83rd Annual Meeting of the Northeastern Section American Society of Plant Biologists

April 13-14, 2019

USDA Forest Service

and

University of New Hampshire

Durham, NH

83rd Annual Meeting of the Northeast Section of the American Society of Plant Biologists Symposium Schedule

Saturday April 13, 2019		
Time	**Activities**	**Location**
10:00-1:00	**Onsite registration (payment in cash or checks only) and check-in**	Huddleston Hall
1:00-1:15	**Welcome; Rakesh Minocha & University of New Hampshire Representative**	Huddleston Hall
1:15-1:30	**ASPB Representative; Shoshana Kronfeld**	Huddleston Hall
1:30-2:15	**Keynote Speaker: Dr. Mariano Alvarez, Duke University** *Transgenerational effects: epigenetics and phenotypic plasticity in response to seasonal variation.*	Huddleston Hall
2:15-3:00	**Keynote Speaker: Dr. Marta R. M. Lima, University of New Hampshire** *Using metabolomics to study plant adaptation to environmental stresses*	Huddleston Hall
3:00-3:30	**Coffee Break**	Huddleston Hall
3:30-4:00	**Keynote Speaker: Dr. Rakesh Minocha, USDA Forest Service, Durham, NH** *Physiological and biochemical monitoring of stress in forest trees*	Huddleston Hall
4:00-4:30	**Keynote Speaker: Dr. Paul G. Schaberg, USDA Forest Service, Burlington, VT** *Adaptations of red spruce to acidic deposition-induced nutrient deficiencies and a changing environment: historic decline followed by surprising recent rebound*	Huddleston Hall
4:30-6:30	**Poster session and Reception**	
4:30-5:30	**Even Poster Numbers**	
5:30-6:30	**Odd Poster Numbers**	
8:30-9:00	**NEASPB Executive Committee meeting**	Huddleston Hall-Annex

Sunday April 14, 2019		
Time	**Activities**	**Location**
7:30-8:30 AM	**Continental Breakfast**	Kingsbury Hall-N101
	Oral Presentations	
8:30-9:00	**Dr. Thomas Davis, University of New Hampshire** *Prospects for de novo domestication of quinoa relatives native to Northern New England in relation to climate change*	Kingsbury Hall-N101
9:00-9:15	**Lina Castano-Duque, Duke University** *Complex genetic variation and physiology of anaerobic germination in rice*	Kingsbury Hall-N101
9:15-9:30	**Jeeyon Jeong, Amherst College** *Ferroportin 3 is a mitochondrial iron exporter necessary for iron homeostasis in Arabidopsis*	Kingsbury Hall-N101
9:30-9:45	**Alisson Kovaleski, USDA-ARS** *Cold hardiness as a proxy for dormancy evaluations explains plasticity of grapevines*	Kingsbury Hall-N101
9:45-10:00	**Josefina Mendez, Yale University** *Increased efficiency of targeted mutagenesis by CRISPR/Cas9 in plants using heat stress*	Kingsbury Hall-N101
10:00-10:20	**NEASPB Business meeting**	Kingsbury Hall-N101
10:20-10:45	**Coffee Break**	Kingsbury Hall-N101
10:45-11:00	**Samuel Mortensen, Northeastern University** *An efficient transient expression method for analyzing gene function in Catharanthus roseus seedlings and its application to study the regulation of the transcriptional repressor ZCT1*	Kingsbury Hall-N101
11:00-11:15	**Mary Tsai, Indigo agriculture** *Effects of Enterobacter cowanii seed treatment on winter wheat yield*	Kingsbury Hall-N101
11:15-11:30	**Vijaykumar Veerappan**, **Eastern Connecticut State University** *Characterization of deregulated anthocyanin pigmentation mutants in the model legume plant Medicago truncatula*	Kingsbury Hall-N101
11-30-11:45	**Anna C. Haber, University of New Hampshire** *Understanding the Role of Polyamines in Rice under Drought and Salt Stress*	Kingsbury Hall-N101
11:45-12:00	**Yan Zubo, Dartmouth College** *Coordination of chloroplast development through the action of the GNC and GLK transcription factor families*	Kingsbury Hall-N101
12:00-12:15	**Closing Remarks and Adjournment**	Kingsbury Hall-N101

Symposium Keynote Speakers

Keynote Speaker 1

Transgenerational Effects: Epigenetics and Phenotypic Plasticity in Response to Seasonal Variation

Dr. Mariano Alvarez[1], Duke University

Gabriela Auge[2], Andrew Bleich[1], Kathleen Donohue[1]

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2. Fundación Instituto Leloir, IIBBA-CONICET, Ciudad Autónoma de Buenos Aires, CP C1405BWE, Argentina

Shifts and cycles in seasonal cues can generate responses within a generation, via phenotypic plasticity, or across generations, via transgenerational effects. The adaptive value of these responses depends on how strong they are, how long they persist, and how stable they are when environments change. While environmental response has a genetic basis, epigenetic states have also been hypothesized to not only contribute to these within- and trans-generational environmental effects but also to provide a general mode of facilitated adaptation. Using Arabidopsis thaliana, we examine the inducibility, temporal persistence, and environmental stability phenotypic and epigenetic changes to understand the evolutionary consequences of plastic response in natural populations.

Dr. Mariano Alvarez received his PhD in 2016 from the University of South Florida working under Christina Richards. His dissertation focused on the ecological genomics and epigenomics of non-model plants, particularly in estuarine systems. During his Ph.D., he received departmental awards as well as scholarships to the Summer Institute for Statistical Genetics. Dr. Alvarez is currently a postdoc in Kathleen Donohue's lab at Duke University in North Carolina, using Arabidopsis thaliana as a model organism to examine the epigenomics and transcriptomics of epigenetic inheritance in response to seasonal variation, which he'll be talking about today.

Keynote Speaker 2

Using Metabolomics to Study Plant Adaptation to Environmental Stresses

Dr. Marta R. M. Lima, University of New Hampshire

Plant adaptation responses to changing environmental conditions are underpinned by changes in plant metabolism. Understanding these metabolic changes is an important step towards developing or adopting crop plants more tolerant to biotic and abiotic stresses. In this regard, continuous advances in analytical and bioinformatic techniques have allowed metabolomics to become an important tool in the realm of plant physiology, with applications ranging from the investigation of crop adaptations to environmental changes to large scale Long-term ecological studies. By characterizing all possible metabolites of an organism under a particular stressful environmental condition, metabolomics goes beyond the analysis of specific compounds, allowing to gain insights into the changes in biochemical pathways that underlie plant adaptation to environmental stress. Several analytical platforms, each with its own advantages and disadvantages, can be used in metabolomics studies. In our research, proton-nuclear magnetic resonance (1H-NMR) spectroscopy has been used to characterize plant responses to biotic and abiotic stresses, namely to study grapevine response to a complex fungal disease (esca), and to investigate soybean adaptation to iron deficient conditions. The application of 1H-NMR spectroscopy to the study of esca disease of grapevine suggested the plants divert resources from primary to secondary metabolism during infection. The 1H-NMR spectroscopy-based metabolomics study of iron-sufficient and iron-deficient soybean leaves revealed leaf metabolic changes in several pathways due to iron deficiency, including changes preceding appearance of foliar symptoms.

Marta Lima is an Assistant Professor in the Department of Agriculture, Nutrition, and Food Systems at the University of New Hampshire. She has a Ph.D. in Biology (2009, University of Minho, Portugal) and a Masters in Epidemiology (2010, Faculty of Medicine - University of Porto, Portugal). After concluding her Ph.D., Dr. Lima carried out postdoctoral studies at the Portuguese Catholic University (2009-2011), at Baylor College of Medicine-USDA/ARS Children's Nutrition Research Center (2011-2014), and at the University of California Davis (2015-2017). Before joining the University of New Hampshire in 2018, she was a visiting scientist at Virginia Tech. Dr. Lima has been working on plant adaptations to biotic and abiotic stresses using an interdisciplinary approach with an emphasis on metabolomics. She has studied plant responses to plant pathogens, iron deficiency, drought, and combined biotic and abiotic stresses. Her research interests lie at the continuum of plant science and human health and nutrition, using metabolomics as a tool to work across these fields. Currently, Dr. Lima's research focuses on the impact of environmental factors on plant physiology and biochemistry, with the goal of addressing issues related to crop productivity, plant health, and nutrient/phytochemical composition of fresh fruit and legume species.

Keynote Speaker 3

Physiological and Biochemical Monitoring of Stress in Forest Trees

Dr. Rakesh Minocha[1], USDA Forest Service, Durham, NH

Stephanie Long[1], and Subhash Minocha[2]

1. Forest Service, Northern Research Station, Durham, NH 03824, USA
2. Department of Biological Sciences, University of New Hampshire, Durham, NH 03824, USA

Continuous input of pollutants from atmospheric deposition can cause soil Al mobilization/toxicity, nutrient depletion, heavy metal toxicity, and nitrogen saturation. These multiple stressors can lead to a decline in plant health and loss of crop productivity. A major goal of our research is to develop a suite of select cellular metabolites as early markers for assessing changes in forest health and productivity in asymptomatic forests. Our objective is to determine the usefulness of mainly foliar nitrogen metabolites, including putrescine, arginine, proline and GABA, phytochelatins, etc. as early markers of abiotic stress in conifer and hardwood trees before the appearance of visual symptoms. This presentation will be a review of several studies conducted over two decades in collaboration with scientists from various institutions from study sites across Northern New England, Pennsylvania, California and India. Data show a strong correlation between soil nutrients and metabolites (polyamines and amino acids) indicating that the latter can potentially be used as reliable and easily quantifiable markers of abiotic stress. In several studies, these changes were accompanied by lower site productivity. However, removal of stress (e.g. Ca supplementation to Ca deficient soils) reversed the metabolic symptoms of the stress and resulted in species-specific growth improvement indicating the usefulness of these metabolites for monitoring site recovery efforts as well. Additionally, at Harvard Forest, MA and Hubbard Brook, NH, both Long-Term Ecological Research (LTER) sites, the changes observed in the above-ground tissues accompanied changes in microbial population diversity and functions.

Dr. Rakesh Minocha received her Ph.D. from the University of New Hampshire, Durham. During the past 3 decades at US Forest Service, she has been involved in developing biochemical indicators for monitoring of environmental stress in forest trees. She has worked in interdisciplinary teams involving scientists from academic, federal, and industrial institutions from all around the world. Her current research interests are in the following areas: 1) Monitoring the effects of acidic deposition, nutrient depletion, and declining health of forest ecosystems; 2) Effects of climatic change and human activity on the health of urban, suburban and rural forests and soil microbes; and 3) Genetic manipulation of polyamine metabolism to elucidate the molecular mechanisms of nitrogen and carbon assimilation in plants.

Keynote Speaker 4

Adaptations of Red Spruce to Acidic Deposition-Induced Nutrient Deficiencies and a Changing Environment: Historic Decline followed by Surprising Recent Rebound

Dr. Paul G. Schaberg, USDA Forest Service, Burlington, VT

Red spruce (*Picea rubens*) is an ecologically and economically important tree species in the eastern United States and adjacent Canada that experienced significant reductions in growth and increased mortality starting in the 1960s. The timing and location of decline (greatest in the west and at higher elevations) spurred considerable research into the possibility that inputs of acid deposition contributed to red spruce decline. Laboratory and field-based studies verified that inputs of acid deposition depleted calcium (Ca) from soils and resulted in tree Ca deficiencies. These deficiencies impaired plant stress response systems and predisposed foliage to winter freezing injury that thinned crowns, and shifted trees into negative carbon (C) balances that reduced growth and resulted in elevated tree mortality. Science on the impacts of acid deposition helped to bolster the passage of the 1990 Amendments to the Clean Air Act that mandated significant reductions in the sulfur (S), and to a lesser extent nitrogen (N) pollution that produces acid deposition. Recently, increases in red spruce growth and regeneration have been noted through much of the region where the species had previously declined. Tree-ring studies have determined that improvements in growth are significantly correlated with reductions N and or S pollution, as well as increases in fall, winter and spring temperatures that extend the functional C capture period and reduce the risk of foliar winter injury. Although the recent rebound in red spruce growth is promising, modeling projections suggest that significant reductions in suitable habitat are likely for red spruce as temperature and precipitation regimes shift over time. Discrepancies between recent positive trends and projected negative trends in red spruce growth, distribution and abundance have generated substantial scientific uncertainty about the trajectory of the species in the decades ahead.

