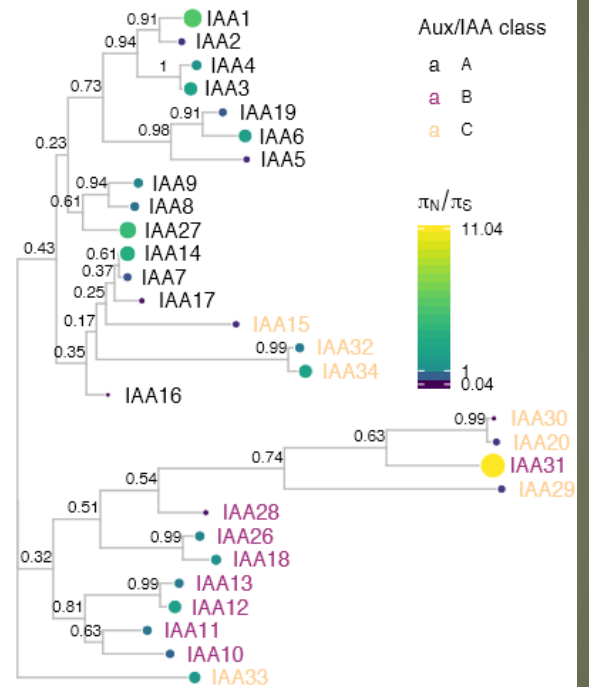
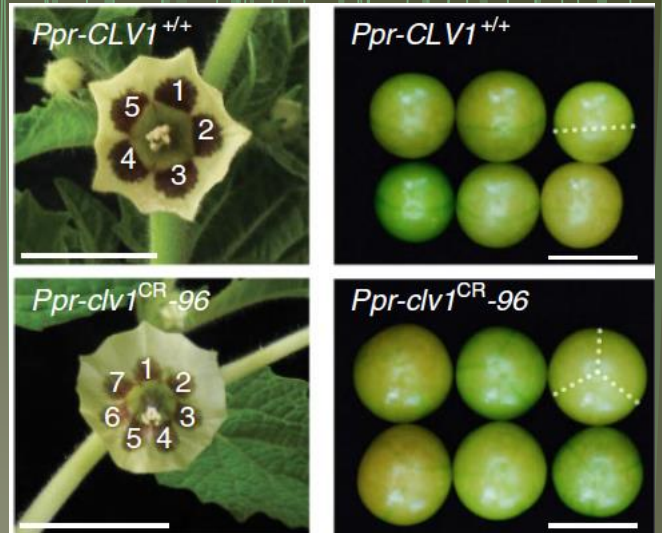


<http://wp.towson.edu/mapmbs/>

# 36<sup>th</sup> Annual Mid-Atlantic Plant Molecular Biology Society



National Wildlife Visitor  
Center  
Patuxent Research  
Refuge  
Laurel, MD

August 12-13, 2019

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**Organizing Committees: Lots of people provide the support and staffing for this meeting!**

**Many thanks to all of them for the fine job they are doing.**

**If you would like to join a committee and help, please let us know. We are always looking for dedicated volunteers!**

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John Hammond  
Ken Haymes  
Savithiry Natarajan  
Chris Clarke  
Sue Mischke

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Jim Saunders

#### **Program booklet:**

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Ben Matthews  
Savithiry Natarajan

# WELCOME

Welcome to the 36th annual Mid Atlantic Plant Molecular Biology meeting.

Thank you for coming!!! It will be great to see many old faces and meet many new faces. We have an even larger, outstanding group of speakers for this year's meeting, and we hope this meeting will be stimulating for all of you and help keep everyone up-to-date in the ever changing, exciting world of plant molecular biology. Our intention for this meeting is to provide an accessible, affordable high quality (and short) meeting in the mid-Atlantic region in a small and informal atmosphere so that scientists at all levels from undergraduate and graduate students to researchers and scientists in industry, universities and government can meet and mingle. We therefore provide lunch and breaks at the meeting so each participant has the opportunity to meet invited speakers and presenters. Many people are involved in the planning and organizing of this meeting (see the previous page), and we thank them all for their efforts in making this another successful and productive meeting. We especially wish to thank our sponsors, who help to defray the cost of the meeting. We always welcome your participation, comments and suggestions. Also, if you are interested please join next year's organizing team and volunteer your services in planning next year's MAPMBS meeting. This meeting was initiated 36 years ago, and several folks have participated all 36 years. Several of us are retired, and we especially hope to encourage more of you younger participants to attend the business meeting (Monday right before lunch) and step up and play a role in continuing this MAPMBS tradition. All are welcome at any stage of the planning and organizing process! We thank you for your continued support and participation in the Mid Atlantic Plant Molecular Biology Society.

