### 2019 Annual Meeting Midwestern Section American Society of Plant Biologists

March 16 – 17, 2019 South Agricultural Sciences Building and Agricultural Sciences Building West Virginia University Morgantown, WV



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### American Society of Plant Biologists Midwestern Section

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#### Short Program

#### Friday, March 15

2:00 - 4:00	Guided Tour of the WVU Evansdale Greenhouse and the International
	Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM)
	(Meet in Greenhouse Lobby)
2:00 – 4:00	Guided Tour of the WVU Arboretum (Meet at Coliseum Parking Lot)

#### Saturday, March 16

.00 – 8:00Registration/Check-in/Poster Set-up/Breakfast	7:00 -
:00 – 8:15Welcoming Remarks	8:00 -
15 – 8:45 Featured Speaker Dr. Gregg Howe	8:15 -
	8:45 -
0:15 – 11:30 Poster Session I (Even #s)/Refreshments	10:15 -
1:30 – 1:00 Oral Session II (T7-T12)	11:30 -
:00 – 2:30 Lunch	1:00 -
30 – 2:30Career Panel	1:30 -
30 – 3:00 Featured Speaker Dr. Steve DiFazio	2:30 -
:00 – 4:30 Oral Session III (T13-T18)	3:00 -
30 – 4:45 Coffee Break	4:30 -

4:45 - 5:45	Keynote Speaker Dr. Elizabeth Haswell
5:45 - 7:15	
7:30 – 10:00	

#### Sunday, March 17

7:00 – 8:00	Registration/Check-in/Breakfast
8:00 – 9:30	Oral Session IV (T19-T24)
9:30 – 10:00	Morning Refreshments/ Remove Posters
10:00 - 10:30	Featured Speaker Dr. Jean-Michel Ané
10:30 – 12:00	Oral Session V (T25-T30)
12:00 – 12:30	Business Meeting, Announcements, Awards

#### **Meeting Locations**

Registration	South Ag Sciences Bldg
All Presentations including Keynote Address	James H. Arbuckle Lecture Hall
Saturday Career Panel Discussion	Lecture Hall G006
Poster Viewing	Agricultural Sciences Bldg
Saturday and Sunday Breakfast	South Ag Sciences Bldg
Saturday Lunch	Agricultural Sciences Bldg
Saturday Coffee Break	South Ag Sciences Bldg
Saturday Dinner	Evansdale Crossing Bldg

### Vegetarian options will be available as part of all meals provided; however, preference will be given to individuals who requested vegetarian meals during meeting registration.

**Posters**: Posters need to be mounted on poster boards between 7:00 am and 9:30 am on Saturday, March 16. Due to space limitations, poster dimensions should be NO larger than 36 in. (width) by 42 in. (height). Pins will be available on-site. Posters need to be removed from poster boards on Sunday, March 17, between 9:30 am and 1 pm. Items remaining on display after 1 pm on Sunday will be discarded.

Photographing, videotaping, or recording of any kind is prohibited (including but not limited to camera phones and digital devices).

**Notice to Oral Presenters**: The preferred format for presentations is PowerPoint. In order to expedite sessions and remain on schedule, we ask you, if possible, to email your presentations to Michael Gutensohn (michael.gutensohn@mail.wvu.edu) by 5 pm on Thursday, March 15th. Alternatively, you may bring a copy of the presentation with you on a USB Flashdrive (labeled with your name) to present at the registration check-in desk. Please name your presentation file with your name, the date you are speaking and the presentation number as listed in the meeting program. For example: Barbara Smith March 16 Talk 10. This will assist us further in making sure your presentation is uploaded to the correct session on the lecture hall computer. We ask all oral presenters to arrive 10 minutes prior to the start of their session to ensure their presentations are loaded and ready. The presentation room will have a computer, projector, screen, remote control and laser pointer for your use.

#### 2019 Annual MW ASPB Meeting, West Virginia University

#### Friday, March 15

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The tour will include a visit to the new 18,615 square-foot Evansdale Greenhouse built in 2012 (https://www.davis.wvu.edu/about-davis-college/centers-initiatives/wvu-evansdale-greenhouse). The 16 bay greenhouse is conveniently located next to the Agricultural Sciences Building. The tour will also include an introduction to the INVAM (https://invam.wvu.edu/), the world's largest collection of vesicular-arbuscular mycorrhizal fungi. Parking will be available in lot 43 across from the main entrance to the greenhouse.

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The WVU Core Aboretum is a 91 acre tract of mesophytic hardwood forest located along the bluffs and bottomlands of the Monongahela River near the WVU campus (https://arboretum.wvu.edu/). The tour will cover a ~2 mile loop, involving some rough and possibly muddy terrain. Parking is available at the WVU Coliseum, and the tour will depart from the Arboretum parking lot at 2 pm. Directions are available here: https://arboretum.wvu.edu/#map

### Schedule of Sessions and Speakers

### Saturday, March 16

7:00 -	8:00	Registration/Check-in/Poster Set-up/Continental BreakfastSouth Ag Sci Bldg			
8:00 -	8:15	Welcoming Remarks			
8:15 –	8:45	<b>Featured Speaker – Dr. Gregg Howe</b> , <i>Michigan State University</i> Plant Immunity to Insect Herbivores: The Dilemma to Grow or Defend			
8:45 –	10:15	Oral Session I			
8:45	T1.	Studying the metabolic crosstalk in the plant terpenoid biosynthetic network <i>Erin Hartzell, Division of Plant and Soil Sciences, West Virginia University</i>			
9:00	T2.	Acylglucose biosynthesis: Specialized metabolic diversity in wild tomato Daniel Lybrand, Dept. of Biochemistry and Molecular Biology, Michigan State University			
9:15	ТЗ.	Transporting polyamines, amino acids and GABA from the chloroplast <i>Menaka Ariyaratne, Dept. of Biological Sciences, Bowling Green State University</i>			
9:30	T4.	Application of CRISPR-Cas9 genome editing technology to discover biological functions of plant cell wall proteins in <i>Arabidopsis</i> <i>Yuan Zhang</i> , <i>Dept. of Environmental and Plant Biology, Ohio University</i>			
9:45	Т5.	Developing a novel protein expression system: Expression of lignolytic enzymes from white-rot fungi in <i>Physcomitrella patens</i> <b>Parul Singh</b> , Dept. of Biology, University of Louisville			
10:00	Т6.	The biological function of Rab-GTT in <i>Physcomitrella patens</i> <i>Hyun Jin Jung</i> , <i>Dept. of Biology, University of Louisville</i>			
10:15 -	- 11:30	Poster Session I (Even # Posters) and RefreshmentsAgricultural Sciences Bldg.			
11:30 -	- 1:00	Oral Session IIModerator – Dr. Susanne Hoffmann-Benning, Michigan State University			
11:30	Т7.	PLAFP as a phosphatidic acid dependent signal for systemic stress response Amanda Koenig, Dept. of Biochemistry and Molecular Biology, Michigan State University			
11:45	Т8.	Persistant water stress inhibits stomatal development in <i>Betula nigra</i> and <i>Cercis canadensis</i> Noel Mano, Dept. of Botany and Plant Pathology, Center for Plant Biology, Purdue University			
12:00	Т9.	Selection of Rhizobacteria with the ability to enhance plant growth under drought and low-nutrient conditions <i>Nathan Nordstedt</i> . Dept. of Horticulture and Crop Science. Ohio State University			
12:15	<b>T10.</b>	<i>In vitro</i> functional analyses of the root-associated bacterial endophytes predict their impact in supporting plant growth in tomato <i>Tri Tien Tran, Dept. of Botany and Plant Pathology, Center for Plant Biology, Purdue University</i>			

12:30 T11.	Maize and cotton primary root growth responses to water deficit: Comparative physiological and metabolic analysis Jian Kang, Interdisciplinary Plant Group, University of Missouri, Columbia
12:45 T12.	Determining and comparing hydraulic responses between trees with different wood types <i>Kelsey Bryant, Dept. of Environmental and Plant Biology, Ohio University</i>
Lunch and Aj	fternoon, March 16
1:00 - 2:30	Lunch
1:30 - 2:20	Career Panel DiscussionLecture Hall G006
2:30 - 3:00	<b>Featured Speaker - Dr. Steve DiFazio,</b> <i>West Virginia University</i> Sex in the Salicaceae
3:00 - 4:30	Oral Session IIIModerator –Dr. Sen Subramanian, South Dakota State University
3:00 T13.	Transcriptomics identifies modules of differentially expressed genes and novel cyclotides in <i>Viola</i> pubescens Anne Sternberger, Dept. of Environmental and Plant Biology, Ohio University
3:15 T14.	Essential roles of <i>Chlamydomonas</i> galactoglycerolipid lipase PGD1 thylakoid membrane remodeling in response to adverse environmental conditions <b>Zhi-Yan Du</b> , Dept. of Biochemistry and Molecular Biology, Michigan State University
3:30 T15.	Functional characterization of nutrient mobilization-related genes in petunia petal senescence <b>Yiyun Lin</b> , Dept. of Horticulture and Crop Science, Ohio State University
3:45 T16.	Profiling genome-wide DNA methylation in identical twin almonds to explore aging and non-infectious bud-failure <i>Katherine D'Amico-Willman, Dept. of Horticulture and Crop Science, Ohio State University</i>
4:00 T17.	Mapping downy mildew resistance QTL in the progeny of two native North American grapevines Gaurab Bhattarai, Dept. of Biology, Missouri State University
4:15 T18.	The subcellular localization of <i>Ralstonia solanacearum</i> K60 Type III Effectors <i>Rachel Hiles, Dept. of Botany and Plant Pathology, Center for Plant Biology, Purdue University</i>
4:30 - 4:45	Coffee Break
4:45 – 5:45	KEYNOTE SPEAKER
	<b>Dr. Elizabeth Haswell</b> , <i>Washington University, St. Louis</i> A Feeling for the Organism: Mechanoperception in the Green Lineage
5:45 – 7:15	Poster Session II (Odd # Posters) and AppetizersAgricultural Sciences Bldg.
7:30-10:00	Banquet Dinner and NetworkingBanquet Dinner and Networking

### Sunday, March 17

7:00 –	8:00	Registration/Check-In/Continental BreakfastSouth Ag Sci Bldg		
8:00 -	9:45	Oral Session IVModerator – Dr. Nicole Waterland, West Virginia University		
8:00	T19.	An evolutionary perspective on recent duplications in the ubiquitin-26S proteasome system <i>Alexander Bochenek, Dept. of Environmental and Plant Biology, Ohio University</i>		
8:15	T20.	Biocontrol fungi in the genus Metarhizium produce ergot alkaloids in a conditionally dependent mann <i>Caroline Leadmon, Division of Plant and Soil Sciences, West Virginia University</i>		
8:30	T21.	Deletion and truncation mutants to probe functional domains of Compromised Hydrolysis of Triacylglycerols 7 (CHT7) protein in <i>Chlamydomonas reinhardtii Chase Lindeboom, Dept. of Biochemistry and Molecular Biology, Michigan State University</i>		
8:45	T22.	Optimizing GmDGAT1b for increasing oil in soybean <i>Kayla Flyckt, Corteva Agriscience, Johnston, Iowa</i>		
9:00	Т23.	Development of enzymes for robust aryloxyphenoxypropionate and synthetic auxin herbicide tolerance traits in maize and soybean crops <i>Clayton Larue, Bayer Crop Science</i>		
9:15	T24.	Hormonal regulation of defense-related secondary metabolism in polyamine-enriched tomato fruit field- tested in contrasting ecosystems <i>Tahira Fatima, Dept. of Horticulture and Landscape Architecture, Purdue University</i>		
9:30 -	10:00	Refreshment BreakSouth Ag Sci Bldg		
10:00 -	- 10:30	Featured Speaker, Dr. Jean-Michel Ané, University of Wisconsin, Madison Discoveries to Improve Nitrogen Fixation in Cereals		
10:30	- 12:00	Oral Session VModerator – Dr. Gustavo MacIntosh, Iowa State University		
10:30	T25.	Exome sequencing highlights the role of historic wild relative introgression in broadening the adaptive potential of modern bread wheat <i>Fei He, Dept. of Plant Pathology, Kansas State University</i>		
10:45	т26	Insights into the genetic architecture of growth defense tradeoffs in a foundation forest tree species		
	120.	Jennifer Riehl, Dept. of Entomology, University of Wisconsin, Madison		
11:00	T27.	Jennifer Riehl, Dept. of Entomology, University of Wisconsin, Madison Novel components of plastids with potential roles in plant stress and defense Zeeshan Banday, Dept. of Molecular Genetics and Cell Biology, University of Chicago		
11:00 11:15	T27. T28.	<ul> <li>Jennifer Riehl, Dept. of Entomology, University of Wisconsin, Madison</li> <li>Novel components of plastids with potential roles in plant stress and defense</li> <li>Zeeshan Banday, Dept. of Molecular Genetics and Cell Biology, University of Chicago</li> <li>Light-induced stabilization of ACS contributes to hypocotyl elongation during the dark-to-light transition in Arabidopsis seedlings</li> <li>Dong Hye Seo, Dept. of Botany and Plant Pathology, Center for Plant Biology, Purdue University</li> </ul>		

**11:45 T30.** Natural fumigation as a mechanism for volatile transport between flower organs *Joseph Lynch, Dept. of Biochemistry, Purdue University* 

#### 12:00 – 12:30 Business Meeting, Announcements, Award Presentations ......James H. Arbuckle Lecture Hall

12:30 pm Meeting ends. Safe travels.

### **Poster Titles**

- P1. A Scanning Electron Microscopy Technique for Viewing Plant-Microbe Interactions at Tissue and Cell Type Resolution Denise Caldwell, Department of Botany and Plant Pathology, and Center for Plant Biology, Purdue University
- **P2.** What is the role of auxin in root-mediated resistance to *Ralstonia solanacearum*? *Katherine Rivera-Zuluaga*, *Department of Botany and Plant Pathology, and Center for Plant Biology, Purdue University*
- **P3.** A Regional Education Program in Sustainable Land Reclamation Management in Central Appalachia Nan Nan, Division of Forestry and Natural Resources, Renewable Materials and Bioenergy Research Center, West Virginia University
- P4. The role of trichome-derived monoterpenes and sesquiterpenes in the interaction of cultivated tomato and potato aphid Fumin Wang, Division of Plant and Soil Sciences, West Virginia University
- **P5.** MiR156 Regulation of Flowering Time of Elevated CO2-Grown Plants *Giavonna Picknally*, *Department of Biology, Saint Joseph's University*
- **P6.** Characterizing the Function of PP2-A13 F-box Protein in Plant Development *Abigail G. Moore, Department of Environmental and Plant Biology, Ohio University*
- P7. Quantitative Expression Reduction of Differentiation and Green Like by iCRISPRi Reveals It's Function in Multiple Metabolic Pathways
   Paymon Doroodian, Environmental and Plant Biology Department, and Interdisciplinary Program in Molecular and Cellular Biology, Ohio University
- **P8.** Epigenetic Modifications are Essential for the Rhythmic Control of volatile Organic Compounds Emission in *Petunia hybrida Shannon Stirling, Department of Biochemistry, and Purdue Center for Plant Biology, Purdue University*
- P9. Time-Course Transcriptomic Analysis of Petunia × hybrida 'Mitchell Diploid' under Water Deficit Stress Using RNA Sequencing Suejin Park, Division of Plant and Soil Sciences, West Virginia University
- **P10.** Using a Poplar Hybrid to Investigate Genetic Control of Associating Insect and Fungal Communities *Sandra Simon, Department of Biology, West Virginia University*

- P11. Analysis of RIN4 homologs in regulating R-mediated immunity in Important Crop Species *Maheen Alam, Department of Biology, Lahore University of Management Sciences*
- P12. Elucidation of Genetic Components for Acidity Tolerance Using Arabidopsis GWAS Panel *Menuka Bhandari*, *Department of Biology*, *West Virginia State University*
- P13. Compartmentation of Putrescine Synthesis in Plants *Kumud Joshi*, Department of Biological Sciences, Bowling Green State University
- P14. Nucleic Acid Programmable Protein Array (NAPPA) Technology as a Functional Genomics Tool for Plant Cell Wall Biosynthesis
   Matrika Bhattarai, Department of Environmental and Plant Biology, Ohio University
- P15. Natural Variation in Arabidopsis thaliana to Discover Candidate Genes in Response to Aluminum Toxicity Arjun Ojha Kshetry, Gus R. Douglass Institute and Department of Biology, West Virginia State University
- P16. The Metabolic and Nutritional Response of *Salix* spp. to Aluminum Stress *Phillip Agosti*, *Biology Department*, *West Virginia University*
- P17. Exploration into Natural Variation for Detecting Novel QTLs for Arsenic Effects in Arabidopsis thaliana Yadira Peña-García, Department of Biology, Gus R. Douglass Institute, West Virginia State University
- **P18.** Are timber management practices impacting the insect pollinators in the Allegheny National Forest? *Craig Larcenaire, Division of Plant and Soil Sciences, West Virginia University*
- P19. Higher order knock-out mutants of Arabidopsis galactosyltransferases: A reverse genetic approach to reveal functional roles of O-galactosylation of arabinogalactan-proteins (AGPs)
   Dasmeet Kaur, Molecular and Cellular Biology Program, Department of Environmental and Plant Biology, Ohio University
- P20. Impact of Simulated Climate Change on Flower Development of Petunia Sarah A. Mills, Division of Plant and Soil Sciences, West Virginia University
- **P21.** Genetic regulation of anticancer and neuroprotective glyceollins in soybean *Asraful Jahan*, *Division of Plant and Soil Sciences, West Virginia University*
- P22. Quantitative Expression Reduction of Differentiation and Green Like by iCRISPRi Reveals Its function in a Novel Stress Responsive Pathway Paymon Doroodian, Environmental and Plant Biology Department, and Interdisciplinary Program in Molecular and Cellular Biology, Ohio University
- **P23.** Cuticular Composition and Gene Expression Analysis of Cutin Genes by RNA-seq in Habanero Pepper Fruits *Tolulope Akinmoju, Department of Biology, Gus R. Douglass Institute, West Virginia State University*
- P24. Candidate Genes for Gravity Signal Transduction Identified from Spaceflight Proteomics *Proma Basu*, Department of Environmental and Plant Biology, Interdisciplinary Program in Molecular and Cellular Biology, Ohio University
- P25. Role of Lipid Binding Proteins and Phosphatidic Acid on Stress Response and Plant Development Briaunna Murray, Department of Biochemistry and Molecular Biology, Michigan State University
- P26. Root-Fungal Associations Enhance the Plant Performance of the Invasive Plant Species, Lesser Celandine *Allison Paolucci*, *Deptartment of Environmental and Plant Biology, Ohio University*