Dr. Paul Schaberg received his BS and MS in Forestry, Ph.D. in Botany, all from the University of Vermont. Dr. Schaberg is a Research Plant Physiologist with the US Forest Service, Northern Research Station and a graduate advisor in the University of Vermont's Rubenstein School of Environment and Natural Resources. His research focuses on the influence of abiotic stress on tree health and productivity. Much of his recent work has involved understanding the influence of acid deposition and climate change on the growth or unexplained decline of numerous tree species in the northeastern US. However, he also studies other issues of regional and national interest, such as the causes of yellow-cedar decline in Alaska, and the restoration of American chestnut and American elm to the Northern Forest.

POSTER SESSION

Poster No.	Presenter	Title of the Poster
P1	**Ahmed G. Meselhy**, Sudhir Sharma, Jenny Patel, and Om Parkash Dhankher	Two Plasma Membrane Intrinsic Protein, OsPIP1;3, OsPIP2;6, are Involved in Arsenic and Boron Transport in Rice
P2	**Denise Butler**, Kristophe Diaz, and Adan Colon-Carmona	An *Arabidopsis thaliana* Centromere-Associated Protein-E (CENPE) Kinesin is Necessary for Proper Cell Division and Differentiation
P3	**Aria Armstrong**, Adrian Lankenau, Kevin Garayalde Batista and John Celenza	Regulation of Defense Compound Production in the Oilseed Crop *Camelina sativa*
P4	**A.S. Chandrakala**, Lin Saho and Subhash C. Minocha	Expression Analysis of Spermidine Synthase 1 (*SPDS1*) gene in response to abiotic stress in *Arabidopsis thaliana*
P5	**Gabriela De Los Santos**, Lauren F. Cole and Carolyn Lee-Parsons	Light Induces the Expression of the Vindoline Biosynthetic Pathway in *Catharanthus roseus*
P6	**Janis Dietz**, Danielle Snider and Pamela Weather	Inhibition of Malaria Transmission by Artemisia: Beyond Artemisinin
P7	**Daniel DiRocco** and Estelle M. Hrabak	Investigating the Role of Protein Phosphatase 2A in the Salt Stress Response in *Arabidopsis thaliana*
P8	**Xin Fang,** Zian Wang, Mingjie Yang, Zitao Zheng, Tianran Pan, Lynda McMaster-Schuyler and Peiyu Zeng	Genetically Development of Drought-Tolerant Switchgrass Cultivar by Agrobacterium-mediated Transformation
P9	**Jeremiah Friedman** and Estelle Hrabak	Characterizing Expression of S-Acyl Transferase Gene in *Arabidopsis thaliana*
P10	**Joseph P. Gallagher**, Harry R. Klein, María Jazmín Abraham Juárez and Madelaine E. Bartlett	Evolution and Function of Duplicate Transcription Factor Genes GT1 and VRS1 in Maize and Brachypodium
P11	**Paola Gutierrez**, Adam Ngo and Azam Noori	Localization and Accumulation of Silver Nanoparticles in Tomato Tissues
P12	**Mingjie Yang**, Tianran Pan, Zitao Zheng, Xin Fang, Zian Wang, Dr. Lynda McMaster-Schuyler, Dr. Peiyu Zeng	Development of Drought and Salt Tolerant Switchgrass Cultivar by Over- expressing CodA Gene via Agrobacterium-Mediated Transformation Method
P13	**Allison Halchak-Lord**, Steven Troy, Laura Van Beaver and Subhash C. Minocha	Effects of Gold and Silica Nanoparticles on Electrically and Chemically Induced Transformation, and Agrobacterium-Mediated Transfection
P14	**Brennan Hyden**, Craig H. Carlson, Ran Zhou, David Macaya-Sanz,	Characterizing the Homomorphic Sex Chromosomes and Sex Determination in *Salix*

	Stephen P. DiFazio, Jerry Jenkins, Jeremy Schmutz and Lawrence B. Smart	*purpurea*
P15	**Alexander Heyl**	Cytokinin Signaling in the Moss *Physcomitrella patens*
P16	**Wenjing Jiang**, Xinyi Yang, Adrian Lankenau, Sanda Zolj, and John Celenza	The Arabidopsis alf3-1 Mutation Causes Autoimmunity in the Root and Identifies a TIR Domain Protein
P17	**Srinivasan Krishnan**, Koustav Maity, Aaron P. McGrath, Leon V. Kochian Geoffrey Chang and Miguel Piñeros	Structure-Function Studies of a Non-Selective Cation Channel Involved in Drought Tolerance
P18	**Sabrina Long** and Zachary Taylor	Isolation and Genotyping of Dormant Yeast Retrieved from Beer off of the SS Oregon Shipwreck
P19	**Xinya Lu**, Ruikang Zhang, Tianran Pan, Zhizhong Yan, Junpeng Xu, Alexandra Smith, Peiyu Zeng and Lynda McMaster-Schuyler	Establishment of Tissue Culture System for Hop Genetic Engineering
P20	**Clayton Ludwig**	Using Chenopodium quinoa as a Source of Genetic Resources to Advance the De Novo Domestication of *Chenopodium berlandieri*
P21	**Shannon McCallan**, Lauren F. Cole, Erin J. Cram and Carolyn Lee-Parsons	The Role of IDD Transcription Factors in the Regulation of Vindoline Biosynthesis
P22	**Lily Mooney** and Estelle Hrabak	Characterization of the Protein S-acyl Transferase Mutant pat 4-4 in *Arabidopsis thaliana*
P23	**Brook T. Moyers**, Amelia Henry and John McKay	Use of Complex Genetic Populations to Build a Better Rice
P24	**Haley Nolen**, Thomas M. Davis and Anissa Poleatewich	Evaluating Genetic Resistance to Downy Mildew in Chenopodium Species for Use in Breeding Programs
P25	**Tianran Pan**, Zian Wang, Mingjie Yang, Xin Fang, Zitao Zheng, Dr. Peiyu Zeng and Dr. McMaster-Schuyler	Genetically Engineering Stress-Tolerant Switchgrass Cultivars by Agrobacterium-Mediated Transformation
P26	**Brennan Senecal** and Estelle Hrabak	Gene Dosage May Explain Phenotypic Differences Between Mutants in Arabidopsis
P27	**Gurpal Singh**, Jessica Rodriguez and Om Parkash Dhankher	Exploring Role of γ-glutamyl cyclotransferases (GGCTs) in Providing Tolerance to Abiotic Stresses via Glutathione Homeostasis
P28	**Madhav Subedi** and Thomas M. Davis	Study of *Chenopodium ficifolium* Genome as a Model Plant for Studying Quinoa Genome
P29	**Lori Tausta** and Scott Strobel	A Conserved Fluoride Channel (FEX) Alleviates

		Fluoride Toxicity in Plants
P30	**Zachary Taylor** and **Sabrina Long**	Isolation and Genotyping of Dormant Yeast Retrieved from Beer off of the SS Oregon Shipwreck
P31	**Hoang Vo**, Bernice Mensah and Aleel K. Grennan	Validation of a Shade Plant Model
P32	**Zian Wang**, Mingjie Yang, Xin Fang and Zitao Zheng	Engineering Drought and Salt Tolerant Switchgrass Cultivars By Overexpressing AVP1, the vacuole Proton Pump (*in progress…*)

P33	**Ruikang Zhang**, Xinya Lu, Tianran Pan, Yanzhi Zhong, Junpeng Xu, Smith Alexandra, Peiyu Zeng and McMaster-Schuyler Lynda	Establishment of Tissue Culture System for Hemp Genetic Transformation
P34	**Alexandra Zink** and Elsa Petit	The Wild Relatives of Grapes in North America: Diversity and Impact of Climate Change
P35	**Rahul Kumar**, Huseyin Yer, Rania El-Tanbouly, Huayu Sun and Yi Li	Screening of Dwarf Perennial Ryegrass Mutants for Rust Under Field Conditions
P36	**Huseyin Yer**, Rahul Kumar, Huayu Sun and Yi Li	Development of Dwarf Perennial Ryegrass Cultivar by Using CRISPR-Cas9
P37	**W. M. Medini Weerasinghe**, Sefali Acharya, Meagan Gagne and Subhash C. Minocha	Designing Poplar for Increased Nitrogen & Carbon Assimilation and Biomass Yield
P38	**Susan L. McEvoy**, Jill L. Wegrzyn and Name Swenson	Sweet Genomes: Sequencing, Assembling, and Annotating Two Maples
P39	**Jocelyn Navarro**, Shannon Manuel and T. Page Owen	Nectary and Digestive Glands of the Carnivorous Pitcher Plant *Nepenthes glandulifera*
P40	**Sanchari Kundu** and Kalyan Ganguly	An In-silico Study of Retrotransposons in a Bamboo Species of South-East Asia, Moso Bamboo (*Phyllostachys edulis*)

Oral Presentations

O-1

Prospects for *De Novo* Domestication of Quinoa Relatives Native to Northern New England in Relation to Climate Change

Dr. Thomas Davis, Sarah Levy, Erin Neff, Madhav Subedi, Haley Nolen, Clayton Ludwig.

Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire

A recent AES-supported, tri-state research project on weed diversity in relation to climate change identified the genus *Chenopodium* as offering opportunity for *de novo* crop domestication for Northern New England agriculture. In this context, we are assessing the representation and potential usefulness of *Chenopodium* species in the northern New England (NNE) states of New Hampshire, Vermont, and Maine. Eleven *Chenopodium* taxa have been reported as present in NNE, including three varieties of allotetraploid *C. berlandieri*, a close relative of cultivated quinoa (*C. quinoa*). However, the potential for misidentification is evident in the annotations of available herbarium specimens, and taxonomic confusion is displayed by various online information sources. Furthermore, several of these taxa are represented poorly or not at all in the USDA Germplasm Repository collection in Ames Iowa. We have collected representatives of five species in NNE: *C. album*, *C. berlandieri*, *C. strictum*, *C. ficifolium*, and the elusive *C. foggii*. A cytomolecular pipeline has been developed to facilitate determination/confirmation of taxon identification, and applied to confirm that *C. foggii* is an AA diploid. As a foundational step in developing local genomic resources for *Chenopodium*, F1 hybrids have been obtained from crosses between diverse representatives of diploid *C. ficifolium*, and locally collected germplasm is being screened for response to downy mildew inoculation. Finally, a study involving locally adapted *C. berlandieri* will be used to help understand the genetics of domestication traits in quinoa.

