selected microsatellite loci. We aimed to determine whether populations growing on serpentine barrens are highly adapted and genetically closer to each other, with genetic exchanges occurring mainly within and between them despite geographic distance, or if they are the result of adaptation of local non-serpentine soil populations. Based on our results, serpentine populations show a high degree of similarity, and they may exchange genetic information mainly with other serpentine populations, though similarity diminishes with increased geographic distance. Non-serpentine populations likewise experience gene flows, especially with closer non-serpentine populations. The least exchange seems to occur between serpentine and non-serpentine populations, which supports the observations that survival on serpentine soils requires very specific adaptations and genetic exchange with non-serpentine neighbors may be selected against.

P46

Introduction of barnase/barstar in soybean produces a rescuable male sterility system for hybrid breeding

Nicole Szeluga[1], Patricia Baldrich[2], Ryan DelPercio[3], Blake Meyers[2], Margaret Frank[1]
[1]Cornell University, Ithaca, NY
[2]University of California - Davis, Davis, CA
[3]University of Missouri - Columbia, Columbia, MO

Hybrid breeding for increased vigor has been used for over a century to boost agricultural outputs without requiring higher inputs. While this approach has led to some of the most substantial gains in crop productivity, breeding barriers have fundamentally limited soybean (Glycine max) from reaping the benefits of hybrid vigor. Soybean flowers self-pollinate before opening and thus are not readily amenable to outcrossing. In this study, we demonstrate that the barnase/barstar male sterility/rescue system can be used in soybean to produce hybrid seeds. By expressing the cytotoxic ribonuclease, barnase, under a tapetum-specific promoter in soybean anthers, we can completely block pollen maturation, creating male-sterile plants. We show that fertility can be rescued in the F1 generation of these barnase-expressing lines when they are crossed with pollen from plants that express the barnase inhibitor, barstar. Importantly, we found that the successful rescue of male fertility is dependent on the relative dosage of barnase and barstar. When barnase and barstar were expressed under the same tapetum-specific promoter, the F1 offspring remained male sterile. When we expressed barstar under a relatively stronger promoter than barnase, we were able to achieve a successful rescue of male fertility in the F1 generation. This work demonstrates the successful implementation of a biotechnology approach to produce fertile hybrid offspring in soybean.

P47

CYP72A349 Regulates Corn Environmental Stress Response

Josie Maguire*, Aimee Torres*, Leeann Thornton
Biology Department, The College of New Jersey, Ewing, NJ

Cytochrome P450s represent the largest family of enzymes responsible for catalyzing hydroxylation reactions in plant metabolism. The CYP72A subfamily is crucial in shaping metabolic diversity in flowering plants, a fundamental aspect of plant evolution and stress tolerance. Corn CYP72As are differentially expressed in response to abiotic and biotic stresses, suggesting distinct roles in plant defense and metabolic pathways. We focused on the role of CYP72A349 in the minor shifts in metabolism that are necessary for corn plants to acclimate to combinations of stresses. CYP72A349 is induced in response to caterpillar feeding, but maize Ds transposon insertion mutants deficient in CYP72A349 (Ds309) do not show a significant phenotype in caterpillar feeding assays. The gene is also upregulated when cold and caterpillar stresses are applied sequentially. Caterpillars fed more on CYP72A349 mutants rather than wild type after plants were cold-stressed, suggesting that CYP72A349 is involved in herbivory defense following abiotic stress. Metabolic profiles of CYP72A349 mutants exposed to salt stress followed by caterpillar feeding showed significant clustering differences compared to wild type. However, a specific secondary metabolite that could cause this feeding difference was not identified. Some CYPs contribute to slight modulations in growth by fine-tuning the balance between bioactive forms of gibberellin. In Arabidopsis, CYP72A9 converts bioactive GA4 to the less bioactive GA1. Additionally, CYP72A349 is homologous to a rice CYP72A gene associated with gibberellin inactivation. We overexpressed CYP72A349 in Arabidopsis to test the hypothesis that it inactivates gibberellin under abiotic and biotic stress as part of the growth-defense trade-off. We are examining the impact of the corn gene on Arabidopsis GA biosynthesis and growth. These approaches are important for revealing the complex metabolic contributions of the CYP72A subfamily to corn stress acclimation.