- P27. Three beta-Glucuronosyltransferases impact physiological and biochemical functions of plant cell wall arabinogalactan-proteins in Arabidopsis thaliana
   Oyeyemi O. Ajayi, Molecular and Cellular Biology Program, Department of Environmental and Plant Biology, Ohio University
- P28. De Novo Domestication of Solanum cheesmaniae by Genome Editing Via CRISPR/Cas9: Harnessing Salinity Tolerance from a Wild Tomato Species Estefania Tavares Flores, Division of Plant and Soil Sciences, West Virginia University
- P29. Caffeine Transport in the Coffee Plant: Understanding Caffeine Distribution and Accumulation at the Subcellular Level
   *Renan T. Pinto*, *Federal University of Lavras*, *Brazil, and Division of Plant and Soil Sciences, West Virginia University*
- P30. Identification of Mobile Transcripts Across the Reciprocal Grafts Involving Watermelon and Bottle gourd by RNAseq Analysis Marleny Garcia-Lozano, Department of Biology, Gus R. Douglass Institute, West Virginia State University
- P31. A Battle of the Regulators: Contrasting Effects of ABA and Transcription Factors on Glyceolin Biosynthesis *Kiersten Jacobs*, *Department of Biology, West Virginia University*
- **P32.** Investigating Floral Transition and Optimizing Germination in *Viola pubescens Brett L. Kalfas, Department of Biological Sciences, Ohio University*
- **P33.** Genetic Improvement of Switchgrass for Energy Production Sanju Sanjaya, Energy and Environmental Science Institute, Department of Biology, Agriculture Experimental Research Station. West Virginia State University
- **P34.** Plant Response Across a Gravitational Gradient Aboard the International Space Station *Alexander Meyers, Department of Environmental and Plant Biology, and Interdisciplinary Program in Molecular and Cellular Biology, Ohio University*
- **P35.** The Effect of Metal Composition and Particle Size on Nanostructure-Toxicity in Plants *Natalie Smith*, *Department of Biology, Missouri State University*
- P36. Molecular Comparison of the Glyceraldehyde 3-Phosphate Dehydrogenase Gene in Common Ragweed and Cuman Ragweed Dave L. Robinson, Department of Biology, Bellarmine University
- P37. Towards an improved metric of *Cryptophonectria parasitica* infection to quantify species based responses to infection Sam Kukor, Department of Environmental and Plant Biology, Ohio University
- **P38.** Gene Expression at the Intersection of Microgravity and Reorientation Implicates New Regulators of Gravity Signaling *Calvin M. Coffin, Department of Environmental and Plant Biology, and Interdisciplinary Program in Molecular and Cellular Biology, Ohio University*
- **P39.** Patterns of Recombination and Segregation Distortion in the Populus Genome *Chanaka Roshan Abeyratne, Department of Biology, West Virginia University*
- **P40.** Evolutionary history and diverse functions of NODULE INCEPTION-LIKE proteins (NLPs) in root development *Narender Kumar*, *Department of Botany and Plant Pathology, Purdue University*

- P41. Comparative GWAS for Seedling Root Variation in Acidic Conditions across the Pepper Species Complexes *Tosin Akinsipe, Department of Biology, Gus R. Douglass Institute, West Virginia State University*
- P42. Functional analysis of MtMATE30, a nodule-specific membrane effluxer in the model legume *Medicago truncatula Lina Yang*, *Division of Plant and Soil Sciences, West Virginia University*
- P43. The Effects of Carbon-Based Nanoparticles on Tomato Germination and Growth Susan Eiben, Department of Environmental and Plant Biology, Ohio University
- P44. Understanding genetic and environmental factors to enable cultivation of winter malting barley in Ohio and across the Midwest
   Ben Eggers, Department of Horticulture and Crop Science, Ohio State University
- P45. Physiological and Metabolomic Responses of Black Cottonwood to Water Stress *Marvin W. Wright*, *Department of Biology*, *West Virginia University*

### **Abstracts of Presentations**

#### Oral Presentations (T1 – T30)

#### T1. Studying the metabolic crosstalk in the plant terpenoid biosynthetic network

**Erin Hartzell**<sup>1</sup>, Laura Henry <sup>2</sup>, Natalia Dudareva <sup>2</sup>, Michael Gutensohn <sup>1</sup> <sup>1</sup> Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV, USA <sup>2</sup> Department of Biochemistry, Purdue University, West Lafayette, IN, USA

Terpenoids are a large and diverse class of metabolites in plants, some of which are vital for basic processes like photosynthesis, respiration, growth and development, while others are important for the interaction of plants with their environment. All terpenoids are derived from the universal building blocks IPP and DMAPP. In plants these are produced by two independent metabolic pathways operating in parallel: the plastidic MEP pathway and the cytosolic/peroxisomal MVA pathway. Although these two pathways are localized in different plant cell compartments, the metabolites they form are routinely exchanged between them. While the multiple steps of both pathways have been studied in detail, comparatively little is known about the metabolic crosstalk between them. In this study we use two Arabidopsis mutants defective in key enzymes of the MEP and MVA pathways. The chs5 mutant is a temperature-sensitive conditional mutant in the MEP pathway gene DXS that shows a pale phenotype due to this pathway providing precursors for chlorophyll and carotenoid formation. In parallel, we have isolated a T-DNA insertion mutant for the MVA pathway gene 5-phosphomevalonate kinase (PMK) showing an embryo-lethal phenotype and reduced male gamete transmission. We have engineered an alternative metabolic route utilizing archaebacterial phosphomevalonate decarboxylase (MPD) and endogenous isopentenyl phosphate kinase (IPK) in the chs5 and pmk mutant backgrounds to study if an enhanced IPP formation in the cytosol can phenotypically complement the defects in these MEP and MVA pathway mutants, respectively. Analysis of chlorophyll and carotenoid contents in MPD / chs5 lines, representing downstream products of the MEP pathway, indicated IPP transfer from the cytosol towards plastids, while sterol contents, representing MVA pathway products, were unchanged. We are now studying if an increased cytosolic IPP pool can also be channeled towards downstream products of the MVA pathway by overexpressing MPD in the pmk mutant background.

#### T2. Acylglucose biosynthesis: Specialized metabolic diversity in wild tomato

**Daniel B. Lybrand**<sup>1</sup>, Bryan J. Leong<sup>2</sup>, Yann-Ru Lou<sup>1</sup>, Pengxiang Fan<sup>1</sup>, Anthony L. Schilmiller<sup>1</sup>, Robert L. Last<sup>1,2</sup>

<sup>1</sup> Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, USA

<sup>2</sup> Department of Plant Biology, Michigan State University, East Lansing, USA

Acylsugars constitute a large class of trichome-localized specialized metabolites that protect Solanaceous species from insect herbivores. Two acylsugar subclasses, the acylsucroses and acylglucoses, have synergistic effects on plant defense. Important crop species such as tomato ( Solanum lycopersicum ) and tomatillo ( Physalis philadelphica ) accumulate exclusively acyl sucroses whereas acylsugars of the wild tomato relative Solanum pennellii comprise primarily acyl glucoses. Here we report extension of the previously published acylsucrose biosynthetic pathway to include acylglucose production. Analysis of an acylglucose-linked quantitative trait locus identified in a backcrossed S. lycopersicum x S. pennellii population revealed a gene encoding a  $\beta$  fructofuranosidase highly enriched in trichomes of S. pennellii but present at low levels in S. lycopersicum tissues. Backcrossed inbred lines of S. lycopersicum and S. pennellii , transgenic expression of the β -fructofuranosidase in acylsucrose-accumulating introgression lines, and CRISPR-mediated knockout of the β -fructofuranosidase gene in S. pennellii further confirmed the role of the candidate gene, designated A CYL S UCROSE F RUCTO F URANOSIDASE 1 (ASFF1), in acylglucose biosynthesis. Characterization of ASFF1 in vitro revealed that the enzyme hydrolyzes the glycosidic linkage of acylsucroses to yield the acylglucoses observed in S. pennellii. While ASFF1 accepts acylsucrose substrates acylated exclusively on the pyranose ring ("P-type" acylsucroses) such as those found in S. pennellii, it does not act on the furanose-ring-acylated compounds ("F-type" acylsucroses) that accumulate in S. lycopersicum. Accumulation of P-type or F-type acylsucroses was previously shown to be controlled by a cyl s ugar a cyl t ransferase (ASAT) enzyme substrate preference and site of acylation activity. The substrate specificity of ASFF1 demonstrates how previously reported differences in ASAT activity between wild and cultivated Solanum species facilitate acylglucose biosynthesis in S. pennellii while pre-empting it in S. lycopersicum. These discoveries may aid efforts to incorporate acylglucoses into the chemical defense repertoire of tomato and other crops.

#### T3. Transporting polyamines, amino acids and GABA from the chloroplast

#### Menaka Ariyaratne, Lingxiao Ge, Paul Morris

Department of Biological Sciences, Bowling Green State University, Bowling Green, OH, USA

Polyamines are low molecular weight aliphatic organic compounds that are present in all living organisms and are essential for cell viability. Polyamine homeostasis in cells is maintained by their biosynthesis and transport. Many cells have a transport system to uptake exogenous polyamines, but only a very few studies have been carried out to identify and characterize polyamine transporters at the cellular level. The objective of this study is to utilize an E. coli mutant system to characterize the transport activity of AtBAT1. The AtBAT1 gene has two splice variants: BAT1.1 and BAT1.2 In the present study, we characterized the function of both AtBAT1.1 and AtBAT1.2by heterologously expressing the proteins in an E. coli mutant deficient in polyamine antiporters and measuring the uptake of radiolabeled substrate into inside-out membrane vesicles prepared from E. coli cells expressing the proteins. Transport assays revealed that both proteins are proton-mediated high efficiency transporters of putrescine (Km = 70  $\mu$ M and 226  $\mu$ M respectively) and spermidine (Km = 53  $\mu$ M and 173  $\mu$ M respectively). Competition assays showed that BAT1.1 functions as a high affinity GABA and arginine transporter. Since BAT1 was originally identified as a broad substrate amino acid transporter, we used competition assays to show that the uptake of spermidine is inhibited by mM levels of alanine, glutamate and agmatine. Transient expression of BAT1.1-GFP in the leaves of Nicotiana benthamiana showed that BAT1.1 was localized to the ER in the veins of leaves and the chloroplast of mesophyll cells. BAT1.2 was found to be localized only to the chloroplast. Based on these evidences, we conclude that BAT1 is a proton mediated high affinity

exchanger of polyamines, arginine and GABA. In addition, it has a low affinity for agmatine, alanine and glutamate. Since the chloroplast serves as a site for the synthesis of arginine, agmatine, GABA, and putrescine, BAT1 can regulate the export of these metabolites from the organelle.

### T4. Application of CRISPR-Cas9 genome editing technology to discover biological functions of plant cell wall proteins in *Arabidopsis*

#### Yuan Zhang and Allan M. Showalter

Molecular and Cellular Biology Program, Department of Environmental and Plant Biology, Ohio University, Athens, OH, USA

Arabinogalactan-proteins (AGPs) are a diverse family of plant hydroxyproline-rich glycoproteins that are known for the abundance of sugars present on their molecular surface. Addition of the various sugars to AGPs requires the action of a large number of distinct enzymes, called glycosyltransferases (GTs). To elucidate the biological functions of the glycan chains in general on AGPs, eight gal actosyl t ransferases (GALTs), namely, GALT2-6 and HPGT1-3, which catalyze the addition of the first sugar, galactose, onto AGPs are being studied. Most higher-order galt mutants were indistinguishable compared to WT with respect to vegetative growth; however, the galt2 galt3 galt4 galt5 galt6 mutant exhibited several abnormal phenotypes in reproductive growth including defective pollen, shorter siliques, and increased seed abortion. This indicates that the AGP glycan chains play a key role in plant reproduction. Glucuronic acid (GlcA), the only negatively charged sugar on AGPs, is added by three glucuronic acid transferases (GLCATs), namely GLCAT14A, GLCAT14B, and GLCAT14C. Some glcat mutants exhibited delayed germination, reduced seed set, misshapen pollen, and defective pollen development. This indicates that GlcA on AGPs is critical in certain stages of plant development. Higher-order genetic mutants of GTs generated in this project will elucidate the functional importance of the Gal residues (added by the GALTs) and the GlcA residues (added by the GLCATs), thus revealing sugar structure/function relationships for the AGPs. Our CRISPR/Cas9 approach provides a simpler and faster way to generate higher-order plants mutants, which is especially useful for editing multi-gene families with members having redundant functions such as cell wall related gene families.

### T5. Developing a novel protein expression system: Expression of lignolytic enzymes from white-rot fungi in *Physcomitrella patens*

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Plant cell walls function in structural support and protection from pathogens, and also represent a potential major renewable carbon source that could be used in bio-ethanol production. The major challenge with processing plant biomass is the presence of lignin, a complex aromatic polymer that is recalcitrant to chemical and biological degradation. However, a group of white-basidiomycetes fungi produce a number of enzymes that can break down lignin from woody and non-woody plants, allowing the fungi to use cell wall sugars as an energy source. Out of five different enzymes we selected from two fungus species, Pleurotus eryngii and Phanerochaete chrysosporium, we cloned laccase from Pleurotus eryngii , aryl-alcohol dehydrogenase and glyoxal oxidase from Phanerochaete chrysosporium . The enzymes were chosen on the basis of their broad substrate specificity, suitable activity and stability, and sufficient genomic information, for further study. To utilize these lignin degradation enzymes, we are expressing them in a novel system using an inducible vector. ggb mutants of the moss Physcomitrella patens grow as individual undifferentiated cells similar to unicellular green algae. *P. patens* doesn't produce lignin, making it a tremendously useful plant system for expressing these delignification enzymes. Our ultimate goal is to bind these active enzymes to nanomembranes for large-scale bioprocessing of lignin-rich biomass for bioenergy and industrial bioproduct applications.

#### T6. The biological function of Rab-GTT in *Physcomitrella patens*

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Protein prenylation, a common lipid post-translational modification, is required for growth and development in eukaryotes. Rab geranylgeranylation involves the addition of one or two 20-carbon geranylgeranyl moieties to Rab-GTP target proteins, which regulate vesicle transport. The reaction is carried out by Rab geranylgeranyltransferase (Rab-GGT), which is composed of three subunits, RGTA, RGTB, and REP. Loss of function of the Rab-GGT α subunit RGTA1 has not been reported in any plant. While knockout of either of the two β subunits RGTB1 or RGTB2 results in no phenotype in the moss Physcomitrella patens, knockout of RGTB1 resulted in loss of apical dominance and gravitropism in the flowering plant Arabidopsis. These results showed that two Rab-GGT β subunits in P. patens are functionally redundant, but RGTB2 in Arabidopsis could not fully compensate for the loss of function of RGTB1 . Therefore, the biological functions of Rab geranylgeranyltransferase remain largely unknown. Previous studies in our lab showed that complete knockout of any P. patens Rab-GGT components (RGTA1, RGTB1 & amp; RGTB2, REP) appeared to be lethal, since no viable single mutant plants of RGTA1 or REP and double mutant plants of RGTB1 and RGTB2 were recovered. Here we have generated P. patens plants containing artificial miRNA constructs targeting each Rab-GGT component by an inducible knockdown system using  $\beta$ -Estradiol as a inducer, and systematically analyzed the phenotype upon β-Estradiol induction. Our results show that knockdown of either RGTA1 or REP, or knockdown of RGTB1 in an rgtb2 background, resulted in defects in tip growth and polar cell elongation. These studies will help reveal the function of Rab-GGT in regulating cell polarity and differentiation.