**You can keep up with MAPMBS on our website:**

**<http://wp.towson.edu/mapmbs/>**

Ben Matthews, chair MAPMBS 2019

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## 2019 MAPMBS 36<sup>th</sup> Annual Meeting Schedule

**Monday, August 12, 2019**

**9:00 Registration and poster set-up**

**9:20 Welcome - Ben Matthews, James Saunders**

**Moderator: Janice Strachan USDA/ARS/BRS**

**9:25 Jose Feijo** University of Maryland  
Ion signaling and chemotropism: when pollen tubes get drunk and can't steer anymore.

**10:00 Peter Abrahamian** Gulf Coast Research and Education Center  
Tracing outbreaks of bacterial spot of tomato from transplants to field using whole-genome analysis.

**10:20 Coffee break Posters**

***If you have a poster 1-4 AND you want to be in the Poster-contest - please be by your poster***

**11:00 Tamara Collum** USDA-ARS-AFRL  
Identification of phloem specific defense responses during Plum Pox virus infection in *Prunus domestica* L.

**11:20 Yasmin Rivera** USDA-APHIS  
Exploring the potential of Nanopore real-time sequencing for detection and diagnostics of regulatory plant pathogens.

**11:55 Fuxi Wang** University of Maryland  
Investigating plant meristem development by studying a suppressor of *tso1* mutants.

**12:15-1:15 Lunch Posters MAPMBS business meeting**  
***If you have a poster 5-10 AND you want to be in the Poster-contest - please be by your poster***

**Moderator: Yiping Qi University of Maryland**

**1:15 Chris Higgins** HortAmericas  
How controlled environment agriculture technology is going to change the future of food production.

**1:50 Clare Casteel** University of California, Davis  
Investigating how vector-borne viruses modulate plant-insect interactions

**2:25 - 3:05 Coffee break**

**Posters**

**If you have a poster 11-13 AND you want to be in the Poster-contest - please be by your poster**

**3:05 Introduction of Keynote speaker: Ben Matthews**

**3:10 The Leslie Wanner Keynote speaker:**

**Joyce van Eck**

Boyce Thompson Institute

Application of gene editing to accelerate improvement of underutilized crops.

**4:10** Close of day; depart the Visitor Center (building closes at 4:30)

{Speaker dinner in evening, for Invited speakers and MAPMBS program committee}

## Tuesday, August 13, 2019

**9:00 Registration & Posters**

**9:15 Session moderator: Ben Matthews**

**9:20 Philip Bates** Washington State University  
Tracing non-textbook pathways of triacylglycerol synthesis in developing transgenic and wild-type oilseeds

**9:55 Nazrul Islam** USDA-ARS-NEA  
Assessing seed quality traits in genome altered soybean by proteomics

**10:15-11:00 Coffee break Posters**

**If you have a poster 14-16 AND you want to be in the Poster-contest - please be by your poster**

**11:00 Clay Wright** Virginia Tech  
The signal and the noise: Understanding and engineering plant signaling with synthetic biology and natural variations

**11:35 Simon Sretenovic** University of Maryland  
Improving plant genome editing with engineered Cas9 variants.

**11:55 Chong Zhang** USDA-ARS-GIVFL  
Characterization of tomato Anthracnose resistance caused by *Collitotrichum* species

**12:15 – 1:15 Lunch break Posters**

**If you have a poster 17& above AND you want to be in the Poster-contest - please be by your poster**

**Session moderator: David Puthoff Frostburg State University**

**1:15 Kevin Hockett** Pennsylvania State University  
Role of Bacterial Antagonism in Plant Host Colonization.

**1:50 Alice Lunardon** Pennsylvania State University  
Genome-wide annotation and analysis of small RNA loci in 48 plant species.