O-2

Complex Genetic Variation and Physiology of Anaerobic Germination in Rice

Dr. Lina Castano-Duque[1], Sharmistha Ghosal[2], Fergie Quilloy[2], Shalabh Dixit[2] and Thomas Mitchell-Olds[1]

1. Biology Department, Duke University, Durham, NC
2. International Rice Research Institute, Los Banos, Philippines

To improve food security in the developing world, the current trend in rice production is to shift from transplanting seedlings to direct sowing of seeds. Following heavy rains, direct-sowed seeds may need to germinate under flooded, anaerobic conditions, but most rice genotypes cannot survive these conditions. To identify complex trait loci associated to anaerobic germination (AG), we integrated phenotypic germination information with a 700,000 SNP data base from the rice 3,000 genome initiative for genome-wide association studies (GWAS). Using 109,440 seeds, we quantified AG% in 2,700 (wet season) and 1,500 (dry season) rice genotypes and performed GWAS, followed by post-GWAS analysis that

encompassed a generalized SNP-to-gene set analysis, metanalysis and a network dense module search. We determined that transcription factors linked to ethylene responses or genes involved in several metabolic processes are significantly associated with AG. SNP-to-gene, meta- and dense module network GWAS analyses identified genes that have shown changes in gene expression in response to AG in previous experiments. We found two significant gene-sets involved in sphingolipids metabolism, whose function in AG has not been characterized. In our network-GWAS analysis we evaluated the top 100 network modules; these modules showed genes involved in a wide variety of metabolic processes and found a fatty acid desaturase that also was significant in the SNP-to-gene set analysis. We determined that anaerobic germination percentages are highest among *indica* subpopulations, and AG is a polygenic trait with complex physiological differences among rice genotypes. We selected several genes of interest that have not been linked to AG before to perform further functional genomics analyses. Currently we are characterizing these genes' relationship to flooding in rice mutants by doing genetic, physiological and biochemical experiments.

O-3

Ferroportin 3 is a Mitochondrial Iron Exporter Necessary for Iron Homeostasis in Arabidopsis

Dr. Jeeyon Jeong[1], Leah J. Kim[1], Jingwen Zhang[1], Fengling Hu[1], Jennifer Gallegos-Iraheta[1], Madeline Clyne[1], Ju-Chen Chia[2], Rong Huang[3], Avery Tucker[1], Claire Castellano[1], Kaitlyn M. Tsuyuki[1], Angie Kim[1], Christopher T. DaVeiga[1], Emily Y. Park[1], Elizabeth M. Parsons[1], Daniel D. Chung, Liangtao Li[4], Jerry Kaplan[4], Olena K. Vatamaniuk[2], Mary Lou Guerinot[5]

1. Department of Biology, Amherst College, Amherst, MA 01002
2. Soil and Crop Sciences Section, School of Integrative Plant Sciences, Cornell University, Ithaca, NY 14853
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4. Department of Pathology, Division of Microbiology and Immunology, School of Medicine, University of Utah, Salt Lake City, UT 84132
5. Department of Biological Sciences, Dartmouth College, Hanover, NH 03755

Mitochondria are organelles of high iron demand, but highly susceptible to iron-induced oxidative stress. Therefore, iron must be tightly regulated in mitochondria, but mitochondrial iron transport is still not well-understood in plants. In this study, we show that the Arabidopsis Ferroportin 3 (FPN3) is a mitochondrial iron exporter critical for iron homeostasis. *FPN3* is highly expressed in the shoots regardless of the iron status, but its expression is iron-regulated in the roots in response to the iron status of the shoots. Gene expression analysis of *fpn3* mutants with a drastically reduced level of *FPN3* implied that shoot iron levels are lower whereas mitochondrial iron levels are higher in the mutants than in wild type. Phenotypic analysis revealed that *fpn3* mutants cannot grow as well as wild type under iron deficient conditions. Unexpectedly, the *fpn3 vit1* double mutant did not exhibit a growth defect under iron deficient conditions. We observed enhanced rhizosphere acidification by *fpn3 vit1* mutants treated under iron

deficient conditions, which explains the double mutant phenotype. However, the increased proton efflux was not accompanied by the induction of *IRT1* or *FRO2*, indicating that it was not caused by a Strategy I- mediated iron deficiency response. Overall, we propose that FPN3 is necessary for optimal growth under iron deficient conditions, and speculate that mitochondria may be involved in storing and buffering iron levels in plant cells.

O-4

Cold Hardiness as a Proxy for Dormancy Evaluations Explains Plasticity of Grapevines

Alisson Pacheco Kovaleski[1], Jason P. Londo[1,2]

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2. School of Integrative Plant Sciences – Horticulture Section, Cornell University-New York State Agricultural Experiment Station, 630 W. North Street, Geneva, NY

Grapevines (*Vitis* spp.), much like other subtropical, temperate, and boreal perennial plants, set overwintering buds during late summer and fall. These buds contain floral tissue that must survive winter temperatures in order to from reproductive structures the following season and, and in case of agricultural plants, a crop. The level of cold hardiness of buds varies throughout the winter, largely based on air temperature. The objective of this study was to evaluate the level of response of different temperatures on the gain of cold hardiness (acclimation) and loss of cold hardiness (deacclimation). For acclimation, *V. vinifera*'Cabernet Sauvignon' buds were collected from the field and prepared into single-node cuttings and placed under different temperature regimes: constant temperatures (2, 7°C) and cycling temperatures (2±5, 5±3, 7±3, and 7±5°C) were used. Samples were collected at regular intervals to determine the effect of temperature on cold hardiness using differential thermal analysis (DTA) to measure low temperature exotherms. The greatest gains in cold hardiness occurred under 2±5°C, whereas under the same average temperature but constant regime (2°C), there was lit t le acclimation. For deacclimation, single-node cuttings of 'Cabernet Sauvignon' were subjected to multiple constant temperatures: 2, 4, 7, 8, 10, 11, 22, 30°C. Buds were collected at multiple t imes during the winter, with different levels of chilling fulfillment towards dormancy. The rates of deacclimation had an enzymatic-like response, where an exponential response was observed at low temperatures and a reduction in the increase of rates at high temperatures. Within a given temperature, rates had a logistic response to field chilling accumulation. The differential responses to temperature between acclimation and deacclimation suggest temperature sensing is necessary for acclimation but not for deacclimation. The logistic response of rates to chilling makes this a useful phenotype to understand dormancy responses in perennial plants.

O-5

Increased Efficiency of Targeted Mutagenesis by CRISPR/Cas9 in Plants Using Heat Stress

Josefina Mendez, Chantal LeBlanc, Fei Zhang, Emma Corcoran, Yamile Lozano, Krishna Chatpar, Vivian F. Irish and Yannick Jacob

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CRISPR/Cas9 has radically improved our ability to introduce mutations in eukaryotic organisms. However, CRISPR/Cas9 is also known to show variable efficiency in creating mutations at both on- target and off-target sites in different organisms. In this study, we find much higher frequencies of CRISPR-induced mutations in Arabidopsis grown under heat stress at 37°C compared to standard temperature (22°C). Taking advantage of quantitative assays based on green fluorescent protein (GFP) reporter genes, we find that CRISPR/Cas9 targeted mutations in the Arabidopsis genome are increased by approximately 5-fold in somatic tissues, and up to 100-fold in the germline after heat stress. Furthermore, in vitro assays demonstrate that Cas9 from Streptococcus pyrogenes (SpCas9) is more active in creating double stranded DNA breaks at 37°C than at 22°C, suggesting a potential contributing mechanism for the in vivo effect of temperature we observe on CRISPR/Cas9. Using temperature-optimized CRISPR/Cas9, we have been able to rapidly create a septuple mutant background by targeting genes coding for histone H4 of Arabidopsis. Further, we find the effect of temperature on the mutation rate is not limited to Arabidopsis, as we observe a similar increase in targeted mutations by CRISPR/Cas9 in Citrus plants exposed to heat stress at 37°C. Droughts, damaging storms, and increasing environmental temperatures associated with climate change are negatively affecting food crop yields worldwide. Thus, improving tools for genetic engineering like CRISPR/Cas9 is needed to contribute to the development of sustainable agriculture in this rapidly changing world.

O-6

An Efficient Transient Expression Method for Analyzing Gene Function in *Catharanthus roseus* Seedlings and its Application to Study the Regulation of the Transcriptional Repressor ZCT1

Samuel Mortensen[1], Diana Bernal-Franco[1,4] , Lauren F. Cole[2], Suphinya Sathitloestsakun[3], Erin J. Cram[1], Carolyn W. T. Lee-Parsons[3,4]

Department of Biology[1], Department of Bioengineering[2], Department of Chemistry and Chemical Biology[3], and Department of Chemical Engineering[4], Northeastern University, Boston, MA

The medicinal plant *Catharanthus roseus* produces two terpenoid indole alkaloids (TIAs), vinblastine and vincristine, used as anti-cancer drugs. However, vinblastine and vincristine are only produced at less than 0.001 % by weight in the plant. The expression of this pathway is tightly regulated and understanding this regulation can lead to strategies that increase TIA

production. Studying gene function in *C. roseus* is limited by the availability of methods. The development of transgenic plants via *in vitro* transformation and regeneration is labor intensive, inefficient, and irreproducible. The development of stable hairy root lines is a commonly used method, but processes which are specific to photosynthetically active tissue cannot be studied in hairy roots. Therefore, transient methods are the preferred method in *C. roseus*. Virus induced gene silencing is used to knock down a gene of interest but no reliable method for overexpressing genes of interest by *Agrobacterium* infiltration into leaves exists for *C. roseus*. Previous transient transformation methods for seedlings showed an uneven and faint expression of the transgene due to the waxy surface of *C. roseus* leaves. We systematically optimized the transient expression method by 1) the infiltration method and age of seedlings, 2) introducing a constitutively active *VirG* gene, and 3) improving construct design by implementing a dual-luciferase system. Routinely, 100 % of the seedlings show transgene expression, and cotyledons are uniformly transformed. As a proof-of-concept, this method was used for transactivation assays of the *STR1* promoter with its known activator, ORCA3, and its known repressor, ZCT1. Furthermore, we used this method to investigate the regulation of *ZCT1* and characterized *cis*- regulatory elements within the *ZCT1* promoter. The *ZCT1* promoter is repressed by itself and not likely activated by ORCA3, in contrast to what was previously expected. Promoter deletion studies highlight the importance of an *activation sequence-1* (*as-1*) element within the *ZCT1* promoter, suggesting a different class of transcription factor being the key activators of *ZCT1*.

O-7

Effects of *Enterobacter cowanii* Seed Treatment on Winter Wheat Yield

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The global population is projected to reach ~9 billion by 2050. It is estimated that the production of wheat, corn, rice, and soybean must increase by 2.4% to meet the growing demand for food (Rey et *al.* 2013). However, due to climate change, there is also increasing need to produce crops that use less irrigation water, require fewer chemical inputs, and can withstand extreme weather and disease threats. Encouragingly, plant tissues are hosts to complex microbial communities – known as the plant microbiome or phytobiome – located in the phyllosphere, endosphere, or rhizosphere that often form mutually beneficial relationships with the host plant. Many of these plant-associated microbes have positive effects on host health, growth, and tolerance to environmental stresses (reviewed in Kephart et al., 2018). Such beneficial microbes can be strategically deployed as seed treatments to increase crop productivity while simultaneously reducing the need for irrigation and chemical fertilizers to sustainably meet production demands despite a changing environment. Indigo's mission is to improve grower profitability, environmental sustainability, and consumer health. To this end, Indigo develops technologies that leverage naturally-occurring plant microbes to sustainably

increase yield, particularly in crops experiencing environmental stresses such as drought stress. Here, we present data on *Enterobacter cowanii*-treated wheat seed (*Triticum aestivum* L.) and trace its performance from lab screening to performance in field trials and commercial acres to demonstrate its effect on yield.