#### T7. PLAFP as a phosphatidic acid dependent signal for systemic stress response

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To survive fluctuating environmental conditions, plants must adjust their development. While there are many known local signals that respond to stress stimuli, systemic coordination via long-distance signaling mechanisms are essential for a rapid distal response. Long-distance transport in plants occurs in the vascular bundles. One component, the phloem is a dynamic tissue that not only transports photoassimilates from source to sink but also bidirectionally traffics metabolites, nucleic acids, proteins, and even lipids. I am investigating PHLOEM LIPID-ASSOCIATED FAMILY PROTEIN (PLAFP) and its role in long-distance signaling in Arabidopsis thaliana . We identified PLAFP and other predicted lipid-binding proteins (LBP) in the phloem. Interestingly, lipids such as oxylipins and phosphatidic acid are found in the phloem sap as well. We propose that phloem LBPs act in longdistance, lipid-mediated signaling to systemically coordinate the plant's response to stress. We have shown that PLAFP binds specifically to phosphatidic acid (PA) and that PLAFP is induced by the same abiotic stresses that induce production of PA. We are investigating the PLAFP-PA movement and the biochemical mechanism by which PLAFP interacts with PA in the phloem. A pull down approach revealed putative PLAFP-interacting proteins, including MAP kinases, which could mediate downstream signaling. RNA-Seq analysis of PLAFP wild type, knock-down, overexpression, and complementation lines indicate which processes PLAFP-PA might coordinate. To better understand PLAFP's ability to solubilize lipids for transport in the phloem, I will mutagenize conserved arginine, lysine, and tryptophan residues, followed by lipid binding assays, to study the biochemical mechanism of binding. We will employ optogenetics to investigate PLAFP systemic movement and the role of PA in complex movement, as well as crystallography to study the structure of the PLAFP-PA complex. This project was funded in part by USDA grant #MICL04147 and NSF grants #1144391/1836680 to SHB and USDA-NIFA NNF 2015-38420-23697 to AK.

#### **T8.** Persistant water stress inhibits stomatal development in *Betula nigra* and *Cercis Canadensis*

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Because plant stomata control transpirational water loss to the environment, stomatal development would be expected to be responsive to reduced water availability. However, changes in stomatal development in response to water stress vary considerably among species, especially in trees. Moreover, the stomatal plasticity of many common North American tree species has not been investigated in detail. We examined the stomatal developmental response of three species, red maple (Acer rubrum L.), river birch (Betula nigra L.) and Eastern redbud (Cercis canadensis L.), subjected to mild and severe persistent water deficit. Birch and redbud leaves reduced the fraction of leaf epidermis that was occupied by stomatal apparatus (stomatal pore index, SPI) through smaller stomatal size and reduced stomatal density, respectively. Birch and redbud had a lower stomatal index in response to water stress. In birch and redbud leaves, photosynthesis rates and stomatal conductance were highly correlated with SPI, such that water stressed leaves could reduce gas exchange and transpirational water loss. Despite the smaller stomatal area fraction, leaves that had developed under water deficit could return to non-stress rates of photosynthesis and stomatal conductance after a period of restored water sufficiency. Under more severe stress, similar trends in stomatal development were observed, with the exception that under severe stress conditions, maple leaf stomata were also smaller. Of the different measures of water relations used in this study, osmotic potential was shown to be the most predictive of stomatal plasticity, being correlated with at least one of size, density, index or SPI in all three species. While tree species may reduce stomatal development due to water stress, this response is not universal, and ultimately does not impede growth potential following resumption of water sufficient conditions. We propose that tree species in temperate North America exhibit pronounced differences in stomatal plasticity in response to water deficit.

#### **T9.** Selection of *Rhizobacteria* with the ability to enhance plant growth under drought and lownutrient conditions

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Low nutrient and post-harvest drought stress are two of the most limiting factors to horticulture crop quality; causing stunted growth and reduced flowering. Plant growth promoting rhizobacteria (PGPR) have emerged as an innovative and sustainable solution to alleviate abiotic stresses in plants via their ability to enhance nutrient uptake, produce plant growth-promoting hormones, and reduce stress hormones such as ethylene. In this study, a collection of 45 Pseudomonas strains were screened for their ability to alleviate abiotic stress when applied to floriculture crops. Polyethylene glycol (PEG) was used for the in vitro selection of osmotic stress tolerant bacteria, a trait correlated with the ability to alleviate drought stress in plants. A second bioassay selected bacteria that produce the enzyme 1-aminocyclopropane-1-carboxylic acid deaminase (ACCd). ACCd reduces the amount of stress ethylene produced by plants during times of abiotic stress by degrading the ethylene precursor, ACC. Of the 45 strains tested in the PEG and ACC-deaminase bioassays, 14 and 6 were selected, respectively, with one strain being selected in both bioassays. A high-throughput greenhouse trial was developed to evaluate the effectiveness of the selected strains to promote plant growth under drought and low-nutrient conditions. Shoot biomass and flower number, two characteristics important to floriculture crop value, were evaluated as indicators of plant growth promotion. Of the 19 bacteria strains tested, three elite strains were selected for their ability to promote plant growth under both drought and low nutrient conditions. The three elite strains were validated in a production scale experiment for their ability to increase growth of Petunia hybrida, Impatiens walleriana, and

Viola wittrockiana. Application of each of the three strains resulted in a significant increase in plant biomass of all three species. This work provides an efficient method to select and validate bacteria for their ability to increase floriculture crop quality.

### T10. *In vitro* functional analyses of the root-associated bacterial endophytes predict their impact in supporting plant growth in tomato

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Plant roots constantly interact with a diversity of microorganisms, including pathogenic and beneficial microbes. The complex community of microorganisms living in close association with the root, the root microbiome, is significant for achieving optimal plant health and productivity. Recent advances in plant microbiome research revealed that plant genotype has a small but significant contribution to root microbiome assembly. Identifying microbial isolates that lead to plant growth promotion or disease suppression traits in greenhouse or field environments is currently a bottleneck in plant microbiome work. We hypothesized that phenotyping multiple in vitro functions of culturable root endophytic bacteria could predict their impact when inoculated on plants in greenhouse conditions. We isolated 183 bacterial endophytes from the roots of a domesticated tomato species Solanum lycopersicum and its wild cousin S. pimpinellifolium grown in a field in central Indiana. Sanger sequencing of 16S rRNA was used to identify the isolates. We tested 30 isolates from each genotype for four in vitro functional traits: auxin production, siderophore production, phosphate solubilization, and antagonism to a soilborne bacterial pathogen of tomato, Ralstonia solanacearum . We also screened for the presence of DAPG or HCN synthesis genes in the genome of the isolates. Hierarchical clustering of the in vitro functional traits suggested four main groups: auxin high-producers, phosphorus solubilizer, siderophore producer, and a group with high antagonism against R. solanacearum. The auxin producers tended to have low antagonistic traits. Isolates with the DAPG biosynthesis gene also tended to be strong siderophore producers. Isolates from each different functional group were inoculated on tomatoes grown in the greenhouse. Preliminary results indicate that isolates with similar in vitro functional traits perform similarly in binary experiments with plants. Understanding how in vitro functional traits of bacteria relate to their impact on plants may offer new opportunities for crop improvement with plant-associated microbes.

### T11. Maize and cotton primary root growth responses to water deficit: Comparative physiological and metabolic analysis

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Water deficit is one of the most significant stresses affecting plant growth and crop production worldwide. Although the relative maintenance of root compared with shoot growth under water deficits has been reported in both maize (*Zea mays L.*) and cotton (*Gossypium hirsutum L.*), it is not known whether similar or different mechanisms are involved in the adaptation of root growth to water deficits. In both species, the response of cell elongation to water deficit varies within the primary root growth zone, with distinct regions of growth maintenance and inhibition. With similar growth patterns and by imposing treatments resulting in similar root water potentials, we hypothesized that comparison of the metabolic responses between the species will enhance our ability to discern changes that are important for the regulation of root growth under drought. Comprehensive metabolite analyses associated with the differential responses to drought in the primary root growth zone in both species were performed. In maize, 111 metabolites increased in abundance (123 for cotton) and 196 declined in

abundance (320 for cotton), indicating a major disruption of normal metabolic activity. However, although the extent of the disruption was similar and there were commonalities in the metabolic response, there were also significant differences that have important implications for the control of growth in water-stressed roots of the two species. Sulfur metabolism exhibited the most striking differences that separate the responses of the two species to drought. Sulfur metabolites tend to increase in abundance in maize while decreasing in cotton. Further investigations into the abundance of glutathione, an essential metabolite in the sulfur metabolism pathways, verified the quantitative differences in the response of the two species. Current research is focusing on the effects of manipulating sulfur levels on cotton primary root growth under drought. Supported by Cotton Inc. grant no. 13-472 to R.E.S.

#### T12. Determining and comparing hydraulic responses between trees with different wood types

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Hydraulic dysfunction and the associated carbon depletion are two of the determining factors in tree mortality. We are interested in examining physiological responses involved in the carbon-water trade-off of trees with different wood types when they experience a decrease in atmospheric water availability. In temperate forests, deciduous trees are either diffuse or ring porous and have diverse hydraulic characteristics and carbohydrate requirements. We are interested in how the sap flux of ring- and diffuse-porous species vary in response to an increasing vapor pressure deficit. We predicted that ring-porous trees would maintain higher sap flux despite increasing atmospheric dryness because of their higher carbohydrate requirements. Previous studies have found conflicting results but were conducted on individuals of differing size and age classes; we seek to resolve this conflict by measuring mature canopy species of similar ages and sizes. We measured sap flux using established protocols from the Granier thermal dissipation method and calculated vapor pressure deficit from recorded temperature and humidity values. Diffuse-porous species included Fagus grandifolia and Acer saccharum ; ringporous species included Quercus rubra and Quercus alba . We found that sap flux significantly varied with vapor pressure deficit (p<0.001) and wood type (p<0.01). Furthermore, we found that wood type significantly affected the relationship between sap flux and vapor pressure deficit (p<0.05). These results support our hypothesis that ring- and diffuse-porous species respond differently as the atmosphere becomes drier. However, contrary to our predictions, the diffuse-porous species maintained a higher sap flux than the ring-porous species as vapor pressure deficit increased. This finding may be due to the substantial late season precipitation. We will continue collecting sap flux measurements over another growing season to further understand this trend and will also measure leaflevel gas exchange and xylem water potential to provide additional lines of evidence.

### **T13.** Transcriptomics identifies modules of differentially expressed genes and novel cyclotides in *Viola pubescens*

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Viola is a large genus with worldwide distribution and many traits not currently exemplified in model plants including unique breeding systems and the production of cyclotides. Here we report *de novo* genome assembly and transcriptomic analyses of the non-model species *Viola pubescens* using short-read DNA sequencing data and RNA-Seq from eight diverse tissues. First, *V. pubescens* genome size was estimated through flow cytometry, resulting in an approximate haploid genome of 455 Mbp. Next, the draft *V. pubescens* genome was sequenced and assembled resulting in 264,035,065 read pairs and 161,038 contigs with an N50 length of 3,455 base pairs

(bp). RNA-Seq data were then assembled into tissue-specific transcripts. Together, the DNA and transcript data generated 38,081 ab initio gene models which were functionally annotated based on homology to Arabidopsis thaliana genes and Pfam domains. Gene expression was visualized for each tissue via principal component analysis (PCA) and hierarchical clustering, and gene co-expression analysis identified 20 modules of tissue-specific transcriptional networks. Some of these modules highlight genetic differences between chasmogamous and cleistogamous flowers and may provide insight into *V. pubescens* ' mixed breeding system. Orthologous clustering with the proteomes of *A. thaliana* and *P. trichocarpa* revealed 8,531 sequences unique to *V. pubescens* , including 81 novel cyclotide precursor sequences. Cyclotides are plant peptides characterized by a stable, cyclic cystine knot motif, making them strong candidates for drug scaffolding and protein engineering. Analysis of the RNA-Seq data for these cyclotides was also highlighted in a maximum likelihood protein cladogram containing *V. pubescens* cyclotides and published cyclotide sequences from other Violaceae and Rubiaceae species. Collectively, this work provides the most comprehensive sequence resource for Viola , offers valuable transcriptomic insight into *V. pubescens*, and will facilitate future functional genomics research in Viola and other diverse plant groups.

### T14. Essential roles of *Chlamydomonas* galactoglycerolipid lipase PGD1 thylakoid membrane remodeling in response to adverse environmental conditions

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Photosynthesis occurs in the thylakoid membrane, where the predominant lipid is monogalactosyldiacylglycerol (MGDG). As environmental conditions change, photosynthetic membranes have to adjust. In this study, we used a loss-of-function Chlamydomonas reinhardtii mutant deficient in the MGDG-specific lipase PGD1 (PLASTID GALACTOGLYCEROLIPID DEGRADATION1) to investigate the link between MGDG turnover, chloroplast ultrastructure, and the production of reactive oxygen species (ROS) in response to different adverse environmental conditions. The pgd1 mutant showed altered MGDG abundance and acyl composition and altered abundance of photosynthesis complexes, with an increased PSII/PSI ratio. Transmission electron microscopy showed hyperstacking of the thylakoid grana in the pgd1 mutant. The mutant also exhibited increased ROS production during N deprivation and high light exposure. Supplementation with bicarbonate or treatment with the photosynthetic electron transport blocker DCMU protected the cells against oxidative stress in the light and reverted chlorosis of pgd1 cells during N deprivation. Furthermore, exposure to stress conditions such as cold and high osmolarity induced the expression of PGD1, and loss of PGD1 in the mutant led to increased ROS production and inhibited cell growth. These findings suggest that PGD1 plays essential roles in maintaining appropriate thylakoid membrane composition and structure, thereby affecting growth and stress tolerance when cells are challenged under adverse conditions.

#### T15. Functional characterization of nutrient mobilization-related genes in petunia petal senescence

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Flower longevity is an important characteristic of floriculture crops. When petals senesce, ornamental plants lose their aesthetic and economic value. However, the regulatory mechanisms involved in flower petal senescence are complex and not well understood. Although nutrient mobilization is considered important in the regulation of flower petal senescence, there is a lack of genetic evidence to substantiate the roles of nutrient mobilization-related genes during this process. A recent transcriptomic study of *Petunia*  $\times$  *hybrida* corollas identified nutrient mobilization-related autophagy (ATG) and nuclease (NUC) genes that are differentially regulated during petal senescence. We hypothesize that regulating the expression levels of these genes will affect the longevity of

petunia flowers, with varying effects under high and low nutrient conditions. To test this hypothesis, individual genes were knocked down using virus-induced gene silencing (VIGS) in *Petunia* × *hybrida* 'Picobella Blue'. The *ATG*-silenced plants were fertilized with 50 or 250 ppm N from 15-5-15 fertilizer at every irrigation, and the NUC -silenced plants were given nutrient solutions with normal or low phosphate levels. Our results indicate that the silencing of *NUC1* resulted in delayed flowering and early senescence, and the silencing of three individual *ATGs* caused changes in flowering time, flower longevity, and flower number in petunia under different fertility levels. The genes identified in this study will be further tested using stable genetic engineering and genome editing. The results of this project will be fundamental for future studies of flower petal senescence and will provide genetic information for future floriculture crop improvement.

#### **T16.** Profiling genome-wide DNA methylation in identical twin almonds to explore aging and noninfectious bud-failure

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All eukaryotes age, but perennial plants seem to defy this phenomenon as they experience cycles of dormancy, vegetative, and reproductive growth while exhibiting prolonged longevity. Despite the negative impacts aging can have on crop production and yield, the process is poorly understood and research neglected for perennial crops such as fruit and nut trees. Almond, an important nut crop produced primarily in the United States, exhibits non-infectious bud-failure (BF), an age-related disorder. This disorder affects vegetative bud development, indirectly affecting kernel-yield, and represents an opportunity to address aging in a commercially-relevant, vegetatively-propagated, perennial crop. Using monozygotic twin almond pairs with discordant BF exhibition, whole-genome bisulfite sequencing was performed to search for differentially methylated regions (DMRs) in the almond genome. Sequencing reads were mapped back to the reference genome, and methylated bases were called to distinguish genome-wide methylation patterns. Identified DMRs and other quantifiable features derived from the bisulfite sequencing are being analyzed to determine their association with phenotypic data from and within the twin pairs, namely BF exhibition. Results from this work will expand our current understanding of aging in a perennial crop while providing additional information about the potential of BF exhibition in breeding and production efforts of almond. Translation of this information will aid in tackling BF as an obstacle to almond breeding and nursery propagation and provide a basis for the interrogation of aging in other Rosaceae crops in which it is an issue but remains unexplored.

#### T17. Mapping downy mildew resistance QTL in the progeny of two native North American grapevines

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Linkage maps and QTL analysis have become essential tools for the positional cloning of agronomically important genes and for marker-assisted selection. In this study, two North American grape species, *Vitis rupestris* and *Vitis riparia*, and their 294 F 1 progeny were used to construct parental linkage maps and to perform QTL analysis for downy mildew resistance. Marker discovery was accomplished by genotyping-by-sequencing (GBS), and resulted in 348,888 single nucleotide polymorphism (SNP) markers. Of these, 11,063 informative markers

(3.17% of original SNP dataset) were retained after filtering for various quality parameters and missing data. A two-way pseudo-testcross strategy was followed for map construction using JoinMap5.0. The 1,115 and 1,177 significant markers (threshold  $\text{LOD} \ge 14$ ) for V. riparia and V. rupestris were grouped into 19 linkage groups covering 1657.4 and 1401.3 cM of genetic distances with an average marker interval of 1.486 and 1.19 cM, respectively. Maps were validated by pinpointing a single significant QTL determining maleness at chromosome 2 in the genetic background of the V. riparia male parent. Phenotype data for leaf downy mildew resistance were collected with both in vitro and natural inoculation of 86 and 136 F 1 progeny, respectively. With both methods, QTL analysis for reduced leaf area coverage by mildew lead to a significant peak at chromosome 10 in V. rupestris explaining 15-45% of the phenotypic variance. For in vitro inoculation, a significant QTL was detected for reduced disease intensity at chromosome 8 of V. riparia also, explaining 15% of the variance. These are the first SNP-based high-density linkage maps of these native North American grape species. The maps are expected to serve as an important resource for breeding modern varieties for environment-friendly grape cultivation. Keywords: Downy mildew, QTL, Linkage map, GBS, Marker-assisted selection

#### T18. The subcellular localization of Ralstonia solanacearum K60 Type III Effectors

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*Ralstonia solanacearum* is a soil-borne bacterial pathogen that causes Bacterial Wilt disease in over 200 plant species in 53 botanical families This detrimental disease is found globally and is caused by various strains of *R. solanacearum*; one example is the K60 strain which is found in the United States. Bacterial pathogens like *R. solanacearum* use their type III secretion system (T3SS) to suppress host immune responses and cause disease. T3SS is composed of a needle-like structure that pierces the host cell wall and secretes type III effector (T3E) proteins into the host cell. The functionality of the T3Es in the K60 strain is not well understood A small list of K60 T3E proteins was selected to be studied; the first step in this experiment is to determine the subcellular localization of these T3Es A green fluorescent protein (GFP) was attached to the C-terminus of the individual T3E, and was transiently expressed in two systems: the hairy root system in tomato and the *Nicotiana benthamiana* leaf system. The subcellular localization of each T3E was observed with the Zeiss LSM 880 Upright Confocal Microscope. Preliminary results suggest that one T3E, RsK60-17, localizes at the plasma membrane, RsK60-15 at the nucleus, and two, RsK60-3 and RsK60-6, localize at actin filaments. Future work will use secretion assays to confirm secretion of the T3Es, as well as yeast two-hybrid and mutant analyses to determine the interacting partners and function of the individual T3Es.