**2:10 Chris Wozniak, Robert Merker, Neil Hoffman** EPA, FDA and USDA-APHIS  
Evolving regulatory pathways for products developed through gene editing as well as those utilizing dsRNA / RNAi among other technologies.

**2:45 \*\*\*\*Poster competition awards ceremony\*\*\*\***

## Meeting schedule

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- 2:55 Yingxiao Zhang** University of Maryland  
Efficient plant genome editing by CRISPR-CPF1 and its variants in rice and maize.
- 3:15 Jiangnan Peng** Morgan State University  
Taccalonolides, novel microtubule-stabilizing agents for the treatment of cancer.
- 3:35 Jianhua Zhu** University of Maryland  
Dissecting the role of alternative splicing in plant abiotic stress responses.
- 4:10** Close of day – posters down; depart the Visitor Center (building closes at 4:30)



| <b>Poster #</b> | <b>Abbreviated Author list</b>   | <b>Abbreviated title</b>   |
|-----------------|--|--|
| <b>1</b>        | Muzi Li , . . . Zhongchi Liu   | Comparative analysis . . .<br>developing fruit   |
| <b>2</b>        | Jinyoung Y. Barnaby . . .<br>Anna M. McClung                                       | Exploring Naturally Existing . . .<br>Climate  |
| <b>3</b>        | Cristina P. Fernandez-Baca . .<br>. Jinyoung Y. Barnaby                            | Identifying genomic . . . rice<br>cultivars  |
| <b>4</b>        | Yun-Ting Kao . . . Bonnie<br>Bartel§   | Peroxisomal ubiquitination. . .<br>peroxisome function   |
| <b>5</b>        | Matthew Fabian, Xiaoning<br>Zhang, and Hua Lu                                      | Elucidating the role . . . regulation<br>in Arabidopsis  |
| <b>6</b>        | Adam Levav<br>Zhaohui Zhong, Simon<br>Sretenovic . . . Yiping Qi and<br>Yong Zhang | Improving plant genome editing<br>with engineered CAS9 variants                                    |
| <b>7</b>        | Gabrielle A. Bate, Mark K.<br>Jeness and Angus S.<br>Murphy                        | ABCB Auxin . . . light-mediated<br>responses   |
| <b>8</b>        | Anna DiBattista, Vinay K.<br>Nagarajan, . . . Pamela J.<br>Green                   | Evidence for endoribonucleolytic<br>cleavage of nonsense-mediated<br>decay (NMD) targets in plants |
| <b>9</b>        | Mark K Jenness, Reuben<br>Tayengwa, Angus S. Murphy                                | AtABCB19 regulates leaf . . . blue<br>light responses  |
| <b>10</b>       | Rakesh K. Upadhyay, . . .<br>Autar K. Mattoo                                       | Polyamine accumulation . . .<br>versus catabolism genes  |
| <b>11</b>       | Nick Johnson and Harsh Bais  | Evasion of plant innate defense . . .<br>Salmonella on Lettuce.                                    |
| <b>12</b>       | Anna Reachmack, . . .<br>Angus S. Murphy   | Investigating the . . . with plant<br>membranes  |
| <b>13</b>       | Shifaa Alshammari . . .<br>Hemayet Ullah   | Arabidopsis scaffold. . .<br>development pathway   |

WHAT IS MY POSTER NUMBER???

## **ION SIGNALING AND CHEMOTROPISM: WHEN POLLEN TUBES GET DRUNK AND CAN'T STEER ANYMORE**

**Jose Feijo**

University of Maryland

Ion homeostasis has been implicated as essential for tip growth. We focus on  $\text{Ca}^{2+}$  and pH signalling on the pollen tubes, the male gametophyte of plants. I will report on molecular mechanisms and quantitative studies of the choreography of these two ions during chemotropic responses necessary for ovule targeting and fertilization. I will also focus on the biology of the  $\text{Ca}^{2+}$  permeable channels Glutamate Receptor-Like (GLR) and their role on the control of apical growth.