P48

Characterization of the Juice Polyphenol Composition of 14 Malus Cultivars

Kamal Tyagi[1], Andy Lui[2], Sheng Zhang[2], Gregory Michael Peck[1]
[1]Horticulture Section, College of Agriculture and Life Sciences, Cornell University, Ithaca, NY, USA
[2]Proteomics and Metabolomics facility, Institute of Biotechnology, Cornell University, Ithaca, NY, USA

Apple polyphenols contribute significantly to the flavor, aroma, color, and potential health benefits of hard (fermented) cider. Previous studies have revealed wide variations in total polyphenol levels using Folin–Ciocalteu assay among 158 Malus cultivars (Wojtyna thesis, 2018). In this study, 14 cultivars representing varying levels of total polyphenol content were further analyzed for polyphenols analysis using RP-HPLC and untargeted LC-MS/MS approaches. Total polyphenol using Folin–Ciocalteu assay ranges from 560.0 to 4,860.0 mg L-1. The RP-HPLC profiling resulted in the identification and quantification of 19 polyphenols, with the most abundant classes being hydroxycinnamate and flavan-3-ols. The RP-HPLC analysis showed that Kola exhibited the highest total polyphenol content of 1,428.5 mg L-1, predominantly composed of chlorogenic acid (1311.0 mg L-1). Following closely, Kaz 95 18-06 showed 1093.2 mg L-1, with flavan-3-ols (860.0 mg L-1) as the predominant polyphenol. Additionally, Zapta has the highest amounts of phlorizin (80.1 mg L-1), a dihydrochalcone. Furthermore, untargeted LC-MS analysis identified 102 polyphenols across these cultivars, including 21 general polyphenols reported in apples and other unknown with potential beneficial effects. In summary, our research contributes valuable insights into the use of each method and identifies a few cultivars for their potential use in hard cider, breeding, and physiological studies.

P49

A Putative Hemicellulose Gene-PtrPARVUS2, Drives Guard Cell and Epidermis Expression in Poplar

Dan Wang*, Heather D. Coleman
Department of Biology, Syracuse University, 107 College Place, Syracuse, NY, 13244 USA

The cell wall is a vital structure in plant cells, serving as a physical barrier and contributing significantly to global organic carbon. Certain tree species, such as poplar, are known for their rapid biomass production and have been extensively studied for their potential in bioenergy production. This study focuses on xylan, a major hemicellulose component in poplar, and specifically on one member of the glycosyltransferase 8 (GT8) family genes, PtrPARVUS2, involved in xylan biosynthesis. PtrPARVUS2 is hypothesized to have tissue-specific activity, and its promoter was analyzed bioinformatically, revealing motifs associated with Gibberellic Acid (GA), Methyl Jasmonate (MeJA), Auxin, and Abscisic Acid (ABA) responsiveness, among others. Hormone treatments indicated that PtrPARVUS2 expression could be inhibited by ABA, 1-naphthaleneacetic acid, a synthetic auxin, MeJA and GA in a short time. Expression pattern analysis in wildtype trees demonstrated higher expression in stem tissues, particularly the bark. The generation of transgenic lines expressing Enhanced Green Fluorescent Protein (EGFP) driven by PtrPARVUS2 promoter confirmed tissue-specific activity, with strong expression in guard cells and stem epidermis, as well as developing xylem. These findings provide insights into PtrPARVUS2 interactions with phytohormone signaling and guard cell wall formation. The study establishes a valuable promoter for targeted biotechnological applications in guard cells and stem epidermal cells.