O-8

Characterization of Deregulated Anthocyanin Pigmentation Mutants in the Model Legume Plant *Medicago Truncatula*

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Anthocyanins are flavonoid compounds produced by plants that provide tolerance to biotic and abiotic stresses. Some of these compounds are well-known for their antioxidant properties and therapeutic benefits to human health. Recently, anthocyanins are of significant interest because of their potential to be used as a natural coloring agent and as therapeutic agents to improve neurodegenerative diseases such as Alzheimer's and Parkinson's. However, plants do not produce adequate quantities for the industrial and pharmaceutical applications. Hence, understanding the transcriptional regulation of accumulation of anthocyanin pigments in plants will enable us to improve crop plants with increased anthocyanin levels, and to metabolically engineer plants and microbes to produce anthocyanins in large quantities. To identify novel regulators of anthocyanin accumulation, we are using a forward genetic approach in the model legume plant *Medicago truncatula*.I have screened Medicago *Tnt1* mutant population and isolated 12 different *Tnt1* insertion mutant lines with deregulated anthocyanin accumulation in vegetative organs. Some of the mutants show loss of anthocyanin pigmentation in leaves, petiole and stem whereas others display increased or enlarged anthocyanin spots in leaves. Furthermore, some mutants also have white/black colored seeds caused by abnormal accumulation of a different flavonoid compound proanthocyanidin. Among all the mutants, some of the mutant phenotypes are novel because there were no previously published reports on these phenotypes. Data on phenotypic characterization and efforts toward identifying the causative genes will be presented.

O-9

Understanding the Role of Polyamines in Rice under Drought and Salt Stress

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Abiotic stresses are important constraints on crop yield. Paddy-grown rice is particularly

susceptible to drought and salt stress, which have negative effects on carbon and nitrogen intake that limit plant growth and grain yield. Polyamines (PAs), mainly putrescine (Put), spermidine (Spd), and spermine (Spm), are important molecules in plant metabolism and have been implicated in abiotic stress responses, both as protectors of plants from stress and preparing the plant for tolerance of stress. This has led to genetic manipulation of PA metabolism aimed at improving drought and salt tolerance in rice and other crops. Prior to overexpressing PA biosynthetic genes, we have profiled the response of a commercial rice variety to drought and salt stress. We found that PAs may be involved in recovery from stress, but levels during stress appear to fluctuate widely. To minimize sampling errors, we also studied differences in PA contents among different parts of the long, morphologically heterogeneous rice leaf. The results show that under drought, Put is increased in the sheath and decreased in the lamina as compared to the control, suggesting that the plant prioritizes protection of the meristematic tissues by compatible solute accumulation. There were no differences in Spd or Spm under drought. Furthermore, PA levels were higher under moderate salt stress than severe stress. In all cases, the PA levels were significantly higher in the leaf blade than the sheath. This research will ultimately increase scientific understanding of abiotic stress tolerance for plant improvement.

O-10

Coordination of Chloroplast Development through the Action of the GNC and GLK Transcription Factor Families

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Fundamental questions regarding how chloroplasts develop from proplastids remain poorly understood despite their central importance to plant life. Two families of nuclear transcription factors, the GATA NITRATE-INDUCIBLE CARBON-METABOLISM-INVOLVED (GNC) and GOLDEN TWO-LIKE (GLK) families, have been implicated in directly and positively regulating chloroplast development. In the frame of this project, we determined the degree of functional overlap between the two transcription factor families in Arabidopsis (*Arabidopsis thaliana*), characterizing their ability to regulate chloroplast biogenesis both alone and in concert. We determined the DNA-binding motifs for GNC and GLK2 using protein-binding microarrays; the enrichment of these motifs in transcriptome datasets indicates that GNC and GLK2 are repressors and activators of gene expression, respectively. ChIP-seq analysis of GNC identified *PHYTOCHROME INTERACTING FACTOR* and brassinosteroid activity genes as targets whose repression by GNC facilitates chloroplast biogenesis. In addition, GNC targets and represses genes involved in

ERECTA signaling and thereby facilitates stomatal development. Our results define key regulatory features of the GNC and GLK transcription factor families that contribute to the control of chloroplast biogenesis and photosynthetic activity, including areas of independence and cross talk.

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POSTER PRESENTATIONS

P-1

Two Plasma Membrane Intrinsic Protein, OsPIP1;3, OsPIP2;6, are Involved in Arsenic and Boron Transport in Rice

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Plasma membrane intrinsic proteins (PIPs) belong to the subfamily of Aquaporins (AQPs) which are involved in transporting water and small molecules including metalloids such as arsenic (As) and boron (B) in plants. Arsenic is a highly toxic element for humans while boron is required by plants but even a slight change in its concentration causes toxicity or deficiency to the plant. We have studied four rice PIP genes (OsPIP1;3, OsPIP2;4; OsPIP2;6 and OsPIP2;7) for their role in As and B transport in plants. Heterologous expression of these PIPs in Xenopus oocytes and yeast showed As and B transport. Transgenic Arabidopsis overexpressing these four OsPIPs showed strong tolerance to AsIII and B, without any significant accumulation in the plant as compared to wildtype controls. Further, to understand their in-planta functions, we knocked down the expression OsPIP1;3 and OsPIP2;6 genes using RNAi. The RNAi lines showed enhanced tolerance to AsIII and a significant reduction in As accumulation in root and shoot tissues. Collectively, these preliminary data indicated a prominent role of the selected PIP genes in As transport and tolerance/sensitivity in rice. Further experiments for As and B influx and efflux assays as well as total As accumulation in mature seeds are in progress. Additionally, we are also exploring the soil amendment assays for reducing the As uptake and accumulation in rice using various sulfur compounds. Rice grown in soil amended with sulfur compounds showed strong tolerance to As and reduced total As accumulation in root and shoot tissues. In order to understand the role of sulfur compounds for ameliorating As toxicity at molecular levels, we plan to use the RNA-Seq approach to identify the differentially regulated genes and gene networks in rice, which will be helpful to develop strategies for further reducing As uptake and accumulation in rice grains.

P-2

An Arabidopsis thaliana Centromere-Associated Protein-E (CENPE) Kinesin is Necessary for Proper Cell Division and Differentiation

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Plant growth and development relies on coordinated and timely division that produces functional cells with different fates. Checkpoints exist within the cell cycle such as the transitions between G1-S phase and G2-M phase that control synthesis and duplication of DNA, respectively. In mitosis, the Spindle Assembly Checkpoint (SAC), another surveillance mechanism, ensures proper chromosome segregation. Very little is known about the components and regulation of the SAC in plants. We have identified a SAC component, Centromere-Associated Protein-E (CENPE) from *Arabidopsis thaliana* that has roles in both meiosis and mitosis. We call the gene AtCENPE2. An insertion mutation, *atcenpe2-1*, effects the proper alignment of chromosomes and causes a slower time to divide. We observed instances of DNA aneuploidy as a result of the chromosome segregation defects and mitotic delay. The *atcenpe2-1* mutant exhibits stunted growth, embryonic defects, and defects in male gametogenesis, and we show that the mutation is transmissible via pollen and ovules. Mutants have altered root apical meristem morphologies, as well as altered transport of auxin, known to regulate both shoot and root growth and development. Additionally, the mutation causes altered cell fate in the leaf, resulting in abnormal development and placement of stomatal pores. This research hopes to add to the understanding of the SAC, hormonal integration and regulation of mitotic progression, and the potential link between cell division and cell fate.

P-3

Regulation of Defense Compound Production in the Oilseed Crop Camelina sativa

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Climate change can impact defense against herbivores and pathogens in multiple ways. For example, geographical ranges of plants, insect pests, and plant pathogens may change in ways independent of each other. Thus a particular plant species may be exposed to different and/or greater numbers of insect pests and pathogens than it had evolved to deter. Identification of which defense pathways are available to a particular species and understanding the interactions between primary and secondary metabolism is an important focus of crop improvement and sustainable agriculture. Thus uncovering mechanisms that modulate defense compound synthesis will elucidate key targets for enhancing synthesis and potentially protecting plants from rapidly changing environments due to climate change. Camelina sativa is an emerging sustainable oilseed crop adapted to northern climates. Enhancing Camelina's chemical defenses will increase its value as a bioenergy crop especially in the face of changing environments due to climate change. As member of the mustard family, Camelina produces anti-herbivore glucosinolates and the phytoalexin, camalexin. However, compared to most other

mustards including Arabidopsis, Camelina's glucosinolate production is reported to be less robust in terms of tissue distribution and chemical diversity. These differences indicate that Camelina may have developed defense strategies distinct from Arabidopsis and suggest ways to improve Camelina's defenses to expand its growth range. Two areas are being studied in this project. First, we are using genomic and transcriptomic methods to identify similarities and differences in defense pathways between Camelina and Arabidopsis. Second, we are using our knowledge of tryptophan primary and secondary metabolism in Arabidopsis to modify the profile of Camelina's tryptophan-derived defense compounds such as camalexin and indolic glucosinolates. Our findings suggest that while Camelina and Arabidopsis share a similar tryptophan-derived defense compound pathways, the two species regulate these pathways differently in response to biotic and abiotic stress.

P-4

Expression Analysis of Spermidine Synthase 1 (*SPDS1*) gene in response to abiotic stress in *Arabidopsis thaliana*

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Spermidine synthase (SPDS) is a key enzyme in the biosynthesis of the polyamine spermidine, which is the substrate of another polyamine spermine. Polyamines play a variety of physiological roles in plant development, growth and stress responses. The enzyme converts the diamine putrescine to the triamine spermidine using decarboxylated S-adenosylmethionine (dSAM) as the source of an aminopropyl group. The enzyme SPDS is encoded by a two-gene family in *Arabidopsis thaliana.* In order to analyze the specificity of tissue- and organ-level expression of these genes, we used the approach of promoter: reporter fusion. Four different fragments of the putative promoter of *SPSD1* (named *AtSPDS1A, AtSPDS1B, AtSPDS1C,* and *AtSPDS1D*) were fused with the *β-glucuronidase* (GUS) gene and used to produce transgenic plants of *A. thaliana* by floral dip method using *Agrobacterium tumefaciens*. The activity of GUS was assayed fluorometrically to quantify the enzyme activity as well as histochemically to localize its distribution in different organs and tissues at different stages of the life cycle of the plant. In vitro-grown 3-week old seedlings were tested for the effects of various forms of abiotic stress [100 mM NaCl, 300 mM sorbitol, 0.1 mM AlCl3, 0.5 mM CdCl2, low pH (4.5) or low temperature (4°C)] for 0 h, 24 h, and 48 h before histochemical and fluorometric assays. The results show (1) high degree of tissue specific expression of the gene during the entire life cycle of the plant, and (2) higher GUS enzyme activity in response to 0.1mM AlCl3, 0.5mM CdCl2, and low temperature stress, with relatively less effect of salinity and low pH stress.

P-5

Light Induces the Expression of the Vindoline Biosynthetic Pathway in *Catharanthus roseus*

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Catharanthus roseus is the only plant in the world known to produce vindoline, a precursor of anticancer agents, vinblastine and vincristine. The last seven genes leading to the formation of vindoline (T16H2, 16OMT, T3O, T3R, NMT, D4H, DAT) have recently been fully elucidated. In order to monitor the regulation of these genes, quantitative PCR (qPCR) using Sybr Green dye was used and thoroughly validated. The proper validation of qPCR primers is necessary, especially when working with a non-model organism such as *C. roseus*, where validated probes are unavailable. First, gel electrophoresis, sequencing, and dissociation curves were used to confirm the primer's target specificity. Next, dilution curves confirmed that the primers were efficient to 100 $\pm$ 10%. Finally, we tested 3 different reference genes to determine which one had the most stable expression under our treatment conditions. These validation steps were necessary in order to subsequently observe the upregulation of the vindoline pathway genes when *C. roseus* seedlings were subjected to light.