## TRACING OUTBREAKS OF BACTERIAL SPOT OF TOMATO FROM TRANSPLANTS TO FIELD USING WHOLE-GENOME ANALYSIS

**Peter Abrahamian**,<sup>a,b,#\*</sup> Sujan Timilsina,<sup>b</sup> Gerald V. Minsavage,<sup>b</sup> Neha Potnis<sup>d</sup>, Jeffrey B. Jones,<sup>b</sup> Erica M. Goss,<sup>b,c</sup> Gary E. Vallad,<sup>a</sup>

<sup>a</sup>Gulf Coast Research and Education Center, Wimauma, Florida

<sup>b</sup>Department of Plant Pathology, University of Florida, Gainesville, Florida

<sup>c</sup>Emerging Pathogens Institute, University of Florida, Gainesville, Florida

<sup>d</sup>Department of Entomology and Plant Pathology, Auburn, Alabama

\*Current address: USDA-ARS Molecular Plant Pathology Laboratory, Beltsville, MD

# Corresponding author: Peter Abrahamian; e-mail: [peter.abrahamian@ars.usda.gov](mailto:peter.abrahamian@ars.usda.gov)

Outbreaks of bacterial spot on tomato (BST) caused by *Xanthomonas perforans* (Xp) are a major concern for sustainable crop production. BST is a common occurrence in tomato transplants grown for field production. We hypothesized that BST outbreaks in commercial fields originate from Xp strains inadvertently introduced from commercial transplant facilities. We used a genome-wide single nucleotide polymorphism (SNP) analysis to characterize Xp strains recovered from tomato transplant facilities and fields in commercial production areas. Xp strains were isolated from symptomatic transplants prior to roguing at two commercial transplant growers. Then the same group of transplants were tracked to commercial fields to recover Xp strains from diseased plants prior to harvest. Whole-genome sequencing was carried out on 84 strains isolated from transplant and field plants from Florida and South Carolina. SNPs were called using three reference strains that represented the genetic variation of the sampled strains. Transplant and field strains clustered together by grower within each phylogenomic group, consistent with expectations. The range of genetic divergence among strains isolated from field plants was similar to the range obtained from strains on transplants. We estimate that at least 60% to 100% of field strains were an extension of the transplant strain population. Current management of BST mainly relies on the frequent application of pesticides during field production. However, the lack of effective pesticides and the development of strain tolerance to certain bactericides limits the ability to control outbreaks in production fields. Better knowledge of probable sources of Xp inoculum during tomato production is helpful to refine management strategies.

## **IDENTIFICATION OF PHLOEM SPECIFIC DEFENSE RESPONSES DURING PLUM POX VIRUS INFECTION IN PRUNUS DOMESTICA L.**

**Tamara D. Collum**<sup>1,2</sup>, Andrew L. Stone<sup>3</sup>, Diana J. Sherman<sup>3</sup>, Elizabeth E. Rogers<sup>3</sup>, Christopher Dardick<sup>2</sup> and James N. Culver<sup>1,4</sup>

<sup>1</sup>Institute for Bioscience and Biotechnology Research, College Park, MD <sup>2</sup>USDA, Agricultural Research Service, Appalachian Fruit Research Laboratory, Kearneysville, WV <sup>3</sup>USDA, Agricultural Research Service, Foreign Disease-Weed Science Research Unit, Frederick, MD <sup>4</sup>Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD  
Tami.Collum@usda.gov

Plant phloem tissues are the main conduit for the movement of hormones, nutrients, proteins, RNAs, and carbohydrates essential for plant growth and development. The phloem is also utilized by plant viruses as the main route for viral long-distance movement and the establishment of systemic disease. Movement of molecules through the plant phloem is regulated, and specific viral-plant interactions are required for viruses to move through the phloem. However, these phloem specific interactions and plant responses are not well characterized especially in agriculturally important perennial crops. In this study, we use translating ribosome affinity purification (TRAP) followed by RNA sequencing to identify phloem specific responses to plum pox virus (PPV) infection in plum (*Prunus domestica* L.) during leaf development. PPV is the causative agent of sharka, one of the most devastating diseases of stone fruits. We found that in four to six-week old leaves the phloem has a disproportionate response to PPV with two- to six-fold more differentially expressed genes in phloem than non-phloem tissues despite similar infection levels. Phloem alterations included genes associated with salicylic acid mediated defense responses and RNA silencing. The results reveal new insights into the dynamics of plant defense responses in phloem tissue types during virus infection. Identified genes also provide new candidates for manipulating the timing of plant defense responses to disrupt the normal virus lifecycle and develop new strategies for disease resistance.