P50

Genetic variation standardization (rsIDs) enhances Trait-Driven Interoperability and Genomic Function Transfer

SharonWei[1], Marcela Tello-Ruiz[1], Vivek Kumar[1], Andrew Olson[1], Kapeel Chougule[1], Doreen Ware[1,2]
[1]Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724
[2]USDA ARS NEA, Plant Soil & Nutrition Laboratory Research Unit, Ithaca, NY, 14853

The Reference SNP cluster ID (rsID) is a unique identifier for groups of genetic variations (GVs) co-located at specific positions in the genome. It facilitates standardized referencing across databases, studies, and publications, primarily in human research for mutation identification and data integration. However, its use in plant research has been limited due to inadequate support. With the maturation of the European Variation Archive (EVA), hundreds of millions of rsIDs have been assigned to plant genomes, including agriculturally significant ones hosted in Gramene (https://www.gramene.org/) and SorghumBase (https://sorghumbase.org/). This development made GV data aggregation and marker-based breeding more feasible. The plant-centered genomic database and browser Gramene has embraced this by ingesting rsIDs from EVA release 5, linking them to QTL, phenotype, and germplasm data. Currently, four crop genomes have integrated rsIDs: Sorghum (41M), Rice (27M), Maize (47M), and Grape (0.3M). For SorghumBase, we also imported GWAS study results from the Sorghum Association Panel population and associated the phenotypes/traits with rsIDs. As the number of sequenced pan-genomes increases, computationally calling GVs on each accession's genome becomes impractical. Instead, mapping rsIDs from the reference genome to pan-genomes proves more feasible and efficient. Gramene tested this approach by implementing the variation mapping pipeline from EVA, allowing accurate mapping across different assemblies of the same genome and genomes of different accessions from the same or closely related species. This method's successful implementation holds significant potential for breeding initiatives. Gramene's efforts are supported by funding from USDA ARS (8062-21000-041-00D).

P51

Phylogenetic Analysis of Cellulose Synthase-like D Genes in Bryophyte Diversification

Kerrina Whelan, Alexandra Walling, Rachel Schwartz, Alison Roberts
University of Rhode Island 45 Upper College Rd, Kingston, RI 02881

Cellulose is an essential component of plant cell walls that provides structural rigidity while contributing to cell growth and expansion. Members of the Cellulose Synthase-like D (CSLD) gene family encode subunits of complexes that generate fibrillar cellulose in plant cell walls during tip growth and cell division. The CSLD gene family has evolved through whole-genome and small-scale gene duplications within several plant lineages. Gene duplications are a major driver of functional novelty in plant evolution, contributing to adaptations that facilitated transition from water to land. The diversification of CSLDs is a useful example for understanding the impacts of gene diversification on development and adaptive radiation. The model moss species Physcomitrium patens has eight CSLD genes, two of which are expressed preferentially in leafy gametophores and are required for normal cytokinesis. However, it is unknown whether this pattern of diversification and functional specialization is shared with other bryophytes. Here we use computational methods to construct a phylogenetic tree of CSLD genes identified in fully sequenced bryophyte genomes, identify patterns in CSLD divergence, and interpret what those patterns reveal about the evolution of this gene family. We provide evidence supporting both early CSLD diversification that predates divergence of major moss lineages and more recent gene duplication events. Parallels between CSLD diversification in mosses and vascular plant lineages are discussed.

P52

Characterization of a Unique Melon Variety Exhibiting a Mesocarp Specific Deficiency in Carotenogenic Metabolic Flux

Emalee Wrightstone[1], Sombir Rao[1], Zhangjun Fei[2], Li, Li[3]

[1]Cornell University, Ithaca NY 14853, USA
[2]Boyce Thompson Institute, Ithaca NY 14853, USA
[3]Robert W. Holley Center for Agriculture and Health, USDA-ARS, Ithaca, NY 14853, USA

Carotenoids are critically important to human nutrition and health. Despite a well-established carotenoid biosynthetic pathway, its regulatory controls remain elusive. Melon (Cucumis melo) fruit exhibits more than a 1,000-fold difference in flesh carotenoid level, making it a remarkable crop model for novel gene discovery. The primary carotenoid of orange-fleshed varieties, β-carotene, accumulates due to a specific allele of Orange (Or) that controls chromoplast biogenesis and posttranslationally regulates phytoene synthase (PSY), the key rate-limiting enzyme of carotenoid biosynthesis. We identified a unique melon variety that contains this orange-fleshed allele but develops a carotenoid deficient mesocarp surrounding a β-carotene rich endocarp. In this study we characterized this unique variety in comparison with an orange-fleshed melon variety. Sequence, transcript level, and protein abundance analyses of PSY and Or revealed no differences between the tissues of these two varieties. Additionally, PSY activity was confirmed by an in vitro bacterial assay. Although PSY is functionally active, a significant deficiency in carotenogenic metabolic flux through PSY was found in the white flesh tissue during fruit development, indicating the presence of a novel factor affecting metabolic flux that we termed “CmCAF” (CAROTENOID ACCUMULATION FACTOR). QTL-seq analysis of F2 bulks detected two QTLs, one of which was fine-mapped to a region containing 42 genes. We hypothesize the white mesocarp flesh results from a regulatory protein affecting PSY activity, which is under investigation. This study presents a thorough characterization of a unique melon variety and provides the foundation to reveal a novel posttranslational regulator of carotenoid biosynthesis in melon fruit.