#### T19. An evolutionary perspective on recent duplications in the ubiquitin-26S proteasome system

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The Ubiquitin-26S proteasome system (UPS) is a vital piece of machinery common amongst eukaryotes. The UPS involves first the ubiquination of proteins leading to degradation within the 26S proteasome. The plant UPS has been largely expanded; particularly so in land plants. Although it is commonly agreed that the large size of the UPS is important for plants to combat various stresses and adapt to their sessile life style, many of its members remain uncharacterized in plant genomes. Looking into the duplication patterns of the UPS in a short evolutionary history may highlight the mechanism of how this system expanded in the plant kingdom. Here, we took advantage of the recent accomplishments in a genome sequencing project of 11 closely-related species in the rice genus and systematically analyzed their UPS members. As a comparison, we also reannotated those members in three Arabidopsis species that split 5-10 million years ago. Through comparing the orthologous relationship of the UPS

members, we expect to categorize ancient and recent duplications within orthologous groups. By analyzing their duplication mechanisms, evolutionary selections, and expression patterns, we will address the role of the UPS in plant adaption and the unique duplication mechanisms of its members.

### **T20.** Biocontrol fungi in the genus *Metarhizium* produce ergot alkaloids in a conditionally dependent Manner

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Ergot alkaloids are important agricultural and pharmaceutical chemicals. Genomic sequence data indicate that fungi in the genus Metarhizium have the capacity to produce lysergic acid-derived ergot alkaloids; however, the accumulation of ergot alkaloids in these fungi has not been experimentally demonstrated. Metarhizium species colonize soil, plant roots, and insects. Because of these properties, some Metarhizium species are used as biocontrol agents. We investigated Metarhizium species grown under different conditions for accumulation of ergot alkaloids by high performance liquid chromatography (HPLC) with fluorescence detection and mass spectrometry. Metarhizium brunneum and Metarhizium flavoviride were cultured saprotrophically on three different media. Accumulation of ergot alkaloids varied by medium and fungus. Metarhizium flavoviride did not accumulate ergot alkaloids on any of the media, whereas Metarhizium brunneum accumulated large quantities of the ergot alkaloids lysergic acid α-hydroxyethylamide (LAH), ergine, ergonovine and chanoclavine-I only on sucrose yeast extract agar. We also investigated the accumulation of ergot alkaloids under the ecologically relevant conditions of mutualistic growth of the fungi on plant roots and parasitic growth in infected insects. We inoculated the roots of corn (Zea mays), bean (Phaseolus vulgaris), and Medicago truncatula with M. brunneum and *M. flavoviride*, and no ergot alkaloids were produced by either fungus on roots of any of the plants. Larvae of the insect Galleria mellonella were inoculated with spore suspensions of M. anisopliae, M. flavovoride, M. brunneum, and M. robertsii. Each of the four species produced ergot alkaloids in infected larvae. The mean concentration of LAH (the most abundantly accumulating ergot alkaloid) in M. brunneum-infected larvae was 154 mM. The data demonstrate that several Metarhizium species have the ability to produce ergot alkaloids of the lysergic acid amide class and that production of ergot alkaloids is tightly regulated and associated with insect colonization.

### T21. Deletion and truncation mutants to probe functional domains of Compromised Hydrolysis of Triacylglycerols 7 (CHT7) protein in *Chlamydomonas reinhardtii*

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Under nutrient-limiting conditions, microbes enter a quiescent state where they temporarily cease growth and accumulate high-value carbon compounds such as triacylglycerols (TAG) that can be used in biofuel and feedstock production. Upon nutrient resupply, TAG is degraded to fuel cellular growth; however, previous work has identified a mutant of the unicellular algae, *Chlamydomonas reinhardtii*, that was delayed in regrowth and degradation of TAG upon refeeding of nitrogen (N) to N deprived cultures. The mutant contained a deletion of a gene termed Compromised Hydrolysis of Triacylglycerols 7 (CHT7). While CHT7 contains a domain known in other species to bind DNA, the mechanism of how it regulates cell life-cycle decision making as well as its functional domain(s) are still largely unknown. To determine what portions of CHT7 are functionally important, we introduced truncation and deletion mutations in the genomic sequence of *CHT7* using site-directed mutagenesis PCR. These constructs were introduced into the Chlamydomonas *cht7* mutant, and the resulting lines

were examined for complementation of the mutant phenotypes such as delayed degradation of TAG and delayed regrowth during N-resupply following N-deprivation. We found that the truncated mutants displayed phenotypes similar to the cht7 mutant indicating the C-terminus region of CHT7 is necessary for its activity. Furthermore, the deletion of one of the predicted protein-protein interaction domains resulted in almost complete loss of CHT7 function. Understanding which portions of CHT7 are functionally important could grant us a better understanding of cell life-cycle decision making that could potentially be utilized in engineering algae for the production of biofuels and feeds.

#### T22. Optimizing GmDGAT1b for increasing oil in soybean

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Soybean derived protein and oil are important commodities for animal feed and human consumption. Improving soybean seed composition by increasing oil and protein content has the potential to add significant value to the crop and improve environmental sustainability. Diacylglycerol acyltransferase (DGAT) catalyzes the final step in triacylglycerol (TAG) biosynthesis. We have previously demonstrated that a soybean DGAT1b variant with 14 amino acid substitutions (GmDGAT1b-MOD) is improved compared to WT and increases oil content by 3 percentage points when overexpressed in soybean (Roesler et al., 2016). Recent work has aimed to identify a GmDGAT1b variant with improved activity and/or stability with four or fewer substitutions, which could be introduced into soy by CRISPR editing. We have utilized a high-throughput Nile red assay to screen GmDGAT1b variants in S. cerevisiae. A subset of these variants was also tested in planta by transient expression in *N. benthamiana* leaf. We have implemented SPE/GC-FID and HPLC-ELSD methods to enable oil analysis for our transient expression system. Using these techniques, we have identified variants with one to four amino acid substitutions that accumulate more oil than WT GmDGAT1b in yeast and *N. benthamiana*. One variant with three substitutions accumulated oil to the same levels as the GmDGAT1b-MOD variant in *N. benthamiana*. These results suggest that GmDGAT1b can be modified by CRISPR editing to improve soybean oil content.

### T23. Development of enzymes for robust aryloxyphenoxypropionate and synthetic auxin herbicide tolerance traits in maize and soybean crops

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Effective management of weedy species in agricultural fields is essential for maintaining favorable growing conditions and crop yields. The introduction of genetically modified crops containing herbicide tolerance traits has been a successful additional tool available to farmers to better control weeds. However, weed resistance challenges present a need for additional options. To help meet this challenge, a new trait that provides tolerance to the aryloxyphenoxypropionate (FOPs) herbicides and members of the synthetic auxin herbicide family, such as 2,4-D, was developed. Development of this herbicide tolerance trait employed an enzyme engineered with robust and specific enzymatic activity for these two herbicide families. This engineering effort utilized a microbial-sourced dioxygenase scaffold to generate variants with improved enzymatic parameters. Additional optimization to enhance in-plant stability of the enzyme enabled an efficacious trait that can withstand the higher

temperature conditions often found in field environments. The enhanced enzymatic and temperature stability parameters of the enzyme variants confer on transgenic maize and soybeans robust herbicide tolerance that is useful in weed management systems using these two herbicide families.

### T24. Hormonal regulation of defense-related secondary metabolism in polyamine-enriched tomato fruit field-tested in contrasting ecosystems

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Secondary metabolites, phenolics and flavonoids, are important biomolecules linked to human health as well as plant defense against abiotic and biotic stresses. Our goal is to improve the nutritional quality along with extended shelf-life of tomato fruit. We hypothesized that specific enhancement of biogenic amines spermidine (SPD) and spermine (SPM) in tomato will lead to higher metabolic activity, providing precursors for flavonoids and polyphenols. We developed genetically engineered tomato plants whose fruit specifically express a heterologous S-adenosylmethionine decarboxylase gene and accumulate higher levels of SPD/SPM. Simultaneously, we also developed engineered lines with deficiency of either the stress hormone methyl jasmonate or the ripeninghormone ethylene, and backcrossed each of these with high SPS/SPM lines to develop double transformants. All these transgenic lines along with azygous controls were tested in two consecutive years under two different agroecosystems, made by black polyethylene (BP) and hairy vetch mulch (HV), at the USDA's Beltsville North Farm. In addition to determining the changes in primary metabolome, we focused also on analyzing the levels of secondary metabolites including polyphenols and flavonoids in these tomato lines. In parallel, we obtained RNA-Seq analysis of the respective tomato lines to discern the relationship between genetic potential vis a vis the content of different polyphenols and flavonoids. Our results have demonstrated specific interactions between genotypes and agroecosystems in modulating the accumulation of polyphenols and flavonoids in ripe tomato fruits. These findings reiterate that higher biogenic polyamines SPD and SPM regulate plant metabolic processes in a positive manner.

### T25. Exome sequencing highlights the role of historic wild relative introgression in broadening the adaptive potential of modern bread wheat

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Introgression from wild relatives is a potential source of beneficial diversity. The contribution of introgression from wild relatives to adaptive evolution of bread wheat remained unknown. Here, we used a newly released wheat genome reference to create a diversity map of the wheat genome including nearly 7.3 million SNPs. The data was generated using targeted re-sequencing of 890 diverse hexaploid and tetraploid wheat accessions including tetraploid wild emmer wheat. We used this data to identify genomic regions showing the signals of introgression from wild emmer. By analyzing the patterns of selection associated with wheat improvement and environmental adaptation, we assessed the contribution of wild emmer introgression to local adaptation, crop improvement, and distribution of different functional classes of SNPs across the wheat genome. Genomic regions targeted by improvement selection and environmental adaptation substantially overlapped with introgressed genomic regions, respectively, suggesting that wild emmer likely contributed beneficial allelic diversity used during the development of locally adapted cultivars By studying wheat phenotypic diversity, we showed that historic gene flow from wild relative played an important role in shaping the agronomic traits in modern wheat and likely broadened its adaptive potential. A detailed map of genome-wide introgression developed in our study can guide targeted deployment of wild relative diversity for wheat improvement

#### T26. Insights into the genetic architecture of growth-defense tradeoffs in a foundation forest tree species

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Physiological tradeoffs in plant allocations to growth versus defense govern the ecology and evolution of treeinsect interactions and influence sustainable production of forest ecosystems. Despite the importance of plant defenses to forest health, a fundamental gap remains in our knowledge concerning the genetic architecture of growth-defense trade-offs. Populus provides an ideal model system to study the underlying genetic architecture of different resource allocation strategies, as phenolic defense compounds (e.g., phenolic glycosides) are strongly and negatively correlated with growth. This study aims to identify the genetic architecture underlying variation in key growth and chemical defense traits in a foundation forest tree species (Populus tremuloides) using genome-wide association analysis combined with complementary methods (e.g., multiple marker association analysis, extreme phenotype sampling, and inclusion of transcriptomic data). A large association mapping common garden of Populus tremuloides was established in 2010 with four replicate blocks of genotypes (N = 515) collected from a north-south transect throughout Wisconsin, U.S.A. We evaluated a suite of relevant tree traits between 2014 and 2017. Sequence capture genotyping of the WisAsp population resulted in the discovery of ~170,000 SNPs. In our preliminary analysis, budbreak and phenolic glycosides showed high broad-sense heritability, while growth traits showed low to moderate heritabilities. Single-marker GWA analyses have revealed only six significant SNPs in both growth and defense traits. Multiple-SNP GWA analysis revealed that for all traits, no more than 40% of the variation explained by our SNP set was attributed to SNPs with larger effects, meaning that these traits are likely controlled by many small to moderate effect genes. Next steps include further GWA analyses with increased sample size and number of markers, as well as inclusion of differential expression data from a subset of trees with extremely low and high levels of constituent phenolic glycosides.

#### T27. Novel components of plastids with potential roles in plant stress and defense

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Plastids are the sites of biosynthesis of key signal molecules of plant immunity. Plastid membranes are embedded with a unique set of channels and transporters that enable transport of nutrients, ions and metabolites. Plant HyPRPs are a family of proteins with N-terminal proximal proline-rich regions (PRRs) and a hydrophobic Cterminal domain. AZI1 is the best-studied HyPRP that employs a special signal-anchor mechanism for plastid envelope targeting. It uses an N-terminal bipartite signal that consists of a short N-terminal hydrophobic domain (HD) followed by a PRR, with characteristics similar to a classical plastid import signal. AZI1 is specifically necessary for systemic immunity. Its pool at the chloroplast envelope is enriched during infection, which is consistent with its role in mobilizing the plastid-derived priming signal azelaic acid (AZA). The chloroplast localization of AZI1 requires the PRR. However, the distinct roles of most other HyPRPs as well as their precise localizations within cells remain largely unknown. We employed an algorithm originally used for plasmodium (PATS) to predict plastid localization of Arabidopsis HyPRPs. We also used TargetP and ChloroP algorithms to accurately predict targeting of HyPRPs, after in silico removal of SP-like sequences. Using confocal microscopy and immunoblotting, we observed the plastid envelope localizations of HyPRPs. However, some of the HyPRPs not predicted to target plastids were experimentally found to localize in plastids. Additionally, some HyPRPs predicted to target to plastids localized to non-plastidic membranes (PM/ER). We hypothesize that, among other factors, the specific length of N-terminal PRRs, the presence of charged residues and the potential for posttranslational modifications has a major influence in determining the plastid localization of HyPRPs.

### **T28.** Light-induced stabilization of ACS contributes to hypocotyl elongation during the dark-to-light transition in *Arabidopsis* seedlings

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Hypocotyl growth during seedling emergence is a crucial developmental transition influenced by light and phytohormones such as ethylene. Ethylene and light antagonistically control hypocotyl growth in either continuous light or darkness. However, how ethylene and light regulate hypocotyl growth, including seedling emergence, during the dark-to-light transition remains elusive. Here, we show that ethylene and light cooperatively stimulate a transient increase in hypocotyl growth during the dark-to-light transition via the light-mediated stabilization of 1-aminocyclopropane-1-carboxylic acid (ACC) synthases (ACSs), the rate-limiting enzymes in ethylene biosynthesis. We found that, in contrast to the known inhibitory role of light in hypocotyl growth, light treatment transiently increases hypocotyl growth in wild-type etiolated seedlings. Moreover, ACC, the direct precursor of ethylene, accentuates the effects of light on hypocotyl elongation during the dark-to-light transition. We determined that light leads to the transient elongation of hypocotyls by stabilizing the ACS5 protein during the dark-to-light transition. Furthermore, biochemical analysis of an ACS5 mutant protein bearing an alteration in the C-terminus indicated that light stabilizes ACS5 by inhibiting the degradation mechanism that acts through the C-terminus of ACS5. Our study reveals that plants regulate hypocotyl elongation during seedling establishment by coordinating light-induced ethylene biosynthesis at the posttranslational level.

### **T29.** Control of multicellularity in the moss *Physcomitrella patens* through modifying ROP-GTPase Activity

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One of the most important innovations of life on earth is the transition of multicellularity from unicellular ancestors, but little is known about such transition. Here we show multicellularity in the moss Physcomitrella patens can be achieved through modifying ROP-GTPase activity by either S-acylation or prenylation. In vascular plants there are two types of ROPs, with type I terminating in a CaaX box and activated through protein prenylation, including farnesylation and geranylgeranylation, and type II terminating with a GC-CG box and activated through S-acylation. In contrast, moss only has type I ROPs, which terminate in a canonical prenylation CaaL box, providing a nice system to dissect the function of the two types of ROPs. To study the effects of ROPs on the cell adhesion, we have stably knocked down ROPs by artificial micro-RNA (amiRNA) and confirmed the essential function of ROPs in cell adhesion and cell polarity as reported. Moreover, overexpression of moss type I ROPs in ggb mutants rescued cell adhesion defects in the mutant, making the single-cell like ggb plant form filamentous cells and gametophores. To understand the conservation of the moss type I ROPs and the vascular plant type I/II ROPs, we showed that overexpressing native Arabidopsis ROP1, terminating in a CaaX box, as well as overexpressing a chimeric type II ROP in which the CaaX box of AtROP1 was replaced by a GC-CG box of the type II AtROP11, both rescued the ggb mutant phenotype. These data showed that control of multicellarity in the moss is via the ROP pathway, which can be activated by either protein prenylation or protein S-acylation. Using SEM and live imaging, we have found that the lost of cell adhesion is caused by uncontrolled and uncoordinated cell expansion these plants.