## **EXPLORING THE POTENTIAL OF NANOPORE REAL-TIME SEQUENCING FOR DETECTION AND DIAGNOSTICS OF REGULATORY PLANT PATHOGENS**

**Yasmin Rivera**  
USDA/APHIS

“Real-time” nanopore sequencing using the MinION device offers the advantage of portability and access to data as it is generated. The research community has proven this technology as useful for sequencing in remote areas as well as obtaining entire genomes in one single read. Plant pathogen diagnostics brings a different set of challenges to the process of library preparation and sequencing. At the USDA CPHST Beltsville Lab, we are evaluating whether this new technology can improve the current diagnostics of plant pathogens of regulatory importance to the US. During this talk we will discuss the sequencing of Plum Pox Virus and other plant viruses using the MinION device and how this technology compares with other sequencing methods.

## **INVESTIGATING PLANT MERISTEM DEVELOPMENT BY STUDYING A SUPPRESSOR OF *tso1* MUTANTS**

**Fuxi Wang**, Wanpeng Wang, Zhongchi Liu

CBMG Department, University of Maryland – College Park 0225 Bioscience Research Building, College Park, MD Email: fxwang@umd.edu

The plant meristems at the root tip and shoot apex give rise to all below and above ground tissues and organs and at the same time maintain their stem cell identity. The balance between cell proliferation and differentiation at the meristem is vital for plant development. In Arabidopsis, previous research demonstrated that TSO1 plays an important role in regulating such a balance. Mutations in TSO1 cause severe development defects, including shoot meristem fasciation, sterility, and short root. It has been shown that TSO1 transcriptionally represses MYB3R1 expression and forms a regulatory module with MYB3R1 to balance the proliferation and differentiation at both the root and shoot meristems. However, MYB3R1 is not the only factor that acts together with TSO1. A new genetic suppressor of *tso1-1* mutant, named A144, was found to suppress shoot apical meristem fasciation phenotype and partially restore the fertility of *tso1-1* mutant. Interestingly, the same suppressor also enhanced the short root phenotype of *tso1-1*. Mapping-by-sequencing has identified a mutation in a cyclin gene as the best candidate and complementation has confirmed this mutation is the causal mutation of A144. The characterization of A144 suppressor phenotype and the study of the suppression mechanism will further our understanding of TSO1 regulatory functions and provide new insights into the plant meristem regulation.

## **TRACING NON-TEXTBOOK PATHWAYS OF TRIACYLGLYCEROL SYNTHESIS IN DEVELOPING WILD-TYPE AND TRANSGENIC OILSEEDS**

**Philip D. Bates**

Washington State University, Pullman, WA

phil\_bates@wsu.edu

Triacylglycerols (TAGs, the main component of plant oils), are the most energy-dense carbon reserves produced by plants, and supply humans with much of the calories and essential fatty acids required in our diet. Because TAGs are composed of long-chain hydrocarbons (fatty acids), plant oils can also replace petroleum in many applications, such as renewable biofuels and as feedstocks for the petrochemical industry. The fatty acid composition of plant oils is directly related to their value for food or industrial purposes. Most common food crops contain just five different fatty acids, however in the plant kingdom >450 different unusual fatty acids are found which provides a vast repertoire of structures that can be used as renewable chemical feedstocks. The rising human population and decreasing amount of arable land has amplified the need to produce plant oil more efficiently, and to produce oils with fatty acid compositions tailored to different end uses. To engineer plant oil compositions, we need a better understanding of the metabolic pathways involved in TAG assembly. Most textbooks indicate TAG is assembled from glycerol-3-phosphate and three acyl-CoAs by four sequential enzymatic reactions known as the Kennedy pathway that was discovered 60 years ago. Since then, many more reactions have been discovered which demonstrate lipid metabolism is a highly interconnected network where TAG assembly overlaps with essential membrane lipid biosynthesis. The path of fatty acid flux through this network directly affects both membrane lipid and TAG fatty acid compositions. However, in most plant tissues acyl flux through the lipid metabolic network is unclear, which directly affects our ability to engineer plant oil compositions. To better understand the major routes of plant fatty acid flux through the lipid metabolic network we utilize in vivo metabolic labeling approaches to: elucidate the major pathways of fatty acid flux in different plants and plant tissues; discover bottlenecks to oil accumulation in plants engineered to produce novel fatty acid compositions; and to produce new hypotheses for engineering the designer plant oils of the future.