P53

Characterizing Basal Calcium Level Maintenance by Calcium Pumps and Its Impact on Signaling in Arabidopsis

Peiqiao Xie, Jian Hua
School of Integrative Plant Science, Plant Biology Section, Cornell University

The basal cytoplasmic calcium concentration ([Ca2+]cyt) is emerging as a critical determinant of plant growth, development, and environmental responses. Recent studies reveal that plasma membrane-localized autoinhibited Ca2+ pumps ACA8 and ACA10 in Arabidopsis thaliana play an essential role in maintaining low [Ca2+]cyt and survival under cold- and heat-induced stress. The loss-of-function mutants aca8 aca10 exhibited an elevated [Ca2+]cyt, resulting in an enhanced autoimmunity with mis-regulated expression of defense response genes. Increased [Ca2+]cyt at low temperature amplified autoimmune responses to trigger cell death and chilling susceptibility, whereas increased [Ca2+]cyt at high temperature induced stomata closure and heat susceptibility. These physiological effects conferred by an elevated [Ca2+]cyt suggest the importance of maintaining a proper resting [Ca2+]cyt in mediating (a)biotic responses in plants. However, the functional implications associated with decreased [Ca2+]cyt in plants remain unclear. Here, I attempt to use overexpression of full-length or autoinhibitory domain-truncated ACA8/10 to attenuate [Ca2+]cyt in order to further characterize the role of these Ca2+ pumps in [Ca2+]cyt regulation. Through a combination of basal Ca2+ imaging and physiological response analyses including stomatal closure measurement, pathogen growth test, as well as cold and heat tolerance assay, this study will shed light on the impact of low [Ca2+]cyt on different developmental and stress-responsive processes in plants.

P54

Identification and Characterization of Genes in Benzylisoquinoline Alkaloid Biosynthesis in Goldthread (Coptis trifolia)

Yoo-Shin Koh[1]*, Yoojeong Hwang[2], Mi-Jeong Yoo[2]
[1]Duke University, Durham, NC 27708
[2]Department of Biology, Clarkson University, Potsdam, NY 13699

Understanding the biosynthetic pathways of phytochemicals can expand our understanding of their production in plants and their potential applications in various fields, including medicine, agriculture, and food science. Coptis species produce many alkaloids, including berberine, palmatine, and coptisine. These alkaloids possess therapeutic effects and have been utilized in traditional medical systems for ages. This work aimed to analyze the transcriptomes of the leaf and root of Coptis trifolia (L.) Salisb. to identify the genes responsible for the synthesis of benzylisoquinoline alkaloid (BIA). Coptis trifolia is distributed in East Asia and North America and is sister to the clade that contains Asian medicinal species. Currently, most of the genes involved in BIA biosynthesis have been discovered in Asian species. Therefore, the transcriptomic data obtained from C. trifolia will help to bridge the existing gap in our understanding of genes associated with BIA biosynthesis. A total of 120 million short reads were acquired using the Illumina NovaSeq sequencer, and these reads were assembled into transcripts using the de novo assembler Trinity. The quantification of transcripts was determined by aligning short reads to the assembled transcripts. Genes that were expressed at higher levels in roots compared to leaves were further examined to identify genes involved in the production of BIA.