#### T30. Natural fumigation as a mechanism for volatile transport between flower organs

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Plants synthesize volatile organic compounds (VOCs) to defend themselves against herbivores and pathogens. Generally, accumulation and emission of VOCs occur from the tissue of their biosynthesis. However, using biochemical and reverse genetic approaches, we demonstrate a new physiological phenomenon: inter-organ aerial transport of VOCs via natural fumigation. Before petunia flowers open, a tube-specific terpene synthase produces sesquiterpenes, which are released inside the buds and then accumulate in the stigma, potentially defending the developing stigma from pathogens. These VOCs also affect reproductive organ development and seed yield, which is a previously unknown function for terpenoid compounds.

#### Poster Presentations (P1 – P45)

### P1. A Scanning Electron Microscopy Technique for Viewing Plant-Microbe Interactions at Tissue and Cell Type Resolution

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Observing pathogen colonization and localization within specific plant tissues is a critical component of plant pathology research. High resolution imaging, in which the researcher can clearly view the plant pathogen interacting with a specific plant cell, is needed to enhance our understanding of pathogen lifestyle and virulence mechanisms. However, it can be challenging to find the pathogen along the plant surface or in a specific cell type. Because of the time-consuming and expensive nature of high resolution microscopy, techniques that allow a researcher to find a region of pathogen colonization more quickly at low resolution and subsequently move to a high-resolution microscope for detailed observation are needed. Here we present paraffin scanning electron microscopy (PSEM), a technique in which paraffin embedded samples are first sectioned to identify a region of interest. Subsequently the same block is recut, deparaffinized, and used in scanning electron microscopy to generate high resolution images of plant-pathogen interactions in specific plant cell types. This method has several additional advantages over traditional SEM techniques, including reduced noise and better image quality. Here we use this technique to show that Fusarium oxysporum f. sp. lycopersici colonization is restricted in resistant *Solanum pimpinellifolium*, and that PSEM works well in additional pathosystems including maize leaves and *Clavibacter michiganensis* subsp. *nebraskensis*, and Arabidopsis leaves and *Pseudomonas syringae* .

#### P2. What is the role of auxin in root-mediated resistance to Ralstonia solanacearum?

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*Ralstonia solanacearum* is a soilborne bacterial plant pathogen, commonly found in the tropics R. solanacearum causes Bacterial Wilt and infects more than 200 plant species, including economically important crops such as tomatoes, potatoes, peppers, and tobacco. R. solanacearum enters via natural openings in plant roots, invades the xylem tissue, and produces large amounts of exopolysaccharides (EPS) that block water transport, resulting in wilted plants. In tomato, the resistance response to R. solanacearum is quantitative and it is the result of many genes, however, the molecular basis still remains to be studied. Studies with grafting experiments, in which resistant tomato rootstocks were grafted to susceptible scions, showed a significant reduction of Bacterial Wilt incidence in the Southern United States as well as Korea and Japan. These studies have demonstrated that resistance mechanisms in the root are key to whole plant resistance Plant hormones are associated with several processes in plant growth and development. Salicylic acid and jasmonic acid are two hormones that play important roles in plant defense responses. The hormone auxin is involved in almost every plant growth and developmental process, but recent studies have shown that auxin also participates in plant-microbe interactions. However, the role of this hormone in tomato root-mediated resistance to R. solanacearum is unknown. Previous work has shown that a tomato auxin transport mutant diageotropica (dgt 1-1), is resistant to R. solanacearum. RNA-sequencing data at 48 hours after inoculation indicated that auxin-related genes like PIN1, ARFs and AA/AUXs are downregulated in roots of the resistant tomato plant. To better understand this complex process, we are using a reverse genetic approach. Additional tomato mutants defective in auxin pathways will be used to assess the role of auxin in root-mediated resistance. Preliminary results have identified another auxin transport mutant and an auxin signaling mutant with delayed symptom development in response to R. solanacearum.

Understanding the role of auxin in defense responses to *R. solanacearum* in tomato is important for Solanaceae crop improvement.

## P3. A Regional Education Program in Sustainable Land Reclamation Management in Central Appalachia

#### Nan Nan, and Jingxin Wang

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To educate a new generation of workforce prepared to lead sustainable land reclamation management efforts in Central Appalachia, by developing a new certificate, while enhancing the number and diversity of undergraduate students pursuing a university degree, and increasing employment opportunities for students through industry partnerships. Audience: Students who are attending two-year colleges and are planning to pursue a higher degree at a four-year university. This will include underrepresented and first-generation college student groups and will also target students in existing natural resources and STEM programs, who want to augment their education in sustainable land reclamation management. Outcomes/Impacts: (1) a sustainable, high-quality, and multidisciplinary education program in sustainable land reclamation management in the central Appalachian region, (2) 10-15 transferred students each year from two-year colleges pursuing a bachelor of science degree, (3) 35-55 students each year to obtain a certificate in sustainable land reclamation management at WVU, (4) hands-on experience and internships available from regional industry partners to reinforce students' learning and employment opportunities.

## P4. The role of trichome-derived monoterpenes and sesquiterpenes in the interaction of cultivated tomato and potato aphid

**Fumin Wang**, Yong-Lak Park, and Michael Gutensohn Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV, USA

Specialized metabolites produced in glandular trichomes, including volatile terpenes, are heavily involved in the interaction of plants with herbivores. In cultivated tomato, Solanum lycopersicum, the characteristic volatile compounds found in glandular trichomes on leaves and stems are primarily terpenes, including a mixture of abundant monoterpenes and a number of less abundant sesquiterpenes. These terpenes are derived from the enzymatic activities of three terpene synthases, namely TPS20, TPS9 and TPS12. Here, the performance and choice behavior of potato aphid, Macrosiphum euphorbiae, were studied using two tomato trichome mutants, hairless (hl) and odorless 2 (od-2), and their respective background accessions Alisa Craig and Castlemart. hl and od-2 were confirmed via combined gas chromatography-mass spectrometry (GC-MS) analysis to differ quantitatively in their production of glandular trichome derived terpenes compared to respective controls. Principle component analysis for clip cage experiments indicated that each performance variable (longevity, fecundity and intrinsic growth rate) of aphid apterae had a strong negative correlation with production of TPS12-derived sesquiterpenes in trichomes of these tomato lines. Addition of pure TPS12-derived sesquiterpenes ( $\beta$ -carvophyllene and  $\alpha$ -humulene) to artificial feeding diets affected aphid survival, investment of gel saliva and honeydew production. In open Y-track olfactometer assays aphid alatae displayed differential choice behaviors to odors from the hl and od-2 mutants compared to controls. The attraction of aphid alatae towards TPS20-derived monoterpenes as well as their dose-dependent repellence by TPS12-derived sesquiterpenes was further confirmed using pure terpene compounds. Therefore, TPS20-derived monoterpenes, including  $\beta$ -phellandrene, appear to be exploited as cue for host plant orientation by aphid alatae, whereas TPS12-derived sesquiterpenes confer both antibiosis and antixenosis resistance to aphid apterae. The identification of these contrasting roles of trichome-derived terpenes in cultivated tomato provide valuable information towards the development of sustainable control strategies for aphids.

#### P5. MiR156 Regulation of Flowering Time of Elevated CO2-Grown Plants

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The transition from vegetative growth to reproductive growth or flowering time is a critical milestone in the life cycle of plants. The timing of this transition can have serious implications on the reproduction of individual plants, the evolutionary fitness of genotypes within populations of plant communities and ultimately the functioning of ecosystems. Recent evidence suggests that the timing of the transition to reproductive growth is changing in response to rising atmospheric carbon dioxide concentrations associated with human-induced climate change. A review of past studies found that more than half of plant species studied exhibit altered flowering time, either delayed or accelerated, when grown at elevated atmospheric carbon dioxide concentrations. The goal of the research presented here is to elucidate the mechanisms that alter the flowering time of the model plant Arabidopsis thaliana when grown at elevated atmospheric carbon dioxide concentrations. We grew one wild-type and six mutant genotypes of A. thaliana at either 400 ppm CO 2 or 1000 ppm CO 2 and examined the timing of the transition from juvenile to adult vegetative growth as well as flowering time. Mutant genotypes contained mutations that led to altered expression of the developmental regulating microRNA, miR156, or genes that are regulated by miR156. We found evidence of the involvement of miR156 in the control of the response of both the timing of the transition from juvenile to adult vegetative growth as well the timing of flowering to growth at elevated atmospheric CO 2. Whereas, plants with mutations in this pathway develop more rapidly when grown at elevated atmospheric CO 2.

### P6. Characterizing the Function of PP2-A13 F-box Protein in Plant Development

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Protein ubiquitination is a eukaryotic organism-specific process that modifies target proteins covalently with one or multiple ubiquitin moieties. The ubiquitylated proteins are either recognized by the 26S proteasome for turnover or subject to finctional changes in cells. Therefore, it is used to mediate protein levels within a cell and recycle misfolded or unused proteins. The process of ubiquitination is a three-step process that transfers ubiquitin from E1 to E2 and finally to lysine residues of a ubiquitylated protein substrate by E3. The largest ubiquitin E3 ligase family, the SCF complex, contains SKP1, CULLIN 1, and F-box proteins, which target various intracellular proteins for ubiquitylation and degradation. In this complex, the F-box proteins help dictate what proteins are targeted. In this project, we are using both genetic and biochemical approaches to characterize the function of one F-box protein, termed PP2-A13. We use Arabidopsis as a model plant to examine its role in regulating plant growth. We have developed loss of and over expression lines up to date. Our preliminary data have shown that over expression of PP2-A13 significantly suppressed early seedling growth. To characterize its potential substrates and/or interacting partners, I performed a yeast two-hybrid library screen and found different prey proteins that interact with PP2-A13 in yeast cells. Furtern demonstrating their interactions with and ubiquitylation by PP2-A13, as well as the resulting phenotypes in both loss of and over expression lines of PP2-A13 will shed light on the novel function of the SCF PP2-A13complex-mediated ubiquitylation pathway in plants.

# **P7.** Quantitative Expression Reduction of Differentiation and Green Like by iCRISPRi Reveals It's Function in Multiple Metabolic Pathways

**Paymon Doroodian**<sup>1,2</sup>, Zhenyu Gao<sup>1,3</sup>, Madhura Yapa<sup>1</sup>, Jacob Sieg<sup>1</sup>, Maria Gomez<sup>1</sup>, and Zhihua Hua<sup>1,2</sup> <sup>1</sup> Enviromental and Plant Biology Department, Ohio University, Athens, OH, USA <sup>2</sup> Interdisciplinary Program in Molecular and Cellular Biology, Ohio University, Athens, OH, USA

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Previous loss-of-function studies on *Differentiation and Green Like (DAL)* hypothesized that DAL regulates RNA editing of chloroplast mRNAs. Its T-DNA null mutant, *dal-1*, is albino and has aberrant functions in multiple RNA editing sites in chloroplasts. However, the detailed biochemical function of DAL is yet unclear. Here, we developed an inducible Clustered Regularly Interspaced Short Palindromic Repeat Interference (iCRISPRi) approach to quantify the *DAL* function by reducing its mRNA in a controlled manner. Using three independent iCRISPRi lines whose *DAL* transcripts were effectively reduced, we further monitored the impact of DAL expression on their growth and RNA editing in chloroplasts.Inconsistent with dal-1 T-DNA null mutant, we did not observe any RNA editing errors in two iCRISPRi lines, although their growth and chlorophyll content were severely reduced along with a significant reduction of *DAL* expression. To tackle the direct biochemical function of DAL, we quantified the growth suppression, RNA editing error, and trancriptomic changes in the third iCRISPRi line upon different levels of *DAL* expression suppression by dCas9-Krab. Our data suggested that DAL is involved in a number of metabolic pathways both in cytoplasm and chloroplasts.

# **P8.** Epigenetic Modifications are Essential for the Rhythmic Control of volatile Organic Compounds Emission in *Petunia Hybrida*

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Volatile organic compounds (VOCs) are low molecular weight molecules produced by plants with diverse functions including attracting pollinators and self-defense. Complex blends of floral VOCs have been observed to be released in a diurnal fashion adapted to the activity patterns of their pollinators. VOC biosynthesis relies on primary metabolic networks, which provide precursors and cofactors. While many of the genes responsible for production of VOCs have been identified, little is known about how their coordinated expression is achieved at a molecular level. Epigenetic control is known to be involved in regulation of many plant biological processes but has yet to be demonstrated in control of VOC production. We investigated the potential role of histone acetyltransferase (HAT) inhibitor reduced total volatile emission and eliminated its rhythmicity. Analysis of gene expression revealed that the inhibitor differentially represses genes involved in VOC production, which highlights the importance of epigenetic regulation in both primary and secondary metabolic networks.

# **P9.** Time-Course Transcriptomic Analysis of *Petunia* × *hybrida* 'Mitchell Diploid' under Water Deficit Stress Using RNA Sequencing

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Petunia is one of the economically important ornamental crops in United States. There is limited information regarding regulation and signaling pathways associated with water deficit stress response in petunias. The long-term goal of this research is to identify genes that regulate the water deficit stress tolerance. As a first step to accomplish our goal, RNA sequencing was performed and time-course transcriptome data were analyzed. Nine-week-old petunias (Petunia × hybrida 'Mitchell Diploid') were irrigated daily or stressed by withholding water. Leaf tissue samples were collected 1, 3, and 5 days after water was withheld. Stomatal conductance was

reduced after five days of water deficit, indicating plants responded to the stress. Nearly 270 million reads were sequenced by Illumina HiSeq 1500, and de novo assembly using Trinity generated 76,601 contigs. Under water deficit stress, 154, 3,611, and 980 genes were upregulated and 41, 2,806, and 253 genes were downregulated on day 1, 3, and 5, respectively. Gene Ontology enrichment analysis suggested the involvement of the hormone signal transduction, especially abscisic acid and ethylene, in water deficit responses. Thirty-four transcription factor families were identified, including members of AP2/ERF, NAC, MYB-related, C2H2, bZIP families. The transcriptome data from this research will provide valuable molecular resources for deciphering the regulatory network in water stress response mechanism as well as allow us to engineer crops with enhanced water stress tolerance.

#### P10. Using a Poplar Hybrid to Investigate Genetic Control of Associating Insect and Fungal Communities

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Development of host plant resistance has been recognized as the most useful and least costly method of pest management. To utilize the natural defenses of host plants it is necessary to tease apart the genetic and biological forces which make them unpalatable to pests. Populus has rapidly become a key genetic model for research of forest trees. It is important in structuring and supporting the ecology of unmanaged habitats and, given their rapid growth and vegetative reproduction, has become a focus for research into biofuel production. F 2 progeny of interspecific hybrids of Populus deltoides x Populus trichocarpa segregate for a wide variety of traits including resistance to pests and pathogens. Quantitative trail loci (QTL) analysis has traditionally been used to map complex traits to genomic markers. With the sequencing completion of the Populus deltoides genome and the published genome of Populus trichocarpa, QTL analysis can be coupled with a comparison of the structure of genetic intervals associating with different traits in both species. This provides the opportunity to better understand the coevolution of pests with their host and examine patterns in areas of the genome that associate with biotic stressors. Trees were observed for the arthropod pest Pemphigus populitransversus galls, Mordwilkoja vagabunda galls, Phyllocolpa sp. leaf folds and Phyllocnistis populiella leaf mines. Individuals were additionally scored for infection of fungal pathogens including Melampsora sp. leaf rust, Septoria sp. leaf spot, and Septoria musiva stem canker. Broad-sense heritability (H 2 c) revealed a significant genetic factor controlling both insect and fungal pest distributions. Several QTL intervals associated with phenotypes that contain numerous candidate genes mediating the biotic interactions. There was an elevated number of recent tandem duplications in the intervals for several biotic phenotypes. Future work will be aimed at understanding the structural variation in genome intervals which underlie biotic trait distributions.

#### P11. Analysis of RIN4 homologs in regulating R-mediated immunity in Important Crop Species

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The ability of pathogens to cause disease and hosts to resist pathogen invasion are components of an ongoing arms race. Plants rely on a multi-layered immune system to guard against pathogenic invasion. Detection of pathogen associated molecular patterns (PAMPs), triggers the first layer of immunity called PAMP-triggered immunity (PTI). However, pathogens suppress this first layer by deploying virulence factors, including type three secreted effector proteins. In addition to suppressing PTI, these effectors potentially trigger a second layer of immunity, effector triggered immunity (ETI), through the activation of disease resistance (R) proteins. These two modes of immunity work in synchrony to provide the plant with a mechanism to halt bacterial infections. The current study focuses on a multifunctional plant protein, RIN4, that regulates both branches of plant immunity in the model plant *Arabidopsis thaliana* (*At*). Different effectors target and modify RIN4 in an attempt to suppress PTI and promote disease in *A. thaliana*. However two R proteins in *A. thaliana* sense these modifications and activate ETI. Interestingly, RIN4 homologs containing the conserved regions present within At-RIN4 that are required for regulating immunity are found in all sequenced plants species ranging from moss to monocots to dicots. It is therefore hypothesized that RIN4 plays a key role in the defense mechanisms of all plants, including important crops. For the purpose of this study, RIN4 homologs will be tested for their ability to complement At- RIN4 by utilizing a heterologous system, *Nicotiana benthamiana*.