P-6

Inhibition of Malaria Transmission by Artemisia: Beyond Artemisinin

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After decades of intensive research, treatment, and preventive measures, malaria incidence, although lowered, remains a threat for millions of people. In the last century, effective pharmaceuticals including chloroquine and artemisinin, used as single or combination therapies have been developed. However, resistance to these drugs has been noted, and transmission to mosquitos by the gametocyte stage of malaria, which continues the infectious cycle, has not been well controlled. Over the centuries, teas and dried powders from various plant species, most notably *Artemisia*, have been used in both Asia and Africa to treat malaria and other fevers. We are turning back to these more complex phytochemical combinations to try and eliminate malaria symptoms and transmission. Munyangi et al. (2019) found in a large double-blind clinical study that teas made from dried *Artemisia annua* and *Artemisia afra* gave better, faster cure of *Plasmodium falciparum* than a combination therapy of artesunate (artemisinin derivative) and amodiaquine. Further, the teas caused gametocyte elimination at the microscopic level. Interestingly, *A. afra* has lit t le or no detectable artemisinin, indicating the presence of other antimalarial compounds. We are studying teas and dried leaf extractions from *A. annua* and *A. afra* on synchronized *in vitro P. falciparum* cultures for gametocyte elimination. We are using very sensitive RT qPCR with probes against early and late stage gametocytes as well as microscopy. This system should allow us to query if gametocytes are indeed undetectable after *A. annua* treatment and what other phytochemicals from the *Artemisia* species are involved in controlling gametocyte development. Future clinical trials with teas or tablets made from dried *Artemisia* leaves on malaria patients in the DCR (Congo) with the same RT qPCR probes will further determine if gametocytes are below molecular detection and transmission is blocked.

P-7

Investigating the Role of Protein Phosphatase 2A in the Salt Stress Response in Arabidopsis thaliana

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Protein Phosphatase 2A (PP2A) is a ubiquitous enzyme in eukaryotes that moderates a large array of cellular signaling processes. PP2A is composed of three subunits: catalytic C subunit, regulatory B subunit, and scaffolding/regulatory A subunit. In *Arabidopsis thaliana,* the A subunit has three isoforms-A1, A2, and A3- that are highly conserved at the protein level with at least 86% conserved amino acid sequences. In addition, all three isoforms are expressed in roots. Taken together, these observations indicate that the three A subunits may be functionally interchangeable. In comparison to wildtype seedlings, *A. thaliana* seedlings with a mutation in the *A1* gene have roots with obvious root cell file rotation that cause root twisting under conditions of moderate salt stress. The twisted root cells result in a characteristic root curling phenotype when seedlings are grown on vertical plates. In contrast, mutations in the *A2* and *A3* genes do not result in any observable root phenotype. We hypothesized that differences in expression may be responsible for mutant phenotype variation. To test this hypothesis, hybrid genes were constructed using promoters from one subunit and coding regions from a different subunit. The hybrid genes were transformed into *a1* mutant *Arabidopsis* for complementation tests. Preliminary results suggest that different promoter-coding region combinations do affect the ability of the transgene to complement the phenotype.

P-8

Genetically Development of Drought-Tolerant Switchgrass Cultivar by Agrobacterium-mediated Transformation

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Switchgrass (*Panicum virgatum* L.) plays an important role in modern society by its use as a model herbaceous energy crop for ethanol production. In addition, it is used as a paper pulp feedstock. Its ability to grow in marginal soil conditions makes it valuable for hundred energy production. In order to optimize the characteristic and increase yield, we are genetically engineering switchgrass by overexpressing the InsP-5-Ptase gene, which is involved in the phosphoinositide pathway in both animals and plants, its protein specially hydrolyzes soluble inositol phosphates and terminates the signal. Inositol-(1,4,5)-trisphosphate (InsP3) is a soluble second messenger in plants that increases the response to diverse environmental stimuli. InsP-5-Ptase can dephosphorylate InsP3 to InsP2, then terminate the signal induced by InsP3. One expression of this gene results in drought resistant properties. We are overexpressing this gene in switchgrass to produce drought tolerance. Transgenic plants have been obtained and are being subjected to molecular and genetic analysis.

P-9

Characterizing Expression of S-Acyl Transferase Gene in *Arabidopsis thaliana*

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Protein S-acyl transferases are found in all eukaryotic cells where they transfer lipid residues onto their substrate proteins. Lipidation can affect protein location, stability, or activity. In humans, dysfunctional PAT proteins are known to be associated with disorders including schizophrenia and Huntington's disease, but lit t le is currently known about the function of PAT proteins in flowering plants. Further information about the expression patterns of *PAT* genes in the model plant *Arabidopsis thaliana* can provide clues about potential *PAT* gene transformations. I used transgenic plants that expressed *PAT* genes as N-terminal translational fusions with the β-glucuronidase (GUS) gene. Using histochemical GUS assays, the expression patterns of two of the *PAT* genes, *PAT3* and *PAT8*, were mapped at t tissue and cellular levels in *Arabidopsis* throughout the life cycle. *PAT3-GUS* was expressed only in anthers and pollen, while *PAT8-GUS* was expressed throughout the life cycle of *Arabidopsis*, including root meristems, vascular tissue, true leaves, and flowers. Additional assays at various developmental time points are needed to complete GUS expression data sets.

P-10

Evolution and Function of Duplicate Transcription Factor Genes GT1 and VRS1 in Maize and Brachypodium

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Developmental genes may diverge and evolve new roles following whole genome duplication (WGD). Class I HD-ZIP transcription factors play a role in growth repression across the flowering plants, suggesting the repeated recruitment of these genes to regulate repression. In the grasses, class I HD-ZIPs *VRS1* and *GT1* are ancient duplicates and important domestication genes involved in repressing inflorescence, floral, and axillary meristem structures. Here, I ask how these growth repression regulators evolved following WGD. I hypothesize that WGD has allowed these genes to be repeatedly recruited for new roles in growth repression. Using both existing mutants and CRISPR/Cas9-edited lines, I profiled the phenotypes of *vrs1* and *gt1* mutants in maize and *Brachypodium distachyon* (Brachypodium). These mutants exhibit loss of apical dominance in both Brachypodium and maize, but also show derepression of floral structures in maize. I have designed CRISPR/Cas9 guides to dissect promoter regions in both maize and Brachypodium. Promoter lesions induced by genome editing will reveal specific regulatory regions that play a role in shared and novel functions. Ongoing gene expression and protein localization analyses will continue to illuminate how these two gene lineages have

diverged following WGD.

P-11

Localization and Accumulation of Silver Nanoparticles in Tomato Tissues

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Silver is widely applied in industry, medicine, and agriculture. Advancements in nanotechnology have provided a growing market for silver which subsequently increases the risk of silver nanoparticles (AgNPs) being released into the environment. The small size of nanoparticles enables them to enter living organisms, including plants, and subsequently humans through the food chain. It is critical to understand the localization and translocation factor of AgNPs in plants to anticipate the risk of human exposure. The current study reports the detected amounts of Ag^+ and AgNPs in tomato (*Lycopersicum esculentum*) tissue exposed to 30 mg/L of AgNPs in an aqueous medium. To better understand the possible mechanism of silver translocation in plants, the expression of active transporter H^+-ATPase; sulfate symporter; and potassium channel as a passive transporter were studied using q-pcr. Furthermore, the confocal microscope was used to visualize H^+-ATPase in plant tissues. Additionally the size of xylem cells in roots were measured using optic microscope to study the effects of AgNPs on vascular tissues. The results showed the presence of both forms of Ag^+ and AgNPs in roots, stems, and leaves. The highest concentration of silver was detected in roots. Molecular analysis showed an upregulation of the potassium channel in exposed plants compared to the control group. It is suggested that potassium channels could be used for silver transportation in plants. Histological study showed that roots exposed to AgNPs had smaller xylem cells compared to the control. In overall AgNPs can be taken up and translocated in plants and affect plant tissues structure.

P-12

Development of Drought and Salt Tolerant Switchgrass Cultivar by Over-expressing CodA Gene via Agrobacterium-Mediated Transformation Method

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Switchgrass (*Panicum virgatum*) is a major forage crop found in the South American prairie. It was recognized as an important forage crop and has received increased interest as a potential major biofuel crop because of its biomass and ability to grow in less optimal soils. Increased osmotic stress is a major inhibitor of plant growth worldwide. Choline oxidase (COD) is an enzyme that catalyzes the production of glycinebetaine (GB). Build-up of GB in cells has been linked to drought and stress tolerance in bacteria and plants. The CodA gene, which codes for choline oxidase A, is able to catalyze the conversion of choline into glycinebetaine (GB). It has been reported that transgenic plants overexpressing codA show stress tolerance at all life cycle stages, from imbibition of seeds, through the growth of young plants and the photosynthetic activity of mature plants, to the production of fruits and

seeds. Currently, switchgrass tissue culture and transformation systems lag behind in genetic engineering technology when compared to other plants, such as tobacco, corn, and canola. In this project, we are attempting to develop genetic engineered drought-tolerant switchgrass cultivars by overexpressing the codA gene via *Agrobacterium*-mediated genetic transformation. Two cultivars of switchgrass, *Alamo* and *Cave- in Rock* were used in this study. The transgenic switchgrass plantlets have been obtained and they are being subjected to molecular and genetic analysis.

P-13

Effects of Gold and Silica Nanoparticles on Electrically and Chemically Induced Transformation, and Agrobacterium-Mediated Transfection

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Nanoparticles possess distinct physiochemical properties that allow them to interact with and modulate the action of macromolecules. The diversity of these properties allows them to be used in areas such as electronics, manufacturing, environmental remediation, bio-sensing and imaging, and drug delivery. While the future of applications for nanoparticles is burgeoning and bright, the question of their unintended effects on the environment must be considered. Research suggests some have genodynamic effects; for example, ZnO, fullerene, and Ni^2 nanoparticles have been shown to increase transformation of human and microbial cells. Increased transformation of cells in clinical, agricultural, and environmental settings presents a concern, as it can propagate pathogenic or antibiotic-resistant genes. The current experiment sought to further characterize the transformative effects of silica and gold, by identifying their effects on HGT in electro- and chemically competent model organisms *Eschericia coli and Vibrio natriegens,* as well as *Agrobacterium*-mediated transformation of poplar cells by the pathogenic soil bacterium *Agrobacterium tumefaciens.* Current data is conflicting on gold and silica's transformative effects on chemi- or electro-competent *E.coli*. Though *E.coli* transformed with silica regularly show greater CFU formation than controls, these effects have not been significant.

P-14

Cytokinin Signaling in the Moss *Physcomitrella patens*

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Cytokinins are members of a class of phytohormones and regulate plant growth and the response of plants to changes in their abiotic and biotic environment. The cytokinin signal transduction is mediated by a variant of the bacterial two-component signaling system and is comprised of four members: the receptors, the histidine phosphotransfer proteins (HPTs), and the type-A and type– B response regulators - all playing different roles in the signaling pathway. In order to understand the evolutionary patterns of these components, an in depth phylogenetic analysis was conducted for each of the four components. This analysis revealed the presence of a two classes of putative Cytokinin receptors in the moss *Physcomitrella patens*. Member of both classes were characterized and their potential to function as cytokinin receptors analyzed.