P55

Investigating the Role of CITF1-Like Genes in Copper and Iron Homeostasis in Brachypodium distachyon

Tetiana-Olena Zavodna, Elizabeth Mahood, Yulin Jiang, Cheng Zou, Qi Sun. Olena Vatamaniuk
Section of Plant Biology, School of Integrative Plant Science, Cornell University, Ithaca, NY, 14853

Copper (Cu) and iron (Fe) are micronutrients that are essential for plant growth and development. Copper, in particular, is needed for the successful reproduction and grain/seed set. However, both copper and iron are toxic when they are accumulated in cells in excess. Thus, plants developed a system to tightly regulate nutrient uptake, transport, and internal distribution. Iron transport processes have been studied in a model dicot Arabidopsis thaliana and a cereal crop, Oryza sativa (rice). Studies of copper transport and its regulation have been mainly addressed in A. thaliana. How copper transport is regulated and how it affects the fertility of grasses is not well known. Based on studies in A. thaliana, a conserved transcription factor, SPL7 (Squamosa Promoter Binding Protein-Like 7), and a member of the bHLH family of transcription factors, CITF1 (Copper-Deficiency Induced Transcription Factor 1) regulate copper uptake and delivery to reproductive organs. The genome of a wheat model, Brachypodium distachyon, possesses three CITF1-like genes that we designated BdCITFL1, BdCITFL2, and BdCITFL3; their function in mineral nutrient homeostasis and possible regulatory role is unknown. Here, we will present results from our recent functional genomics studies showing that, unlike AtCITF1, BdCITFL1 regulates the homeostasis of both copper and iron.

P56

Temporal Dynamics of Microbial Carrying Capacity in Arabidopsis thaliana Phytospheres

Michael Zulch, Joe Larkin,
Boston University, Boston, MA

The symbiosis between plants and microbes is primarily facilitated by a vital exchange of nutrients and energy. This study investigates the temporal dynamics of growth and energy transfer between Arabidopsis thaliana and Bacillus subtilis growing among its roots. By directly measuring the rosette surface area and exudate accumulation of Arabidopsis thaliana in inoculated and uninoculated miniature phytospheres, we explore how the plant's microbial carrying capacity changes over time. Our findings indicate that the population size of Bacillus subtilis which a single Arabidopsis plant can support is proportional to its surface area integrated over time. This finding adds insights to the quantitative and temporal nature of the plant-microbe energy exchange and highlights the impact of plant morphology on microbial carrying capacity.

LIST OF PARTICIPANTS

First Name	Last Name	Affiliation
Logayn	Abushal	University of Massachusetts
Noor	AlBader	Cornell University
Dylan	Ashe	University of Massachuesetts, Amherst
Maria	Babar	University of Massachusetts
Kaylee	Bagdan	Skidmore College
Gabriella	Ballestas	Clarkson University
Vishwa Jyoti	Baruah	Cornell University
stephane	bentolila	Cornell University
Elise	Boisvert	Cornell University
Shelby	Boulanger	University of Massachusetts
Trey	Bourassa	University of Massachusetts Amherst
Lia	Bozza	University of Rhode Island
Janeen	Braynen	Cold Spring Harbor Laboratory
Emily	Brewer	Boyce Thompson Institute
Ed	Buckler	USDA-ARS; Cornell University
Kylie	Campana	University of Massachusetts
Lauren	Cardarelli	Bridgewater State University
Vishal	Chaudhari	Cornell University
Ju-Chen	Chia	Plant Biology Section, Cornell University
Lauren	Cole-Osborn	Northeastern University
Kate	Dooling	Cornell University
Steven	Ellis	National Science Foundation
Audrey	Fahey	Cold Spring Harbor Laboratory
Serena	Fan	St. Bonaventure University
Leila	Feiz	Boyce Thompson Institute
Joshua	Gendron	Yale University
Greg	Gregory	University of Massachusetts
Samuel	Griffin	St. Bonaventure University
Iesh	Gujral	St. Bonaventure University
Maureen	Hanson	Cornell University
Sam	Hazen	UMass Amherst
Abhijit	Hazra	Cornell University
Michelle	Heeney	Cornell
Cameron	Heinig	St. Bonaventure University
Anna	Hermanns	Cornell University
Katherine	Hlywa	University of Rhode Island
Cynthia	Holland	Williams College
Jian	Hua	Cornell University
Jayne	Ingle	University of Rhode Island
Mohammad	Irfan	Cornell University