#### P12. Elucidation of Genetic Components for Acidity Tolerance Using Arabidopsis GWAS Panel

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Acidic soil is phytotoxic and it affects mineral uptake in plants which directly influence crop production. Soil acidification caused by natural processes and release of industrial pollutants is an ever increasing global concern. Appalachian Mountains coal industry causes high degree of acidic drainage with higher concentration of toxic metals in surrounding environment. In this study, we employed wealth of natural variation found in Arabidopsis thaliana ecotypes using genome-wide association studies (GWAS) followed by reverse genetics to uncover genes involve in acidity stress. The variation in root traits in response to low pH across the set of 240 accessions was screened to identify the Quantitative Trait Loci and associated SNPs at the 5 th and 10 th day of exposure to acidic conditions. Efficient Mixed-Model Association eXpedited (EMMAX) along with Bonferroni correction was used for detecting SNP associations. Our GWAS mapped a total of 29 genes with probability level of  $-\log 10$  (P- value)  $\geq 6$  for root length. MapMan analysis revealed that most of the associated genes were transporters, heat shock proteins and kinases and found to be localized either in cytoplasm or plasma membrane or vacuolar membrane. The functional characterization of the candidate genes using reverse genetic approach is currently in progress.

#### P13. Compartmentation of Putrescine Synthesis in Plants

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Polyamines are essential metabolites in all living organisms. Three plant pathways for the synthesis of putrescine have been described. The differential expression of rate-limiting enzymes in these pathways suggests that each of these pathways may have different roles. In *A. thaliana*, arginine decarboxylase 2 and agmatinase are localized to the chloroplast, and function in concert to synthesize putrescine. Localization of the other two pathways for putrescine synthesis has not been established. In this study, transient expression assays of GFP-tagged ornithine decarboxylase from soybeans and rice, showed that both genes were localized to the endoplasmic reticulum in the mesophyll cells of *Nicotiana benthamiana*. Similarly, the *A. thaliana* genes, arginine decarboxylase 1, agmatine iminohydrolase, and N-carbamoylputrescine amidohydrolasewere also

localized to the endoplasmic reticulum. Thus, two pathways for putrescine synthesis are localized to the endoplasmic reticulum, while a third pathway is localized to the chloroplast. We hypothesize that regulatory mechanisms exist to sense polyamine levels within the ER, and that exclusion of putrescine synthesis from the cytoplasm makes it easier to utilize cytoplasmic levels of putrescine as a signaling molecule in response to various stresses.

### P14. Nucleic Acid Programmable Protein Array (NAPPA) Technology as a Functional Genomics Tool for Plant Cell Wall Biosynthesis

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The plant cell wall is a complex network composed of various types of polysaccharides. glycosyltransferases (GTs) are the enzymes responsible for making these polysaccharides. A large number of GT genes have been identified by genome analysis, but the biochemical function is not established for most of them. Making a highthroughput tool for enzyme assays available would speed up the assignment of GTs biochemical functions. Therefore, the goal of this project is to adapt nucleic acid programmable protein array (NAPPA) to enzyme assays to allow large scale investigation of the biochemical function of GTs, as well as protein-protein and protein-DNA interactions. As a proof-of-concept, four non-processive GTs (AtFUT1, AtFUT6, AtXXT1, and AtGUX1) and five polysaccharide synthases for mixed-linkage glucan (MLG), xyloglucan, homogalacturonan, mannan, and xylan were tested. Enzyme assays were performed in 96-well plates coated with anti-tag antibodies (anti-GST and anti-Halo) at various dilutions, which allow attachment of synthetized tagged GTs from DNA plasmid (via cell-free expression system) on the well surface. The effect of tag position (C-terminus GST-tag versus N-terminus Halo tag) on enzyme activity was also investigated. The synthesis of GTs was assessed by western blotting. Our preliminary data showed that tag location affected the activity of AtFUT1, whereas the activity of other GTs and polysaccharide synthases was not affected. Protein-protein interactions (PPIs) tests confirmed interactions between components of a xylan synthase complex (i.e. TaGT43, TaGT47, and TaGT75) that were previously demonstrated via biomolecular fluorescence complementation (BiFC) technique [1]. 1. Jiang N, Wiemels RE, Soya A, Whitley R, Held M, Faik A (2016). Composition, Assembly, and Trafficking of a Wheat Xylan Synthase Complex. Plant Physiol. 170(4):1999-2023.

## P15. Natural Variation in *Arabidopsis thaliana* to Discover Candidate Genes in Response to Aluminum Toxicity

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Aluminum, a biologically inactive soft metal is one of the most abundant elements on earth's crust. It exists in different soluble cationic form in acidic soil. At pH below 5.0, trivalent (Al 3+) form of aluminum is available to the plants. Micromolar concentration of Al is toxic to plants. It primarily affects root growth further limiting nutrient and water uptake. To adapt to the toxicity of Al in soil, plants may reduce Al uptake or sequester excessive Al 3+ ions in less sensitive cell compartments. They may develop tolerance mechanism against high levels of Al. Plants also have evolved with secretion of organic acids in the soil that chelates Al 3+ which then becomes unavailable to plants. Several genes are known to regulate Al tolerance in plants however; molecular mechanism of plant response to Al in acidic soil is yet to uncover. To study Arabidopsis natural variation in response to high Al levels under acidic growth conditions, we analyzed a set of eight root traits. Root phenotypic data were collected for seedlings of 203 Arabidopsis accessions on 5 th and 10 th day of exposure to 10 µM Al at pH 4.5 followed by genome-wide association study (GWAS). A GWAS was performed using

easyGWAS tool. Mapped genes are involved in signal transduction, stress responses, metal transport, and unknown functions. Reverse genetic approach is being employed to functionally test the candidate genes as the major genetic determinant for the variation in Al tolerance in the A. thaliana populati on.

#### P16. The Metabolic and Nutritional Response of Salix spp. to Aluminum Stress

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Aluminum (Al) in soils is toxic to plants at pH 5. The Al stress syndrome varies among species, and the impact on woody biofuel crops has yet to be fully characterized. Three lines of willow (Salix spp.), chosen for their genealogy and divergent responses to Al, were exposed to 200µM Al in hydroponic culture to evaluate their metabolic and nutritional responses. Roots and leaves of plants exposed to Al for short (8 h), intermediate (32 or 80 h), and long (10 d) durations were analyzed by gas chromatography-mass spectrometry (GC-MS) or inductively coupled plasma-optical emission spectroscopy (ICP-OES). Rootexudates were also collected to quantify resistance strategies related to rhizodeposition. The sensitive parent genotype was found to have a metabolic profile, an ion allocation pattern, and an exudation profile distinctly different from the resilient parent and their hybrid offspring. Various key metabolites were found to correlate strongly with growth in resilient lines despite Al exposure. Morphological responses to Al stress was likewise dependent on genotype.

## P17. Exploration into Natural Variation for Detecting Novel QTLs for Arsenic Effects in *Arabidopsis* thaliana

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Arsenic (As) is a widespread non-essential and generally toxic metalloid for all living organisms. Arsenic pollution causes special concern with respect to its toxic effects in plants. Considering the adverse effects of Asassociated risks, it is important to investigate and focus on the genetic mechanisms that plants have developed for As uptake, accumulation, distribution, and tolerance. Previous studies have identified several As responsive genes involved in its metabolism, translocation, sequestration, etc. In order to identify additional mechanisms, we employed genome-wide association (GWAS) approach and utilized the natural variation in Arabidopsis thaliana ecotypes to determine the phenotypic effect of As(V) on different seedling root traits. The GWAS analysis identified a total of 818 significantly associated SNPs markers correlated with As stress at a probability level of  $-\log(P-value) \ge 4.00$ , which corresponded to 214 candidate genes controlling different root traits under a 100 µM As treatment. MapMan analysis revealed that these candidate loci are involved in heavy metal transport, protein ubiquitination, cellulose synthesis, and ATP synthesis. Further, a set of 68 genes was selected for functional characterization using reverse genetic approach. To test whether the candidate genes are responsible for As response, presently we are characterizing transfer DNA (T-DNA) insertion mutants of these genes in the Col-0 background. The identified alleles that account for natural variation in As(V) tolerance encode pentatricopeptide repeat (PPR) superfamily protein, leucine-rich repeat (LRR) family protein, and several other previously uncharacterized transcription factors. The discovery of these genes functioning as the major determinant of the variation in responses to As could help to breed or select crops with high As tolerance.

### P18. Are timber management practices impacting the insect pollinators in the Allegheny National Forest?

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Black cherry (*Prunus serotina*) is an important timber species in the Allegheny National Forest (ANF), and in recent years, land managers have noticed a decline in natural regeneration. One hypothesis regarding this decline is a lack of pollination of the flowers. Our objectives for this study were to identify the primary insect pollinators of black cherry flowers, and to describe how the insects change between timber management sites. We have collected potential pollinators using colored pan traps in three different timber stand treatments (i.e. unmanaged, shelterwood, and removal cuts) in two locations of the ANF. The traps were made of three 12-oz cups with white, and fluorescent yellow and blue interior color. The canopy traps were hung using a slingshot and suspended on a platform. The understory traps were secured on a wooden stake at the base of the tree. A total of 16,230 insects were captured with the five most abundant orders being flies (66%), beetles (13%), bees/wasps (8%), moths (7%), and thrips (7%). The results of this study showed that insect community structures were statistically different (P < 0.05) between uncut/undamaged and shelter wood treatments. There was also a statistical difference (P < 0.05) in the number of insects captured between the sampling periods of before, during, and after peak flowering of black cherry. This study will help to better understand insect pollination of black cherry and aid in timber harvest management practices.

## P19. Higher order knock-out mutants of Arabidopsis galactosyltransferases: A reverse genetic approach to reveal functional roles of O-galactosylation of arabinogalactan-proteins (AGPs)

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Cell walls are indispensable components for plant survival and have great commercial importance. Understanding the synthesis, structure, and function of cell wall components, such as the hydroxyproline-rich cell wall proteins, is required to engineer cell walls for future commercial use. Arabinogalactan-proteins (AGPs) are a family of abundant cell surface proteins composed of ~90% sugar and ~10% protein; they are implicated to function in a variety of plant developmental processes by unknown molecular mechanisms. AGP biosynthesis involves a series of post-translational modifications resulting in the addition of complex sugar chains to AGP core proteins. Eight Hyp-galactosyltransferases (Hyp-GALTs) namely, GALT2, GALT3, GALT4, GALT5, GALT6, HPGT1, HPGT2, and HPGT3, have been characterized so far for O -galactosylation of hydroxyproline (Hyp) residues in Arabidopsis AGPs, but their partially redundant roles remain unclear. We hypothesize that higher order mutants will show more severe phenotypes with aberrant biochemical compositions, indicating the critical importance of galactose sugars added by the GALT s to the biological functions of AGPs. To assess the functional contributions of Hyp-GALTs, we plan to biochemically and phenotypically characterize vegetative and reproductive traits of higher order GALT gene knock-out mutants. To date, we have identified a homozygous triple mutant (galt5 hpgt2 hpgt3), quadruple mutants (galt2 galt5 hpgt1 hpgt3, galt2 galt5 hpgt2 hpgt3 ), and a quintuple mutant (galt2 galt5 hpgt1 hpgt2 hpgt3 ). Preliminary studies indicated that roots and reproductive traits were affected in the higher order mutants, specifically in the quintuple mutant which showed stunted growth and reduced yield. β-Yariv precipitated AGPs were significantly reduced in the flowers, stems, siliques, rosette leaves and cauline leaves of higher-order mutants, with the highest reduction being demonstrated in the quintuple mutant (ranging from 70-80% less than wild type). Biochemical characterization (carbohydrate analysis) of these mutants will be conducted to relate biochemical AGP alterations to their observed physiological phenotypes.

#### P20. Impact of Simulated Climate Change on Flower Development of Petunia

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Plants are sessile organisms and are often exposed to environmental stresses. In order to cope with such stresses, plants utilize morphological and physiological mechanisms to mitigate the impacts of stresses for survival. Environmental stress significantly affects plants' growth and development, so it is important to understand how plants respond to multiple abiotic factors. The goal of this research was to investigate the effects of elevated CO 2, temperature and water deficit stress on flower development. The main effects and interactions of these abiotic factors were examined. Petunias were grown in growth chambers at two levels of CO 2 (400 and 800 µmol·mol -1), two temperature regimes (21/18 and 28/25 °C day/night), and two irrigation levels (0.15 and 0.30 m  $3 \cdot m - 3$ ). The moisture levels of the growing media were maintained by an automated irrigation system. The number of flowers, flower size, flowering time, growth index, and biomass were examined. Flower number was reduced on plants grown under elevated temperature and water deficit at ambient CO 2. The size of the flowers decreased under elevated CO 2. Reductions in flower number and size may render plants less attractive to pollinators, which could be detrimental to yield and seed production. Flowering time was also delayed with elevated CO 2, but accelerated with higher temperature. The altered pattern of flowering time under climate change conditions could result in an asynchrony between crops and their pollinators. Growth index increased at the elevated temperature, while the highest biomass was observed from plants grown at ambient conditions.

#### P21. Genetic regulation of anticancer and neuroprotective glyceollins in soybean

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Glyceollin phytoalexins are the pathogen-elicited secondary metabolites that belong to the isoflavonoid family of molecules of soybean (Glycine max L. Merr). They show anticancer and neuroprotective activities in mammals therefore they are very important for pharmaceutical industries. They are also induced in soybean in response to abiotic factors like, UV irradiation, heavy metals and jasmonate. However, glyceollins are accumulated in low amount with traditional elicitors and the chemical synthesis of this compound is not economical. Therefore, finding novel elicitors and/or engineering the regulation of biosynthetic pathway could be potential ways to enhance their bioproduction in soybean. To achieve these goals, we first aimed to identify a novel abiotic elicitor and secondly identify a transcription factor that positively regulates glyceollin biosynthesis genes. We screened a panel of abiotic conditions and identified low pH medium as the novel elicitor of glyceollin I in soybean roots. Soybean seedlings treated with pH 3.0 medium accumulated the highest amount (1700 µg gt -1 FW) of glyceollins in root tissues compared to leaf, cotyledon and hypocotyl. Based on a comparative transcriptomics approach, we identified and functionally characterized the GmNAC42-1 transcription factor as a positive regulator of glyceollin biosynthesis. Overexpression and RNAi silencing of GmNAC42-1 in soybean hairy roots resulted in a significant increase and decrease of the accumulation of glyceollins, respectively. qRT-PCR results showed that the overexpression and knockdown of GmNAC42-1 resulted in an increase and decrease in the transcript levels of biosynthesis genes such as G4DT and IFS2. Yeast one-hybrid results showed that GmNAC42-1 TF directly bound the promoters of glyceollins biosynthesis genes IFS2 and G4DT. So, these results clearly indicated that acidity stress is a novel elicitor of glyceollins and GmNAC42-1 regulates the elicitation of glyceollins in soybean by transcriptional regulation of biosynthesis genes.

### P22. Quantitative Expression Reduction of Differentiation and Green Like by iCRISPRi Reveals Its function in a Novel Stress Responsive Pathway

**Paymon Doroodian**<sup>1,2</sup>, Zhenyu Gao<sup>1,3</sup>, Madhura Yapa<sup>1</sup>, Jacob Sieg<sup>1</sup>, Maria Gomez<sup>1</sup>, and Zhihua Hua<sup>1,2</sup>

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Previous loss-of-function studies on Differentiation and Green Like (DAL) hypothesized that DAL regulates RNA editing of chloroplast mRNAs. Its T-DNA null mutant, dal-1, is albino and has aberrant functions in multiple RNA editing sites in chloroplasts. However, the detailed biochemical function of DAL is yet unclear. Here, we developed an inducible Clustered Regularly Interspaced Short Palindromic Repeat Interference (iCRISPRi) approach to quantify the DAL function by reducing its mRNA in a controlled manner. Using three independent iCRISPRi lines whose DAL transcripts were effectively reduced, we further monitored the impact of DAL expression on their growth and RNA editing in chloroplasts. Inconsistent with dal-1 T-DNA null mutant, we did not observe any RNA editing errors in two iCRISPRi lines, although their growth and chlorophyll content were severely reduced along with a significant reduction of DAL expression. To tackle the direct biochemical function of DAL, we quantified the growth suppression, RNA editing error, and transcriptomic changes in the third iCRISPRi line upon different levels of DAL expression suppression by dCas9-Krab. We surprisingly found that the mutants significantly upregulated their responses to two defense hormones, salicylic acid and ethylene, and that the corresponding innate immune response was boosted while the chloroplast RNA editing machinery retained normal. In opposite, multiple sugar metabolic pathways were nearly shut down. To demonstrate these responses, we were able to apply different concentrations of sucrose to partially rescue the growth inhibition of iCRISPRi mutants and discovered that their hydrogen peroxide was highly elevated. Collectively, our data suggest that DAL is a key element in multiple enzymatic complexes involved in oxidation reduction in addition to its role in chloroplast RNA-editing.