P-15

Characterizing the Homomorphic Sex Chromosomes and Sex Determination in *Salix purpurea*

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Purple osier willow (*Salix purpurea* L.) is a dioecious, perennial shrub that has a female heterogametic, ZW sex system. Shrub willow is used as a bioenergy crop alternative to fossil fuels through combustion to generate heat and/or electricity. Previous genetic mapping and gene expression profiling in *S. purpurea* has delimited the sex determination region to chr15. The most recent PacBio assembly of the genome completed by JGI and HudsonAlpha includes distinct Z and W haplotypes of chr15, with >2 Mb of unique W sequence. The SDR is a region of low recombination, thus there are distinct polymorphisms in the genetic structure of the Z and W haplotypes, including differences in annotated gene models in the putative SDR. This includes potential functional and structural differences in transcription factors that are polymorphic between the Z and W haplotypes, which also display sex-specific patterns of expression in RNAseq data. RNAseq and eQTL analysis of 190 F2 individuals is being used to identify promising potential candidates for master regulator genes of sex. Polymorphisms between the Z and W haplotypes are being used to develop reliable predictive markers for sex. A phylogenetic analysis of sex determination regions across the Salicaceae is revealing evidence of translocation in the SDR and switches between ZW and XY systems related to sexually antagonistic genes in the evolution of *Populus* and *Salix* species.

P-16

The Arabidopsis alf3-1 Mutation Causes Autoimmunity in the Root and Identifies a TIR Domain Protein

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Plant defense responses vary depending on the pathogen and intensity of the attack and are mediated primarily through two levels of defense. PAMP- triggered immunity (PTI) is triggered in response to host recognition of pathogen-associated molecular patterns (PAMPs). However, pathogens can evade PTI by secreting effector molecules into the host cell that block PTI. In turn, effector molecules can be inhibited by a second line of plant defense called effector-triggered immunity (ETI). In ETI the plant uses effector-specific resistance proteins to block the effector. ETI results in gene expression changes that lead to the hypersensitive response (HR), a form of cell death, and to the plant-wide systemic acquired resistance. Previously we identified a dominant *Arabidopsis thaliana* mutant, *alf3-1* (*aberrant lateral root formation 3*), whose primary and lateral roots die unless the

growth medium is supplemented with auxin or the plants are grown at high temperature. Based on gene expression profiling, we found that many immune and defense response genes were expressed more highly in *alf3-1* compared to WT. Consistent with these findings, *alf3-1* mutants have greatly increased sensitivity to SA as well as increased production of defense compounds such as camalexin, indolic glucosinolates and phenylpropanoids. In addition we found that the vast majority of defense-related phenotypes dysregulated in *alf3-1* returned to WT levels when the mutant was grown under rescuing conditions. Using whole genome sequencing we found that the *ALF3* gene encodes an uncharacterized TIR domain protein and we have found that this gene's expression is induced in the root by treatment with SA. Because characterized plant TIR domain proteins have been shown to function in plant innate immunity, we hypothesize that *alf3-1* is a gain-of-function mutation that causes an HR in roots even in the absence of a pathogenic trigger.

P-17

Structure-Function Studies of a Non-Selective Cation Channel Involved in Drought Tolerance

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Membrane transport proteins mediate the passage of ions and other hydrophilic molecules across plant membranes. These substrates are essential for plant homeostasis, growth, development, and stress responses. Although many of these proteins have been shown to underlie key agronomic traits involved in plant abiotic stress response, the fundamental biochemical, functional, and structural characteristics of these proteins are largely unknown. Here were present recent results obtained for a non-selective cation channel from crop-plant, involved in mediating Ca^{2+} signaling during drought stress response. We have successfully purified the protein from *Pichia pastoris*, and demonstrate their functionality using droplet-interface bilayer technique, and determined their oligomeric state in proteoliposomes by subunit-counting using Total Internal Reflection Fluorescence Microscopy. This work was funded by NSFPG Award #1444435; http://crops.ucsd.edu/.

P-18

Isolation and Genotyping of Dormant Yeast Retrieved from Beer off of the SS Oregon Shipwreck

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One hundred and thirty-three years ago, the SS Oregon collided with a schooner off the coast of Long Island, carrying beer from Liverpool, England. SUNY Cobleskill was able to obtain one bottle of beer that had been salvaged on a diving excursion of the shipwreck. Our objective is to determine if yeast is present and develop conditions for optimal yeast growth. If present, we will isolate the original yeast found in the beer and grow the organism for genotypic comparison to currently used brewing yeasts. Various sugar agars, using glucose, dextrose, and maltose are being used for yeast isolation and culture. Isolating the ancient yeast is a work in progress, with various conditions being tested to facilitate recovery. Conditions include growing samples on the previously mentioned agar types and supplementing with malt extract (used as wort during the fermentation process) in the presence of oxygen. All measures have been taken to maintain aseptic techniques while handling the yeast to prevent contamination. In the future, after isolating the ancient yeast, various samples will undergo gel electrophoresis and will be sent out for genotyping. Comparing this ancient yeast to currently used yeast strains may offer information regarding evolution among yeast strains and may indicate some information regarding historic beers.

P-19

Establishment of Tissue Culture System for Hop Genetic Engineering

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Hop flowers (*Humulus lupulus* L.) are used in various beverages, including beer, in which they impart a bitter, citric, or zesty flavor. They are also used in Chinese herbal medicine for gastric distress. The current challenge for New York State Hops is fungal disease in increasingly unfavorable climate conditions. Genetic transformation can provide an approach to develop new disease resistant hop cultivars. Heritage hops, collected from various wild hop stands in the Schoharie Valley, New York, were used to develop a tissue culture system which will be used for *Agrobacterium*-mediated transformation. Explants from various plant tissues have been examined for optimal callus induction. Explants from shoot internodes are showing promising potential for embryogenic callus induction. Hop plants were grown in medium containing Murashige & Skoog salt with Gamborg vitamins and different growth hormone treatments to optimize the tissue culture system. In our lab, several gene constructs are available for genetic engineering to create disease resistant and drought tolerant hops.

P-20

Using Chenopodium quinoa as a Source of Genetic Resources to Advance the De Novo Domestication of *Chenopodium berlandieri*

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Quinoa, endemic to the Peruvian Andes, has been cultivated for thousands of years and is well known for its high nutritional content, and lack of gluten. Quinoa demand in the United States is ever increasing, however, quinoa cannot be successfully grown in the northeast because it lacks resistance to downy mildew and cannot tolerate high heat or humidity. Quinoa has a native relative in the northeast known as pitseed goosefoot that is able to thrive in this climate, and has more resistance to downy mildew than quinoa. Pitseed goosefoot, however, lacks important domestication traits. This project focuses on developing a variety of pitseed goosefoot using quinoa genetics that is agriculturally viable for production in the northeast. This hybrid is intended to share many beneficial domestication traits with quinoa, while retaining important naturalized traits from pitseed goosefoot. This effort has the possibility produce a highly nutritious, locally grown food that benefits local farmers, and has a lower carbon footprint than current quinoa production and distribution methods.

P-21

The Role of IDD Transcription Factors in the Regulation of Vindoline Biosynthesis

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This project aims to investigate the role of the Indeterminate Domain transcription factors (IDDs) in regulating the vindoline biosynthetic pathway, a precursor to the anti-cancer compound vinblastine in *Catharanthus roseus*. The expression of the pathway from tabersonine to vindoline is associated with leaf development. Because IDDs are involved in the regulation of leaf development, we are exploring the role of IDDs in regulating the vindoline biosynthetic pathway. The promoter and 5' untranslated region (UTR) of the seven genes in the pathway was amplified and sequence confirmed (1 kb). With these sequences, we determined that the IDD binding motif occurred 7 times in the promoters, twice as likely as it would occur by chance. Additionally, 6 of these motifs occur in pairs 30-160 bp apart, indicating possible dimer formation. The presence of these binding motifs support the role that IDDs could regulate the vindoline pathway genes. To experimentally determine if IDDs regulate the downstream pathway, plasmids containing IDDs of interest driven by a constitutive promoter were cloned, transformed into *Agrobacterium tumefaciens*, and vacuum infiltrated into *C. roseus* seedlings. If our hypothesis is correct, an increase in IDD expression should lead to an increase in transcript levels of the vindoline pathway genes. Transcript levels will be measured by qPCR targeting the 7 genes, and will be presented.

P-22

Characterization of the Protein S-acyl Transferase Mutant pat 4-4 in *Arabidopsis thaliana*

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The palmitoyltransferase (PAT) family in eukaryotes catalyzes S-acylation (i.e., covalent attachment of 16-carbon fatty acids to cysteine residues in substrate proteins). Acylation can affect membrane localization, stability, or activity of these substrates. All PAT proteins contain a conserved active site (DHHC-CRD domain) and usually have four transmembrane domains. Our long-term goal is to characterize the functions of *PAT* genes in the model plant *Arabidopsis thaliana* using a reverse genetic approach. *pat4-4* is a mutant allele of *PAT4* created by insertional mutagenesis with a fragment of foreign DNA (T-DNA). The junctions between the genome and the T-DNA were sequenced to reveal that, in addition to the inserted T-DNA, there was a 50 bp deletion involving part of intron 3 and exon 4 that removes part of the third transmembrane domain. RNA was isolated from seedlings, leaves, flowers, and siliques of wildtype plants and of *pat4-4* plants and reverse transcribed to cDNA. Expression of the wildtype *PAT4* gene was detected in all tissues tested but full-length cDNA was not detected in *pat4-4* plants. The *PAT4* expression pattern was refined using a histochemical GUS assay. Since *PAT4* is expressed in pollen, we investigated whether *pat4-4* mutants have a pollen-associated phenotype by fertilizing wildtype plants with pollen from a *pat4-4* heterozygote. Preliminary results indicate no effect of the *pat4* mutation on pollen transmission.

P-23

Use of Complex Genetic Populations to Build a Better Rice

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Rice is a water-intensive crop and the primary calorie source for many developing countries. The challenge of increasing rice yields must be met under the additional constraints of a growing human population and changing climate, which increase both economic and physical water scarcity. The rice Global MAGIC population is a new, highly recombinant multi-parent population developed at the International Rice Research Institute (IRRI), with 16 parents from the major rice subspecies *indica* and *japonica*. We examined the genetic architectures of establishment, root, and yield component traits following seedling stage drought stress across three growing seasons and reproductive stage drought stress during a single season. We find major genotype by environment interactions: most loci only have a significant effect on trait variance in a single environment, and many loci exhibit dynamic reaction norms. This pattern of highly plastic genetic architecture suggests that effectively meeting breeding targets in drought tolerance may require targeting selection to specific environments, rather than developing a general "jack-of-all-trades" genotype that could be resilient across many environments.

P-24

Evaluating Genetic Resistance to Downy Mildew in Chenopodium Species for Use in Breeding Programs

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Quinoa (*Chenopodium quinoa*), is an Andean pseudocereal that is considered to be a key potential crop for New England agriculture because of its high nutritive properties and adaptability to harsh environments. However, its susceptibility to downy mildew, caused by *Peronospora variabilis* presents a significant obstacle. The long-term goal of this research is to develop a resistant variety of quinoa to be grown in North America. Field trials conducted at UNH evaluated downy mildew disease severity on ten different *Chenopodium* accessions representing four species. Accessions were planted in a 2-factor randomized split plot design with three replicate plots per treatment. Disease severity analyses were performed biweekly based on a 0-5 scale; disease severity for each treatment was compared and significant differences in disease severity was observed among treatments. It was found that *Chenopodium berlandieri* var. *macrocalycium* ecotypes collected from Rye Beach, NH and Appledore Island, ME exhibited the lowest mean disease severity of the season. *P. variabilis* was isolated from each of the ten accessions and ITS and COX2 sequences were compared. Phylogenetic analyses suggested no effect of host species; however, New Hampshire isolates formed a clear cluster when compared with Pennsylvania isolates. The results of this study provide the framework for identifying potential New England native sources of resistance to downy mildew within the genus, and provides preliminary information needed to further investigate resistance at the genomic level in *Chenopodium* spp.