Monirul	Islam	University of Massachusetts Amherst, MA, USA
Courtney	Jahn	National Science Foundation
Georg	Jander	Boyce Thompson Institute
Molly	Johnson	Northeastern University
Magdalena	Julkowska	Boyce Thompson Institute
Yana	Kavulych	Cornell University
Bahman	Khahani	University of Massachusetts
Maja	Klosinska	Millersville University
Yoo-Shin	Koh	Duke University
Shuyao	Kong	Cornell University
Ellie	Kreider	The College of New Jersey
Elise	Krespan	Syracuse University
Valeria	Lacouture	University of Massachusetts
Carolyn	Lee-Parsons	Northeastern University
Darren	Leopold	The College of New Jersey
Patricia	Leyva	Cornell University
Erin	Lincoln	Merrimack College
JIPING	LIU	USDA-ARS; Cornell University
Xing	Liu	Cornell University
YANG	LIU	Cornell University
Jose	Lombana	Cornell University
Josephine	LoRicco	Skidmore College
Harper	Lowrey	Yale University
Rocky	Lu	The College of New Jersey
Josephine	Maguire	The College of New Jersey
Simon	Malcomber	National Science Foundation
Heather	Marella	Bridgewater State University
Katie	McGillivray	University of Massachusetts
Emma	Meehan	Northeastern University
Alexandre	Miaule	Cornell University
Boaz	Negin	Boyce Thompson Institute
Andrew	Nelson	Boyce Thompson Institute
Azam	Noori	Merrimack College
Matthew	Norman-Ariztía	Cornell University
Christos	Noutsos	SUNY Old Westbury
Lilijana	Oliver	Cornell University
Serene	Omran	University of Massachusetts
Lexi	Papamechail	Bridgewater State University
Samantha	Pelletier	Cornell University
Veronica	Perez	Cornell University
Miguel	Pineros	USDA ARS
Kajal	Purohit	Merrimack College
Sombir	Rao	Cornell University

Maryam	Rashidzade	University of Massachusetts, Amherst
Claire	Ravenburg	Cornell University
Erin	Rehrig	Fitchburg State University
Sophia	Rinaldi	University of Massachusetts
Alison	Roberts	University of Rhode Island
Adrienne	Roeder	Cornell University
Luke	Rogers	The College of New Jersey
Pratyush	Routray	Cornell University
Bhaswati	Sarmah	Cornell University
Solomon	Scheiner	SUNY Old Westbury
Nathan	Schuessler	East Stroudsburg University
Sudhir	Sharma	University of Massachusetts Amherst
Hanna	Shatokhina	Cornell University
Alina	Shkurikhina	University of Massachusetts Amherst
Aliyah	Siddiqui	The College of New Jersey
Yogita	Singh	University of Massachsetts
Jacob	Smeraldo	St. Bonaventure University
Louisa	Smieska	Cornell High Energy Synchrotron Source
Nate	Smith	Cornell University
Natalie	Soens	Northeastern University
Nicole	Szeluga	Cornell University
Leeann	Thornton	The College of New Jersey
Ryan	Thummel	Cornell University
Aimee	Torres	The College of New Jersey
Thi	Tran	University of Massachusetts
Kamal	Tyagi	Cornell University
Seren	Villwock	Cornell University
Dan	Wang	Syracuse University
Doreen	Ware	Cold Spring Harbor Labs
Sharon	Wei	Cold Spring Harbor Labs
Erica	Weinstein	University of Massachusetts
Kerrina	Whelan	Mass Health
Klaas	Wijk	Cornell University
Emalee	Wrightstone	Cornell University Plant Breeding and Genetics
Peiqiao	Xie	Cornell University
Lilin	Xu	Cornell University
Samantha	Yanders	Cornell University SIPS, Plant Biology Section
Michelle	Yoo	Clarkson University
Xinyu	Yuan	Cornell University
Tetiana-Olena	Zavodna	Cornell University
Shiqi	Zhang	East Stoudsburg University
Xiao-Ning	Zhang	St. Bonaventure University
Michael	Zulch	Boston University