#### P23. Cuticular Composition and Gene Expression Analysis of Cutin Genes by RNA-seq in Habanero Pepper Fruits

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The cuticle is the major barrier against uncontrolled water loss from leaves, fruits and other primary parts of higher plants. The cuticles of pepper fruit have much greater thickness and amount per surface area, a trait thought to play a role in fruit water loss and the associated maintenance of commercial postharvest fruit quality, as well as resistance to pathogens and insects. Understanding the synthesis and secretion of cutin is necessary in utilizing cutin to improve crop productivity and plant ecological adaptation. In this study, GC-MS analysis was used to determine the cutin composition of two cultivars of Capsicum chinense (PI224448 and PI257145). Additionally, we performed de novo transcriptome sequencing to explore differentially expressed genes between high and low cutin content cultivars and to identify genes related to cutin deposition in pepper fruits. Cutin monomers containing C16 or C18 aliphatic fatty acids, their derivatives, glycerol and phenolic compounds were quantified, the highest cutin content was for (PI257145) with 1284 mg g -1 dry weight (DW) and the lowest content was for (PI224448) with 232 mg g -1 dry weight (DW) of fruit tissue. A total of 146,569 unigenes were obtained, among them, 1689 unigenes showed significant differences in expression between high and low cutin content cultivars. The unigenes involved in cutin biosynthesis, regulation and export were identified by homology search against the CDS sequences from Arabidopsis thaliana. Fourteen putative genes involved in cutin biosynthesis will be further analyzed for their expressions using RT-qPCR. The results described here will be valuable for future studies of the physiological function and identification of genes

associated with fruit cutin synthesis in pepper fruit, and as a starting point for breeding improved fruit quality in pepper.

#### P24. Candidate Genes for Gravity Signal Transduction Identified from Spaceflight Proteomics

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Spaceflight provides a salient control in the study of gravitropism. As the receptor in any cellular signaling model is usually a membrane bound protein, a proteomic study comparing the proteins in seedlings germinated on ISS to Earth can help identify such a receptor for gravity. For this study Arabidopsis Wild Type Col-0 seeds were sterilized and plated on 60mm petri plates. These plates were packed in spaceflight hardware and flown to the International Space Station (ISS). A duplicate set of WT Col-0 seeds were kept on Earth as a ground control. After return from the ISS, proteins were extracted and fractionated into membrane and soluble fractions. Both fractions were analyzed using labelled tandem mass spectrometry at Donald Danforth Plant Science Center. Differential abundance analysis revealed 163 soluble proteins and 167 membrane proteins between spaceflight and ground samples (p < 0.05). To date only two other proteomics experiments have compared the spaceflight proteome of Arabidopsis seedlings. One compared the soluble proteomes and identified 546 differentially abundant proteins while the other compared membrane proteomes and identified 149 proteins. As our study had compared both soluble and membrane proteomes therefore identifying the proteins that were common across all three datasets gave us insight into the expression pattern of proteins that are commonly perturbed in all spaceflight experiments. This intersection dataset identified two membrane proteins and twelve soluble proteins. A network analysis was done on the proteins using String DB which identified two interactions. One was due to the proteins (AT3G62870 and STV1) working together during translation. The other was between PCAP1, a membrane bound protein with a phosphatidyl inositol binding site, and a soluble protein (PATL2) which is a membrane trafficking protein. These proteins therefore can be candidate genes for gravity signal transduction. This work was partially funded by NASA grant # NNX13AM48G to SEW and DRL.

#### P25. Role of Lipid Binding Proteins and Phosphatidic Acid on Stress Response and Plant Development

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Plants have evolved to respond to the changes in their environment, developing mechanisms to better their fitness. For example, plants use long distance signaling to communicate and exchange vital information throughout the whole plant. The vascular system supports systemic transport and contains two main components, the phloem and xylem, used for photoassimilate and water/mineral distribution respectively. Our understanding of the phloem has expanded from a predominant role in assimilate transport to a signaling network for development and stress response. We have identified several lipid-binding proteins (LBPs) in the phloem that may play a role in long-distance signaling. We propose phloem-localized LBPs act in signal lipid transport for systemic coordination of stress response. The protein-lipid complexes may move via the phloem to distal organs where they can be sensed by receptors and act as a signal to initiate changes in gene expression and plant development. We would like to dissect the mechanisms behind lipid-protein phloem transport, while also looking at protein-protein interactions. My research in the lab focuses on PHLOEM LIPID-ASSOCIATED FAMILY PROTEIN (PLAFP), a phosphatidic acid (PA) binding protein. Both PLAFP and PA are found in the plant phloem. We generated and genotyped plants expressing HA-tagged and His-tagged PLAFP and are now using these lines for phenotyping and to understand the effect of PLAFP-PA on plant development and stress

response. This project was funded in part by USDA grant #MICL04147 and NSF grants #1144391/1836680 to SHB.

## P26. Root-Fungal Associations Enhance the Plant Performance of the Invasive Plant Species, Lesser Celandine

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Lesser celandine (*Ranunculus ficaria*) is an invasive spring ephemeral found throughout the northeastern United States. Although this species continues to be a problem, the mechanisms behind its variable success is widely unknown. One possible mechanism that could explain the performance of lesser celandine is the presence of beneficial fungal-root associations. This study focuses on determining the community composition and influence of endophytic root fungi colonization on the variable success of lesser celandine. We hypothesized that plant performance will be correlated to community composition of endophytic root fungi. Sites (n = 64) were chosen in Rocky River Metroparks, Ohio along a 35-meter disturbance gradient from the river. Terminal restriction fragment length polymorphism and cloning were used in conjunction to determine the differences in the community composition of endophytic root colonization for each site. These results were then compared to plant biomass and reproductive output to determine differences in plant success across test sites. We determined that lesser celandine that was colonized by fungal communities consisting of ericoid mycorrhizae and dark septate endophytes had a higher biomass (0.63 g) than plants that were colonized by fungal communities consisting of parasitic endophytes (0.29 g) (t-test, df = 60.9, P & lt; 0.0001). Plant performance was not strongly associated with arbuscular mycorrhizal associations (PERMANOVA, P = 0.0498). Fungal colonization was not associated with reproductive output of lesser celandine (t-test, df = 61.8, P < 0.05).

## P27. Three beta-Glucuronosyltransferases impact physiological and biochemical functions of plant cell wall arabinogalactan-proteins in *Arabidopsis thaliana*

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Arabinogalactan-proteins (AGPs) are plant cell wall glycoproteins that are extensively post translationally modified by conversion of proline to hydroxyproline (Hyp) and by addition of arabinogalactan polysaccharides (AGs) to Hyp residues. Here, we evaluated the biochemical and physiological roles of three beta-glucuronosyltransferase (GLCAT) genes, namely GLCAT14A, GLCAT14B and GLCAT14C, involved in the transfer of glucuronic acid (GlcA) to AGPs. Preliminary results indicate that genetic knock-outs of GLCAT14A, GLCAT14B and GLCAT14C impact the biochemical and physiological roles of these genes in *Arabidopsis*. Quantification of Yariv-precipitated AGPs isolated from wild-type and various glcat mutants in different tissues showed variations between wild-type and glcat14a/b/c single mutants and glcat14a glcat14c and glcat14b glcat14c double mutants. Comparative phenotypic analysis showed that glcat14a/b/c single mutants and glcat14a glcat14c and glcat14b glcat14c double mutants impacted germination, root length of light grown seedlings, hypocotyl and root length of etiolated seedlings, seed set, plant height and number of siliques when compared to wild-type (control) plants. The results obtained in this study will contribute to the better understanding of the biological roles of these genes and AGPs in plant growth and development.

#### P28. De Novo Domestication of *Solanum cheesmaniae* by Genome Editing Via CRISPR/Cas9: Harnessing Salinity Tolerance from a Wild Tomato Species

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Agriculture is the economic sector that utilizes most of the fresh water resources available globally. Salinity is a major concern in arable lands due to increased limitations of water supply as well as continuous irrigation. Salt stress has major impact on crop development and yield, limiting cultivation in marginal lands and agricultural use of saline water worldwide. Tomato is a glycophyte crop that demands high amounts of water and does not tolerate salinity. The use of wild relatives as a source of salinity tolerance in breeding could be used, but this trait is complex and polygenic, and conventional breeding approaches have been limited to successfully deliver high-salinity tolerant varieties. We propose the use of the genome editing technique to domesticate tomato de novo as a high-salinity tolerant crop from the wild relative, *S. cheesmaniae*. From previous data, we chose the accession LA0421 originally from the seashores of the Galapagos Islands as the genetic baseline for knocking out genes involved in domestication traits, including plant architecture, flowering, yield, fruit size, and nutrition. This accession has been characterized for high productivity in saline conditions. Therefore, we are using a multiplex knock-out CRISPR/Cas9 strategy to create loss-of-function alleles for the genes BIF, cycB, J2, EJ2, MULTI, SP, SP5G , and FW11.3 in order to create a novel tomato halophyte crop harboring inherent high-salinity tolerance along with desirable cultivation traits. This research is a proof-of-concept to create crops that could potentially use (at least partially) seawater hydroponics for food production.

## P29. Caffeine Transport in the Coffee Plant: Understanding Caffeine Distribution and Accumulation at the Subcellular Level

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Coffee is one of the most consumed beverages in the world and it represents an important commodity in many tropical countries. Caffeine is the most studied compound in this beverage, and this alkaloid has been associated both to negative and positive effects in human health as well as beverage quality and the plant's biotic stress response. There is indication that caffeine accumulates in the same tissue of synthesis. Surprisingly, the subcellular transport and intracellular accumulation mechanisms of caffeine are not yet well understood at the genetic level. Therefore, we aim to identify and characterize alkaloid membrane transporters focusing primarily on Coffea canephora, the coffee diploid species that accumulates the highest caffeine levels with an annotated genome sequence. Through gene annotation and transcriptome data analysis, we have selected four putative alkaloid transporter genes for further study. Their expression profiles were assessed in leaves from field-grown plants presenting contrasting (three-fold change) caffeine content according to the development stage of the leaf. The expression level of two of these genes showed a high correlation with caffeine content. We are currently assessing the correlation between caffeine content and gene expression at six developmental stages of the coffee bean. Membrane transport assay of selected genes will be performed in a bacterial heterologous system. Finally, we aim at generating coffee plant knockouts via CRISPR-Cas9 genome editing to assess the impact of lack-of-function for each transporter potentially involved in accumulation of caffeine and other alkaloid present in the coffee bean, such as trigonelline. These mutants will also be valuable to better

understand the impact of caffeine content on beverage quality and tolerance to biotic stress of C. canephora . The identification of caffeine transporter systems in C. canephora beans may contribute to develop varieties for a more sustainable coffee production of better cup quality coupled with its natural resilience to biotic and abiotic stresses.

### P30. Identification of Mobile Transcripts Across the Reciprocal Grafts Involving Watermelon and Bottle gourd by RNA-seq Analysis

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Grafting has been used for a long time not only to provide resistance to different abiotic and biotic factors, but also to improve fruit quality of scions without undergoing a long-term breeding process. Beyond its use in horticultural production, grafting has received attention as a research tool to study the mobility of different molecules over long distances. Although the phenomenon of mRNA movement appears important for the plant, their biological role and their influence on their targeted tissues remain undeciphered. In this study, mobile transcripts were identified in watermelon/bottle gourd heterograft by RNA-seq analysis. Libraries representing different tissues were sequenced using paired-end chemistry with Nextseq500 to generate about 25 million reads per sample. A total of 424 bottle gourd transcripts were identified in stem, leaf and fruit of watermelon, thus they were denominated as mobile. In addition, 137 mRNAs were observed to move from watermelon to roots of bottle gourd. The transcripts included potential signaling factors that have been reported as mobile in other plants such as mRNAs encoding elongation factor, eukaryotic translation initiation factor, translationally controlled tumor protein, among others. Reverse-transcription-PCR and sequencing were used to confirm the mobility of the transcripts. These analyses revealed the mobility of two transcripts, Lsi05G008360.1, encoding a phloem filament protein and Lsi03G020080.1, encoding a protein classified in heavy metal transport/detoxification superfamily. Further studies have to be performed in order to provide evidence of their biological role.

### P31. A Battle of the Regulators: Contrasting Effects of ABA and Transcription Factors on Glyceolin Biosynthesis

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The innovations being studied within this project must be refined and separated in order to investigate the concept down to its fundamentals apropos to two genes (MYB and NAC). The first study involves overexpressing the MYBX gene within roots of soybeans. This is to test whether it can prevent the hormone abscisic acid (ABA) from inhibiting the biosynthesis of the soybean phytoalexins, named glyceollins. The implementation of abiotic stressors was established to see which lead to the production of these phytoalexins. In previous studies, it was found that stress (UV-B light, pathogens, etc.) can stimulate glyceollin production within soybeans (Srivastava et.al 2011). These treatments were used to identify transcription factors that regulate the expression of glyceollin biosynthesis. The purpose of this was to ultimately enhance the bioproduction of these glyceollin molecules within soybean plants. However, it was found within the study leading up to this one, that dehydration stress suppresses glyceollin production whereas acidic medium having a pH of 3 (when it's usually around 5.8) stimulated glyceollin biosynthesis (Jahan et al. 2019). The study carried out was a comparative transcriptome analysis. The focus was to identify the gene-overlap when pH elicited and dehydration suppressed glyceollin biosynthesis. NAC42 and MYBX were transcription factors found to positively regulate glyceollin biosynthesis. Subsequent experiments found that the presence of (ABA), a plant

hormone induced by dehydration, blocked the biosynthesis of glyceollins. Now, the idea for the present study is to investigate the relationship between the ABA hormone and the positive glyceollin regulators NAC42 or MYBX. It is hypothesized that overexpression of NAC42 or MYBX will negate the negative effects of ABA on glyceollin biosynthesis. The mechanism of how does this hormone interact with NAC42 and MYBX at the genetic and biochemical levels is what we aim to find out.

#### P32. Investigating Floral Transition and Optimizing Germination in Viola pubescens

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Viola pubescens, like many other members of Viola (violets), has a mixed breeding system with two distinct flower types: chasmogamous, open flowers with petals that promote cross-pollination, and cleistogamous, closed flowers that strictly self-fertilize. In V. pubescens, the two flower types are produced at different times of the season and never overlap. This temporal separation is hypothesized to be influenced by environmental factors. To test this hypothesis, environmental data including light quantity, canopy cover, temperature, soil moisture, soil pH were collected and compared to floral counts across the flowering season in native V. pubescens populations. Analysis of the data indicate that temperature and light quantity are statistically correlated with flower type and provide thresholds for specifying which flower type is most likely to develop. Ongoing research includes mimicking the light and temperature thresholds in growth chambers to preferentially induce chasmogamous or cleistogamous flowers. Seed germination is a problem for production of plants for flowering studies. V. pubescens seeds over-winter and germination/flowering occurs the following spring. To promote timely flowering for growth chamber experiments protocols are being optimized to reduce germination time. These protocols include variations in scarification techniques, imbibing concentration of gibberellic acid, length of imbibement time, and incubation temperatures. In-chamber results show intermediates between chasmogamous and cleistogamous flower types, and germination results show that higher concentrations of gibberellic acid promote faster germination. In conclusion, these experiments could facilitate the use of V. *pubescens* as a model to understand the molecular components leading to floral transition.

#### P33. Genetic Improvement of Switchgrass for Energy Production

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While plant oils as fuel have several advantages over other fuels, the current supply of these energy-rich compounds is constrained by low oilseed crop yield, limited availability of arable land, and the conflict arising out of the need to use arable land for both food and biofuel production. Sustainability is also a challenge: bioenergy crops typically are grown under monoculture systems, which are difficult to sustain. Developing breakthrough technology for a bioenergy crop well-suited to growing on marginal Appalachian surface coal mine lands has the potential to address these issues Switchgrass (*Panicum virgatum L.*) is a perennial, warm-season, dedicated bioenergy crop in the US. Under different agro-climate conditions, switchgrass is capable of producing higher biomass that can be used as a bioenergy feedstock. Recent studies have shown promise growing switchgrass, on the West Virginia's reclaimed surface mine lands that are capable of producing greater biomass than a target yield on agriculture lands. We cloned the cDNA of Arabidopsis transcription factor *WRINKLED1 (WRII)* involved in oil biosynthesis pathway using gene-specific primers. A fragment of 1463 bp containing the complete open reading frame was cloned into a binary vector consisting of a maize ubiquitin promoter (ZmUbi1) and hygromycin selection marker gene. Using optimum transformation condition developed in our lab, we have successfully generated putative transgenic plants. Further, *in vitro* rooted plants

were subjected to hardening processes in growth chamber before transfer to soil condition. Putative transgenic switchgrass plants exhibited a high rate of survivability under growth chamber conditions and undergone normal growth and developmental stages. Molecular and biochemical analysis tools for the identification of integration and expression of *WRI1* in the putative transgenic plants will be developed.

#### P34. Plant Response Across a Gravitational Gradient Aboard the International Space Station

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A plant's ability to sense and respond to gravity is critical to its proper growth and development. As humans begin to explore extraterrestrial environments, plants will be a vital source of food and bioregenerative life support. However, plants have evolved exclusively under the 1g conditions of Earth and may not be optimally adapted for beyond-Earth cultivation. Gaining an understanding of how plants will grow under the conditions of low earth orbit (0g), Earth's moon (0.16g), and Mars (0.38g) is critical to the success of human space exploration. EMCS-PGP (European Modular Cultivation System- Plant Gravity Perception) is an experiment designed to assess the phenotypic and transcriptomic effects of fractional gravity on Arabidopsis seedlings. Over 1,200 seedlings were mounted into EMCS seed cassettes and sent to the International Space Station. The cassettes were loaded onto the EMCS rotor and subjected to centrifugal force equating to g levels between 0.006g and 1g. Root and hypocotyl bending were assessed by still images collected during the experiment, and the seedlings were then frozen and returned to Earth for molecular analysis. Seedlings were dissected into root tip, root, hypocotyl, and cotyledon fractions. RNA was extracted from each sample and sent for sequencing to assess tissue- and g level-specific transcriptomic effects.