P-25

Genetically Engineering Stress-Tolerant Switchgrass Cultivars by Agrobacterium-Mediated Transformation

Tianran Pan, Zian Wang, Mingjie Yang, Xin Fang, Zitao Zheng, Dr. Peiyu Zeng, Dr. McMaster- Schuyler

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Switchgrass (*Panicum virgatum L.*) is known as a C4-type grass, native to central North America. As one of the dominant species of central North American tall grass, switchgrass has been identified to have many growth advantages, with low maintenance and low harvesting costs. In recent years, switchgrass has been developed as a fuel crop with outstanding ability to reinforce soil quality, giving it high value as a potential fuel crop. Under current growth conditions, water shortage and unfavorable weather conditions are crucial factors which influence switchgrass production. Previous studies have indicated FNR (ferredoxin-NADP oxidoreductase)-FLD (flavodoxin) proteins can enhance plant stress tolerant ability. The FNR and FLD both show effects in detoxification processes of ROS (reactive oxygen species) poisoning. FLD is acting as an antioxidant which can effectively inhibit the production of ROS in chloroplasts. As a result, the proteins, FNR and FLD, improve

plant's tolerance to ROS, but also increase plant's tolerance under adverse conditions such as drought and salt. In this research, we are attempting to over-express the FNR-FLD genes in switchgrass by *Agrobacterium*-mediated transformation. The transgenic switchgrass events have been obtained and are undergoing molecule analysis and genetic analysis.

P-26

Gene Dosage May Explain Phenotypic Differences Between Mutants in Arabidopsis

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Protein phosphatase 2A (PP2A) is an enzyme that removes phosphate groups from proteins and is involved in signal transduction pathways in plants and animals. As one of the most abundant phosphatases in eukaryotes, PP2A plays critical roles in responses to light and to multiple hormones and also regulates key enzymes of primary metabolism. The PP2A heterotrimer consists of one A subunit (scaffolding), one B subunit (regulatory), and one C subunit (catalytic). Five distinct isoforms of the C subunit of PP2A are encoded within the genome of the plant *Arabidopsis thaliana*. The C3 and C4 proteins are almost identical and both are expressed in roots, but *c3* and *c4* mutants respond differently to salt stress. Cells of *c4* mutant roots are twisted while the cells of *c3* mutant roots remain straight like wildtype roots. We hypothesized that variations in gene expression might account for the phenotypic differences between *c3* and *c4* mutants. A hybrid gene consisting of the *C4* promoter driving the expression of the *C3* gene (*C4::C3*) was constructed and transformed into *c4* mutants. This transgene tests whether the *C3* coding sequence, when expressed from the *C4* promoter, can complement loss of the *C4* gene. Some of the transformants had straight roots. Genotyping of the transformants is in progress to see if straight roots correlate with presence of the transgene.

P-27

Exploring Role of γ-glutamyl cyclotransferases (GGCTs) in Providing Tolerance to Abiotic Stresses via Glutathione Homeostasis

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Plants employ various mechanism to adjust to abiotic stresses and one such pathway is Glutathione (GSH) homeostasis. GSH, a tripeptide made of cysteine, glycine, and glutamate, is a key antioxidant produced in all living organisms. It protects the cell from the damaging effect of reactive oxidative species (ROS) produced as a result of exposure to abiotic stresses and xenobiotics like heavy metals and pesticides. A steady level of GSH is maintained in the cell via GSH homeostasis, the process of synthesis of glutathione, degradation, and recycling of its component amino acids via γ-glutamyl cycle**.** Recently, we have identified a small family of gamma-glutamyl cyclotransferase (γ-GGCTs) in Arabidopsis consisting of three GGCTs (GGCT1, GGCt 2;1 and GGCT2;2). Previous studies using knockout t-DNA mutant and overexpression (OE) lines of glutamyl cyclotransferase (GGCTs) in

Arabidopsis proved the role of gene GGCT2;1 in degrading GSH in plants and increasing tolerance to arsenic and cadmium along with other abiotic stresses. Both GGCT2;1 OE and t-DNA mutants lines showed increased biomass under heavy metal stresses. While t-DNA lines exhibited higher GSH synthesis by increased nitrogen uptake, OE lines had similar growth via glutamate recycling, therefore, suggesting a role in nitrogen use efficiency (NUE). Currently, we are exploring the role of other paralogs of GGCTs – GGCT1 and GGCT2;2. Our preliminary work in phenotyping t-DNA lines *ggct1* and *ggct2;2* showed differences in sensitivity towards heavy metals including As, Cd, and Mercury (Hg). In contrast to *ggct2;1*, both *ggct1* and *ggct2;2* mutant lines were sensitive to As, Cd, and Hg. These preliminary results showed the GGCT1 and GGCT2;2 differ in their mode of action and may have different substrate preferences than GGCT2;1. We are currently conducting further experiments to characterize these genes for the exact role in Glutathione homeostasis and providing tolerance to heavy metal and abiotic tolerance.

P-28

Study of *Chenopodium ficifolium* Genome as a Model Plant for Studying Quinoa Genome

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Chenopodium quinoa wild (Quinoa) is a widely popular plant consumed for its nutritious grains that contain an excellent balance of vitamins, amino acid, carbohydrate, fibers, lipids, and minerals. It has an excellent ability to tolerate drought, frost and saline soils. Therefore, it is able to grow even in the marginal conditions and diverse agroecosystems such as coastal and highland areas.

Despite these useful agronomic traits, quinoa has some serious production constraining traits that limit its sustainable production, such as excessive branching, proneness to lodging (bending over of plants), susceptibility to disease and sensitiveness to a higher temperature. As a result, the effort for re-domesticating quinoa in Northern New England has not been successful.

The development of agriculturally suitable quinoa variety requires study of native *chenopodium* species as a potential breeding partner for quinoa. Quinoa is an allotetraploid plant that was formed by the hybridization of two different diploid species about 3.3 to 6.3 million years ago in North America. *C. ficifolium,* also known as fig-leaved goosefoot is one of the diploid ancestors of quinoa that is native to the New England region and possesses traits that are well suited for this region. The study of *C. ficifolium* genome will allow us to study the genome of quinoa and also provides information on the evolutionary history of both the species. This requires formation of segregating F2 population of *C. ficifolium*, linkage mapping and QTL analysis for any traits of interest. Currently, the F2 population is being tested for segregation of plant height, branching angle, flowering time, number of branches and chlorophyll content as useful traits for quinoa breeding.

P-29

A Conserved Fluoride Channel (FEX) Alleviates Fluoride Toxicity in Plants

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Fluoride is ubiquitous in the environment in water, soil and air, yet we know very little about how biological organisms avoid fluoride toxicity. Entering either through leaf stomata or through the roots, fluoride causes ion depletion, enzyme inhibition and stress responses which ultimately lead to cell and tissue death. Environmental f actors which increase fluoride concentrations include volcanic action, marine aerosols and human activities like coal burning and brick and glass manufacturing. Fertilizers produced from phosphate rock (mostly fluoroapatite), perlite and vermiculite increase fluoride contamination in soil mixtures. The only known fluoride-specific detoxification mechanism is a channel that effluxes fluoride ions out of the cell. The efficacy of this channel has been demonstrated in bacteria and yeast. Homologs to the yeast channel (FEX for Fluoride Export) have been identified throughout the plant kingdom. We show that the plant FEX homologs function to rescue a yeast knockout strain from fluoride toxicity. Characterization of expression levels confirms publicly available microarray data that indicates FEX is expressed at a low level in all tissues. To evaluate the role of plant FEX we have used Crispr/cas9 to make a frameshift mutation in *Arabidopsis*. Initial characterization of this mutant shows increased sensitivity to fluoride including an inability to produce viable seed, impaired growth and ultimately death. Further characterization of plant FEX expression will aid in understanding and combatting fluoride toxicity.

P-30

Isolation and Genotyping of Dormant Yeast Retrieved from Beer off of the SS Oregon Shipwreck

Zachary Taylor and **Sabrina Long**

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One hundred and thirty-three years ago, the SS Oregon collided with a schooner off the coast of Long Island, carrying beer from Liverpool, England. SUNY Cobleskill was able to obtain one bottle of beer that had been salvaged on a diving excursion of the shipwreck. Our objective is to determine if yeast is present and develop conditions for optimal yeast growth. If present, we will isolate the original yeast found in the beer and grow the organism for genotypic comparison to currently used brewing yeasts. Various sugar agars, using glucose, dextrose, and maltose are being used for yeast isolation and culture. Isolating the ancient yeast is a work in progress, with various conditions being tested to facilitate recovery. Conditions include growing samples on the previously mentioned agar types and supplementing with malt extract (used as wort during the fermentation process) in the presence of oxygen. All measures have been taken to maintain aseptic techniques while handling the yeast to prevent contamination. In the future, after isolating the ancient yeast, various samples will undergo gel electrophoresis and will be sent out for genotyping. Comparing this ancient yeast to currently used

yeast strains may offer information regarding evolution among yeast strains and may indicate some information regarding historic beers.

P-31

Validation of a Shade Plant Model

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The ability to survive and thrive in the shade is not limited to just those plants found in deep woods. Shade plants are also found in your garden, agricultural fields, as well as in meadows. There are still gaps in knowledge about how these plants develop in shade conditions due to the lack of a strong model system. One possible model to study this developmental process is to use plants with artificially enlarged chloroplasts, one of the major traits of shade leaves. *Arabidopsis thaliana* plants with a mutation in a key protein in chloroplast division is a possible model to study this response to low growth light. The mutant and wild type *Arabidopsis thaliana* plants were grown under different light conditions to confirm that larger chloroplast plants are indeed representative of shade plants. Their phenotype, leaf anatomy, and protein content could help us better understand how plants grow in the shade.

P-32

Engineering Drought and Salt Tolerant Switchgrass Cultivars by Overexpressing AVP1, the vacuole Proton Pump (in progress…)

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Switchgrass (*Panicum virgatum*), a robust perennial C4-type grass, has been proposed as a dual purpose crop for both forage and bioenergy uses. Switchgrass grows on barren land and produces high perennial productivity with low maintenance costs. It is an ideal biomass resource for biofuel production, however, because it cannot self-pollinate, genetic modification of switchgrass has been considered to be a more effective method to obtain switchgrass cultivars with desirable traits. Several reports indicate that overexpression of *AVP1* in *Arabidopsis*, tomato, and rice could enhance plant performance against salt and drought stress conditions. The vacuolar H+- pyrophosphatase gene (*AVP1*) discovered in *Arabidopsis* encodes a H+-translocating (pyrophosphate-energized) inorganic pyrophosphatase (H+-PPase) that functions as a proton pump on the vacuolar membrane. It has been reported that overexpression of *AVP1* in *Arabidopsis* could lead to a higher proton electrochemical gradient, which increases vacuolar solute accumulation in plant leaf tissue, reducing water potential, and resulting in increased drought and salt tolerance. In addition, overexpression of *AVP1* results in root enlargement, which helps transgenic plants absorb water more effectively under drought conditions. We are overexpressing the *AVP1* gene in switchgrass. The overall goal of this project is to introduce the drought and salt tolerant trait into this agriculturally important crop. We have successfully generated several transgenic events which are now being subjected to molecular and genetic analysis.

P-33

Establishment of Tissue Culture System for Hemp Genetic Transformation

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Hemp (*Cannabis sativa L*.) belongs to the *Cannabaceae* family and recent interest in its use in the United States has prompted licensing at out institution for its study. Although cannabis as a drug and industrial hemp both derive from the species *Cannabis sativa* and contain the psychoactive component tetrahydrocannabinol (THC), they are distinct strains with unique phytochemical compositions and uses. Hemp has lower concentrations of THC and higher concentrations of cannabidiol (CBD), which decreases or eliminates its psychoactive effects. Thus, the legality of industrial hemp varies widely between countries. The seeds, fiber and raw materials of hemp are used in textiles, oil, paper making, and the pharmaceutical industry. To fulfill the requirement of quality hemp production in agriculture and industry, we are developing disease resistant and dough tolerant cultivars using a genetic engineering approach. Our expertise in *Agrobacterium*- mediated plant transformation has resulted in successful cultivars in other plant species. The purpose of our research is to engineer and develop new cultivars of hemp with disease resistance and drought tolerant properties by *Agrobacterium*-mediated transformation methods. A successful hemp tissue culture system is necessary, and we are evaluating various explants for callus induction and to optimize protocols for future genetic engineering experiments. Leaves, stems or cotyledons are used as explants for callus induction. The Murashige and Skoog medium (MS medium) are used in callus induction experiments. Several constructs are currently available in our lab for developing disease resistance and drought tolerant properties.

P-34

The Wild Relatives of Grapes in North America: Diversity and Impact of Climate Change

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Over the last century, we have observed an increase in global temperatures including the warmest years as far back as meteorological data extends, with the global mean temperature projected to continue rising. Phenology, the timing of biological events, has proved to be one of the most responsive aspects of nature to rising temperatures and one of the simplest to study. Evidence for a wide range of plant species suggests that spring phenophases are especially vulnerable to warming temperatures, generally occurring earlier as a result. My project uses the grape (*Vitis* sp.) as a model system to study these effects. Grapes are very significant from an agronomic perspective and the sustainability of their agriculture is linked to better use of locally-adapted and more diverse crops. The traditional European grapes (e.g. Pinot Noir, Cabernet Sauvignon) are cultivars of a single species, *Vitis vinifera*. We are located on the Northeast coast of the United States, one of the major centers of diversity for grapes, making this region an important

source of *Vitis* germplasm for viticulture. Crop wild relatives are a valuable source of genetic variation for domesticated populations but in the face of climate change, the suitable habitat of these species is expected to greatly decrease or even disappear. Because the phenology of *Vitis* species is heavily dependent on temperature, it is important to understand how the diverse *Vitis* species of this area respond to ongoing changes in climate. Herbarium data serves as a reliable record for studying shifts in flowering phenology in response to climatic changes and herbaria specimen have been used successfully to connect changes in spatial distribution and phenology timing with changes in climate. My project aims to understand how the flowering time of *Vitis* is affected by climate change and how this effect varies between different species and regions.

P-35

Screening of Dwarf Perennial Ryegrass Mutants for Rust under Field Conditions

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Perennial ryegrass (*Lolium perenne,* L*.)* is an important cool-season turfgrass species which is widely cultivated around the world. Perennial ryegrass is very fast to establish, which makes it very favorable for ornamental use. Crown rust (*Puccinia coronata Corda f. sp. lolii*) is one of the most devastating fungal diseases of perennial ryegrass, causing significant economic losses worldwide. Rust infections can be controlled by fungicide chemicals, but the approach is pollutive and expensive. However, breeding rust resistance cultivars of perennial ryegrass may provide a better solution. We have isolated 16 dwarf mutants from an ethyl methanesulfonate (EMS)- and gamma-treated population of "Fiesta 4" cultivar of perennial ryegrass. Under a field condition, we have observed that the wildtype "Fiesta 4" plants were severely infected by rust with an average score of 4.9 (a score of "5" indicates all leaves were infected by rust while "0" is indicative of no infection). Meanwhile, under the same condition, some mutant plant lines scored 0 on average, and the others had an average score of 1-3. It appears that the rust resistance is related to the degrees of dwarfness of the mutant lines. We are currently further evaluating a small number of the rust resistant mutant lines under growth chamber conditions. The mutants identified from this study may provide valuable resources for understanding of the mechanism of rust resistance observed in our dwarf mutants and also for breeding of novel rust resistant perennial ryegrass cultivars.

P-36

Development of dwarf perennial ryegrass cultivar by using CRISPR-Cas9

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Perennial ryegrass (*Lolium perenne* L.) is one of the most popular and important bunch types cool-season turfgrass species. Because of its rapid establishment, attractiveness, and finer leaf texture, it is grown in many diverse areas such as

home lawns, national park areas, athletic fields, golf course, and fairways. Mowing is an energy-intensive cultural practice to maintain turf field. Development of dwarf perennial ryegrass helps to decrease mowing frequency requirement and therefore reduce maintenance costs and disease problems. Increased gibberellic acid (GA) content enhances plant height growth but reduction in GA concentration can cause dwarfism. *GA20oxidase* is a very important gene of the GA biosynthesis pathway. Knockout or knockdown mutations of *GA20oxidase* gene in plants can reduce GA production and therefore cause dwarf phenotype. We have found four copies of *GA20oxidase* gene in the perennial ryegrass transcriptome genomic data. CRISPR-Cas9 assisted genome editing is a very efficient and quick way to create targeted mutation in the genome. In our study, CRISPR-Cas9 is being used to create knockout or knockdown mutations of GA20oxidase genes to develop dwarf perennial ryegrass lines. Eight sgRNAs were designed to target all four *GA20oxidase* genes in "Fiesta4" cultivar of perennial ryegrass. The CRISPR-Cas9 and sgRNA constructs were delivered into perennial ryegrass callus tissues using an *Agrobacterium*-mediated transformation method. About 400 plants were regenerated and transferred to a greenhouse. We are currently evaluating plant height growth of these putative mutant plants. Meanwhile, we are conducting DNA sequencing analysis of the sgRNA targeted regions of the GA20oxidase genes in these plant lines. Successful creation of dwarf mutants could help to develop low mowing frequency cultivars of perennial ryegrass.

P-37

Designing Poplar for Increased Nitrogen & Carbon Assimilation and Biomass Yield

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An important component for plant growth and regulation is nitrogen (N). N fertilization plays an important role in increasing crop yield. However, oxidized and reduced form of N are the most common and most costly input nutrient that often limits carbon (C) assimilation in plants. Moreover, N is also a cause for environmental pollution by leaching and run offs into streams and lakes. Conversely, C is a predominant component of plants which plays a major role in inorganic N assimilation in leaves and distribution of assimilated C between organic acids, starch and sugars. Polyamines (PAs) are aliphatic amines that are present in all living organisms and are an obligatory requirement for cell survival. In higher plants, the most prevalent PAs are spermidine (Spd), and spermine (Spm), and their diamine precursor, putrescine (Put). A variety of roles have been proposed for PAs in plant growth, development, and stress response. Past research in our lab has found that by genetically manipulating the PA biosynthetic pathway, accumulation of both N and C can be enhanced. Using the hybrid poplar plant (*Populus nigr*a x *maximowiczii* - NM6), our research focuses on producing transgenic plants with genes that regulate the PA biosynthesis with the expectation that they would increase N and C assimilation, leading to increased growth and biomass accumulation. We are also testing whether foliar application of N would increases N assimilation in transgenic plants, thereby further promoting higher C assimilation and biomass production.

P-38

Sweet Genomes: Sequencing, Assembling, and Annotating Two Maples

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Acer negundo (box elder) and *Acer saccharum* (sugar maple) represent the first two members of the genus, Acer, to be characterized at the genome level. Box elder is widely spread across North America and is commonly used as a fast-growing ornamental and shade tree in urban areas. Sugar maple is found in the Eastern U.S. and is commercially valuable for both its wood and sap. Sugar maple populations are currently declining in much of their natural range due to abiotic stressors, while box elder has proved more resilient to similar impacts. This resource will contribute to the relatively small collection of hardwood genomes sequenced and annotated to date, and provide a basis for investigations of their adaptive potential across their natural range. The sequencing design consists of deep long read coverage (90x) from Pacific Biosciences SEQUEL and short reads (150bp PE) with a single insert size from Illumina HiSeq. These diploid, highly heterozygous trees have moderate genomes relative to sequenced plants, and which we are currently estimating at 440Mbp and 590Mbp, respectively. The emphasis on long reads in the sequencing design is intended to circumvent some of the challenges associated with this complexity. The final genome assemblies result from assessments of multiple software packages and scaffolding methods. Annotation of these genomes will involve a combination of existing and novel approaches intended to evaluate gene prediction methods. This will leverage extensive tissue-specific RNA-Seq data generated for both species.

P-39

Nectary and Digestive Glands of the Carnivorous Pitcher Plant *Nepenthes glandulifera*

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The carnivorous pitcher plant, *Nepenthes glandulifera*, contains glands that function to attract and digest insects. *N. glandulifera* requires arthropods to obtain the necessary mineral nutrients to thrive in nutrient-poor habitats. This study analyzes the morphology and cellular ultrastructure of the nectary and digestive glands to further understand the secretory mechanisms and uptake functions of this understudied carnivorous plant. Light and transmission electron microscopy were utilized to study the lid, peristome, and petiole, where the nectary glands are located, and the pitcher base where the digestive glands are located. Preliminary results indicate both glands are apoplastically isolated from surrounding tissue. The outer cell wall of the digestive gland contains staining patterns suggesting microchannels, for enhanced transport, which was lacking in the nectary glands. In addition, nectary glands contain a higher number of Golgi apparatus than the digestive glands. A novel structure was found in the nucleus of the digestive gland,

tentatively identified as a chromatubule, providing nuclear stability and increasing the nuclear surface area for more efficient nucleocytoplasmic transport.

P-40

An In-silico Study of Retrotransposons in a Bamboo Species of South-East Asia, Moso Bamboo (*Phyllostachys edulis*)

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Retrotransposons are hallmark for the study of plant evolutionary history and their identification provides a 'snapshot' for the plant DNA configuration. Bambusoideae family includes some of the most common forest plants of South-Asian region and they are highly used in house hold building, paper production and they are potential bio-fuel source for future. Retrotransposons are less characterized in the Bambusoideae family. In this study, In-silico characterization of the nucleotide sequences of protease, reverse transcriptase and integrase gene *Phyllostachys edulis* (Moso bamboo) was undertaken. From GenBank sources; we downloaded the nucleotide and the amino acid sequences of fourteen retrotransposon or retrotransposon-like sequences. Multiple sequence alignment of the amino acid sequences for characterization of aspartyl protease in retrotransposons against the amino acid sequence of a retroviral protease, was performed in MEGA7 and the catalytic active site- Aspartic acid, Serine, Glycine (DSG) was identified. Multiple sequence alignment of the amino acids in reverse transcriptase protein of retrotransposons of P. edulis (ADB85411.1 and ADB8525.1), ASLV retrovirus (AFV99544.1) and *Zea mays* (AAL75483.1) revealed a highly conserved motif- YXDD in the C-terminus of reverse transcriptase protein. Multiple sequence alignment of the amino acid sequences of integrase of retroviruses [Mo-MuLV], [R-MuLV], [ASLV], retrotransposons of *P. edulis* and IS elements in bacteria identified the conserved D, D (35) E region. The conserved nature of the D, D (35) E region suggests a structural similarity between retroviral integrase and bacterial transposase. D, D (35) E region is essential for the integrase processing and joining indicating the presence of single active site. Using Sequence 2 Logo, a graphical representation of the conserved DSG motif, YXDD and D, D (35) E was generated. The current in-silico study is a small step towards understanding structure and function of the retrotransposons in *P. edulis*.

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