### P35. The Effect of Metal Composition and Particle Size on Nanostructure-Toxicity in Plants

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Silver nanoparticles (AgNPs) have consistently been shown to have a detrimental effect on bacteria, yeast, and plants. The interaction of AgNPs with plants have received considerable scientific attention, because it is through plants that these structures can enter the food chain and potentially accumulate in humans and animals. We have previously shown that chronic exposure of *Arabidopsis thaliana* seedlings to AgNPs triggers a gene expression pattern indicative of cell wall reorganization and defense response to oxidative and biotic stress. To gain insight into the mechanism of phytotoxicity, we tested the effects of Ag + ions and non-ionic silver and gold particles by exposing seedlings to low concentrations of silver nitrate, AgNP, or gold nanoparticles (AuNPs). To test if particle size influenced the response by the plant, AgNPs and AuNPs were tested at either 20 nm or 80 nm sizes. AgNO 3 -exposed seedlings exhibited significantly differential gene expression for a fraction of key biomarker genes. While AuNP exposure did not impact gene expression, AgNP exposure at 20 nm and 80 nm lead to the differential expression of 226 and 212 genes, respectively. Gene ontology enrichment analysis revealed that these genes were overwhelmingly involved in biotic and mechanical stress-like response. The size of AgNP particles had little influence on gene expression. Taken together, our data suggest that the chemical nature of AgNPs is an important factor in phytotoxicity. This research was funded by the U.S. Army Engineer Research and Development Center – Environmental Laboratory through the Environmental Quality and Technology Program; Contract No. W912HZ-15-2-0032 P00002.

#### P36. Molecular Comparison of the Glyceraldehyde 3-Phosphate Dehydrogenase Gene in Common Ragweed and Cuman Ragweed.

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There are at least 21 different species of Ragweed (*Ambrosia spp.*) growing in the U.S. To date, we have collected genomic DNA from 15 of these species. In a preliminary study, we have isolated a gene for Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) from three of these species: Common Ragweed (*Ambrosia artemisiifolia L.*) collected in Louisville, KY, Cuman Ragweed (*Ambrosia psilostachya DC.*) collected outside of Montgomery, AL, and Coastal Ragweed ( Ambrosia hispida Pursh), collected in Key West, FL. GAPDH is an important enzyme in plants, as it plays a critical role in the glycolytic pathway. The genes for eight different GAPDH enzymes have been isolated in Arabidopsis thaliana, with some isozymes being either NAD + or NADP + -dependent and located in the cytosol, and others being NADP + -dependent and found in plastids. Both Common and Cuman Ragweed species yielded two alleles each for the GAPDH gene homologous to the GAPC-2 gene of Arabidopsis. All four of these sequences encode an identical protein sequence (201 amino acids) which includes the active site of the enzyme. While the intron structure of the 1178 bp genomic sequences for each of these alleles was similar, there is a 45-bp gap in the intron of one allele (from *A. artemisiifolia*), that is identical to one of the alleles of *A. psilostachya*. This intron variation might, therefore, be ancient. We are utilizing the 15 Ambrosia species we have collected so far to study the evolution of other important genes.

### P37. Towards an improved metric of *Cryptophonectria parasitica* infection to quantify species based responses to infection

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At the beginning of the twentieth century, the fungal pathogen Cryphonectria parasitica, the causal agent of chestnut blight, ravaged the native chestnut population throughout North America. To this day, the existence of the blight continues to suppress chestnut development and resurgence. The American chestnut was a foundational forest species, so its disappearance poses a major barrier to arboreal biodiversity. In this study, we aim to develop a better measure of infection severity in saplings and use this metric to understand how chestnuts respond to concurrent infection and drought stress. Such a metric will help in the restoration of blight resistant BC3F3 hybrid chestnuts (American x Chinese) to forests. Our experiment was conducted comparing infected saplings of American, Chinese, and BC3F3 hybrid chestnuts under droughted and well-watered conditions. At the end of the field season, all specimens were harvested, and three metrics of infection severity were measured. We used the traditional metric of scoring cankers (1-5), used image analysis software to determine canker area, and used a ratio of canker area to stem basal diameter. We determined that the preferred metric was the ratio of canker size to basal diameter, as it better represents canker sizes relative to the size of the stem on which they developed. An applied minimum ratio cut-off of 0.05 cm<sup>2</sup>/mm yielded a sample size of N=45 stems and showed American chestnuts had significantly larger cankers than Chinese (p-value < 0.001). Additionally, a significant difference was found between Americans and hybridized chestnuts (p-value < 0.001), implying these hybrids were resistant. While no significant difference was detected among treatments, average canker ratios tended to be higher in drought treatments. With this ideal metric in mind, we plan to further study the effects of the drought-pathogen interaction this upcoming summer, expanding both our scope and sample sizes.

## **P38.** Gene Expression at the Intersection of Microgravity and Reorientation Implicates New Regulators of Gravity Signaling

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Gravity is among the most critical environmental cues in shaping plant growth; however, its ubiquity on earth limits available options in the study of the plant gravity response. NASA has circumvented this obstacle by hosting experiments in the microgravity environment of the International Space Station. The collection of gene expression data via RNAseq and microarray analyses from Arabidopsis seedlings grown in space has provided a wealth of data regarding how plants respond to a zero gravity environment. When compared to similar data from terrestrial plants exposed to a new gravity vector, several novel components in the gravity signaling pathway have been implicated. Specifically, the transcription factors ERF104, which is upregulated on earth in response to a new gravity vector and downregulated in microgravity, and IQD21, which displays the inverse expression pattern. A third gene, CIB1, was shown to be upregulated in both scenarios. As a known regulator in a diverse array of plant systems, CIB1 was included as a more general participant in the gravity response. Here, we present phenotypic characterizations of mutant lines for theses transcription factors and an update on the generation of transgenic lines overexpressing HA-tagged fusions for each respective transcription factor. These lines will enable ChIP-Seq to identify the binding sites of these three factors in the Arabidopsis genome, and consequently allow us to infer their downstream effects. This research will help to contextualize and clarify the gravity response pathway, as well as shed light on the complex web of interconnected signaling events it entails.

#### P39. Patterns of Recombination and Segregation Distortion in the Populus Genome

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Black cottonwood (*Populus trichocarpa*) is a pioneer tree species identified as a promising renewable feedstock for bioenergy and bioproducts. Populus can be used to study many aspects of perennial development related to phenology, wood formation, vegetative propagation, and dioecy that cannot be studied using conventional plant model systems such as Arabidopsis. Breeding techniques to identify elite parent trees face considerable challenges: lengthy sexual reproduction cycles (of at least 4 years), difficulty to design Recombinant Inbred Lines (RIL), highly heterozygous genomes, and poor juvenile-mature correlation for many traits, necessitating expensive long-term field trials. Genomic selection incorporates genomic information into breeding strategies to alleviate these issues. Prediction models can be improved by detailed understanding of how recombination and segregation vary across the genome, among individuals and between sexes. Linkage information also allows the creation of genetic maps that can be used to identify Quantitative Trait Loci (QTL) for phenotypes of breeding relevance (height, diameter, bud set, and disease resistance). Those QTL can also be used to improve prediction model training. Here, we have re-sequenced the genomes of 49 families (N = 821 offspring), corresponding to a full factorial cross of seven females and seven males. Using benchmark software GATK4, we have called biallelic SNPs. Using the pedigree information, we have revealed patterns of segregation in the genome and selected high-quality markers under Mendelian segregation. We have phased and imputed the progeny genomes and recovered the gametic haplotypes of the fourteen parents, allowing us to estimate fine-scale genomic

patterns of recombination. By means of the R package Onemap, fourteen genetic maps have been created, and specific patterns of variation have been revealed.

# P40. Evolutionary history and diverse functions of NODULE INCEPTION-LIKE proteins (NLPs) in root development

#### Narender Kumar, Anjali Iyer-Pascuzzi

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NLPs are plant-specific transcription proteins have been identified based on the similarity of a conserved DNAbinding RWP-RK domain from the NODULE INCEPTION (NIN) protein in legumes. NLP genes are widespread in genomes of land plants including algae Chlamydomonas reinhardtii and slime mold Dictyostelium discoideum . NLPs have been primarily characterized as master regulators of nitrate responses. However, recently, NLPs were also reported to have roles in root development, seed germination, and biotic and abiotic stresses. Model plants Arabidopsis thaliana and Solanum lycopersicum genome encode nine and six NLPs, respectively. The number of NLPs varies from species to species and seems to have evolved through segmental duplication rather than tandem amplification. As the variation in gene copy numbers may affect the organism fitness and adaptability, my goal is to investigate NLPs evolutionary history, variation in numbers, and their role in developmental mechanisms of plants. We retrieved 334 NLPs from 32 plants genome. All genomes were obtained from publicly available genome database. After improving the quality of the alignment, 228 NLPs were used to estimate the phylogeny, and all NLP sequences clustered into four clades in the phylogenetic tree. NLP sequence from slime mold Dictyostelium discoideum was used as an outgroup. Based on the reconciled phylogeny, we have estimated 100 independent gene duplication and 129 gene loss events during the diversification of NLP gene family. However, there has not been any recent NLP gene duplication in Arabidopsis thaliana and Solanum lycopersicum plants. The functional importance of NLPs in the Arabidopsis thaliana and Solanum lycopersicum root development are under investigation.

#### P41. Comparative GWAS for Seedling Root Variation in Acidic Conditions across the Pepper Species Complexes

**Tosin Akinsipe**<sup>1</sup>, Lakshmi Abburi<sup>1</sup>, Purushothaman Natarajan<sup>1</sup>, Thangasamy Saminathan<sup>1</sup>, Padma Nimakayala<sup>1</sup>, Umesh Reddy<sup>1</sup>

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Soil acidity is one of the major abiotic factors known around the world to limit plant growth. The distribution of major nutrients in soil is determined by the soil's pH, and an imbalance in soil pH can impede absorption of nutrients by the plant roots. The phenotypic variation of root architecture can be used to select the traits and genes associated with their tolerance or susceptibility to low pH. In this research, the seedling root growth traits in low pH was observed in three robust diversity panels belonging to three cultivated species of pepper. Population containing 100-110 lines of three pepper species, *Capsicum annuum, Capsicum baccatum* and *Capsicum chinense*, were grown in germination pouches with reduced pH (3.0) of the nutrient solution. The winRHIZO software was used to phenotype root length, surface area, average diameter root volume, tips, forks and crossings. ANOVA revealed significant variation for root traits in both control and treatment among diversity panels of *C. annuum, C. baccatum*, and *C. chinense*. Study revealed that the means of various root traits were higher in *C. chinense* than the other species in the study. GWAS was conducted for all the root traits in various panels separately using 20000 SNPs. We will present phenotypic variance for various traits, SNP associations and important linked genes.

# P42. Functional analysis of MtMATE30, a nodule-specific membrane effluxer in the model legume *Medicago truncatula*

**Lina Yang**<sup>1</sup>, Terry McManus<sup>2</sup>, Gregory Boyce<sup>1</sup>, Igor Kryvoruchko<sup>3</sup>, Michael Udvardi<sup>3</sup>, William P. Petros<sup>2</sup>, Vagner A. Benedito<sup>1</sup>

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*Medicago truncatula* is a well-established model organism that allows the study of intrinsic features of the legume family. Remarkably, legumes are capable of carrying out nitrogen fixation through a mutualistic association with rhizobial bacteria. Symbiotic nitrogen fixation (SNF) is established through a nutritional exchange, including reduced carbon (dicarboxylates) from the plant for reduced nitrogen (ammonia) from endosymbiotic bacteroids enclosed in the symbiosomes of nodule cortical cells. These exchanges must occur through transporters located in the symbiosome membrane, although the genetic identities of most membrane transporters are unknown. We identified a nodule transporter MATE transporter exclusively expressed in nodules. During nodule development, MtMATE30 starts to express, reaches a peak in young, mature nodules (14 dpi) and maintains consistent expression in older nodules. MtMATE30 is also highly expressed in all nodule zones except in nodule meristem, confirmed by promoter-GUS analysis and in situ hybridization. RNAi knockdown mutants for MtMATE30 were developed did not show clear nodule phenotype compared to the wild type. Since MtMATE30 canonically belongs to a phylogenetic clade that includes members with affinity to diverse alkaloids, we tested efflux activity with alkaloids present in nodules. Trigonelline is a widely distributed pyridinic alkaloid in plants and is largely found in legume nodules. We confirmed the presence of trigonelline in Medicago nodules through LC-MS. MtMATE30 affinity to trigonelline was confirmed using the acrB bacterial system by HPLC. Given that trigonelline is structurally related to nicotinamide, we hypothesize the former could be used for storage in nodule cells in order to be readily converted into NADH during periods of high energetic demands during symbiotic nitrogen fixation. Subcellular localization of this transporter in nodule cortical cells and metabolic profiling of mutant nodules edited via CRISPR/Cas9 system are underway.

#### P43. The Effects of Carbon-Based Nanoparticles on Tomato Germination and Growth

#### Susan Eiben, Sarah Wyatt

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Carbon nanoparticles (CNPs) have been a recent technology of interest in many disciplines including electronics, medicine, and materials science. Exposing plants to CNPs has previous shown to yield a variety of effects including increased fruiting, increased photosynthetic rate, the upregulation of stress response genes, DNA damage, reduction in growth, or even no significant growth differences. A key response of interest found delayed flowering in rice plants, which was attributed to the reduction of water flow velocity through the vasculature from CNP aggregates. Reducing water loss via transpiration, by replicating this mechanism, may allow for plants to tolerate unfamiliar climates, such as semiarid climates on Earth or low pressure conditions on Mars. Using tomato plants, and four different sizes of CNPs, I am investigating the effects on growth and germination. In addition to traditional growth chamber research and measurements, I have added components to investigate possible ecological implications and space-based implementation. Considering not only the potential benefits, but also considering unintentional and lasting impacts, is vital while exploring emerging technologies.

## P44. Understanding genetic and environmental factors to enable cultivation of winter malting barley in Ohio and across the Midwest

#### **Ben Eggers**

Department of Horticulture and Crop Science, Ohio State University, Columbus, OH, USA

Ohio is the fifth largest producer of craft beer in the nation, with an economic impact of \$2.7 billion. The Stockinger Lab is currently developing Ohio winter-hardy malting barley varieties to aid in the rapidly expanding craft beer industry. Winter-hardiness in barley is necessary for the survival of Ohio winters, while quality traits are essential for malting and brewing processes. The complex nature of winter-hardiness and malting quality traits are further complicated by the environment, which can greatly alter trait manifestation. In addition, varieties need high-quality agronomic traits to insure the success of farmers producing malting barley. During the 2013-14 polar vortex, a variety of germplasm were evaluated for winter-hardiness survival and selected for survivability under suboptimal environmental conditions. Resulting in detection of different survival percentages across the lines. Ten individual malting quality component traits were measured in the winter-hardy lines grown over multiple years and across locations in Ohio. Using this data, we can determine how much variation within these traits is due to environmental factors. We found that malting quality traits are affected differently by the environment, specifically the year by location interaction. Linking genetic and phenotypic data will improve clarity of genetic and environmental interactions. Therefore, we will be conducting a Genome Wide Association Study to better determine the genetic differences of traits affected by environment and genetics. This information will also be used to identify Quantitative Trait Loci, areas in a genome that correlate to the traits of interest, to accelerate future breeding and commercialization efforts.

#### P45. Physiological and Metabolomic Responses of Black Cottonwood to Water Stress

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Drought severity and frequency are increasing with global temperatures. These droughts have detrimental effects on plant life by reducing photosynthetic capabilities and increasing the accumulation of reactive oxygen species, which cause a decrease in carbon fixation and therefore lower biomass accumulation. This reduction is a matter of concern for biofuel feedstock production using woody biomass on marginal sites. Populus trichocarpa (Black Cottonwood) is a species commonly used as a plantation crop for biofuel production. However, its physiological responses to water limited conditions remain largely under-studied. We aimed to assess the drought resistance of 358 genotypes of P. trichocarpa and identify the physiological responses contributing to their resistance. While some data analysis is still in process, preliminary findings show that trees in the drought treatments had lower water potentials (p<0.001), higher stomatal resistance (p<0.001), and higher SPAD values (p=0.010). Low performers under drought exhibited higher stomatal conductance on average than high performers (p=0.026), indicating that the high performers had greater stomatal control and perhaps water use efficiency. There were also significant differences between low and high performers on SPAD (p<0.001) and specific leaf area (p<0.001) reflecting genotypic responses to low water availability. As we continue data analysis we hope to confirm these results with other measures, as well as begin to identify the genotypes with the greatest overall drought tolerance. Once identified, more intensive evaluations can be undertaken to elucidate the basis of drought resistance and these genotypes can then be used in plantations where drought is an expected factor.

### List of Presenters

Last Name	First Name	Talk or Poster	Last Name	First Name	Talk or Poster
Abeyratne	Roshan	P39	Kumar	Narender	P40
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Ajayi	Oyeyemi	P27	Larue	Clayton	Т 23
Akinmoju	Tolulope	P23	Leadmon	Caroline	Т 20
Akinsipe	Tosin	P41	Lin	Yiyun	Т 15
Alam	Maheen	P11	Lindeboom	Chase	T 21
Ariyaratne	Menaka	Т 3	Lybrand	Daniel	Т2
Banday	Zeeshan	Т 27	Lynch	Joseph	Т 30
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Bhattarai	Gaurab	Т 17	Moore	Abigail	P6
Bhattarai	Matrika	P14	Murray	Briaunna	P25
Bochenek	Alexander	Т 19	Nan	Nan	P3
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Caldwell	Denise	P1	Ojha Kshetry	Arjun	P15
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Flyckt	Kayla	Т 22	Robinson	Dave	P36
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