## **INVESTIGATING HOW VECTOR-BORNE PATHOGENS MODULATE PLANT DEFENSE**

**Clare L. Casteel** <sup>1,2</sup>

<sup>1</sup>Department of Plant Pathology, University of California, Davis, CA, 95616, USA

<sup>2</sup>Department of Plant Microbe Biology and Plant Pathology, Cornell University, Ithaca, NY, 14850, USA

Numerous studies have demonstrated that vector-borne pathogens influence host characteristics, resulting in altered host-vector contact and enhanced transmission. My lab seeks to determine the molecular mechanisms that underlie this phenomenon and utilize this knowledge to develop innovative control strategies. Previously, I demonstrated that Turnip mosaic virus (TuMV) increases insect vector attraction to and reproduction on infected plants. Changes in host physiology which mediate host-vector interactions were due to the expression of a single viral protein, NIa-Pro. Recently, we determined that NIa-Pro relocates from the nucleus to the vacuole of the plant cell in the presence of the insect vector. Importantly, NIa-Pro needs to relocate in order to inhibit plant defenses during infection. We also demonstrate that this phenomenon occurs for other potyviruses, suggesting a conserved role for the protein in vector–host interactions. These results suggest that plant viruses respond actively to the presence of insect vectors, promoting insect performance and transmission only when needed. Progress on this work will be presented.

## APPLICATION OF GENE EDITING TO ACCELERATE IMPROVEMENT OF UNDERUTILIZED CROPS

Van Eck J<sup>1,2</sup>, Reem N<sup>1</sup>, Swartwood K<sup>1</sup>, Lippman Z<sup>3,4</sup>

<sup>1</sup>The Boyce Thompson Institute, Ithaca, NY; <sup>2</sup>Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, NY; <sup>3</sup>Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; <sup>4</sup>Howard Hughes Medical Institute, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

jv27@cornell.edu

The availability of gene editing technologies, especially CRISPR/Cas, has greatly advanced gene function studies and provides the long-term benefit of approaches to precisely manipulate phenotypes to advance crop improvement. These improvements have the potential to secure agricultural productivity by enhancement of characteristics such as yield and resilience to stresses imposed by climate extremes. Traits of interest to us are those that when modified can transform a plant species that is underutilized because of undesirable agronomic characteristics into one with potential to diversify options for agricultural production. Our early work with the Solanaceae family member tomato (*Solanum lycopersicum*) as a model centered on investigation of gene function as it relates to plant architecture, productivity, and fruit-related characteristics. Results from this work led us to believe that gene editing could be exploited to fast-track improvement, in a sense fast-track domestication of underutilized plant species. Our subsequent work has transitioned to other solanaceous species, including the closest tomato wild relative, *Solanum pimpinellifolium*, and members of the distantly related *Physalis* genus to determine if what we learned from our earlier work with tomato is translatable to improvement or domestication of these species. Within the *Physalis* we are working with two different species, *Physalis pruinosa* (groundcherry), which is a diploid and *Physalis peruviana* (goldenberry) a tetraploid. Through application of CRISPR/Cas-mediated gene editing, we have observed timely improvements of undesirable phenotypes that cements our belief that this technology can indeed be exploited to turn an underutilized species into one with desirable agronomic characteristics within a realistic timeframe. To date, we have targeted a number of genes to affect characteristics such as plant growth habit and fruit size. We observed a more compact growth habit in both tomato and groundcherry by targeting the Self Pruning gene (SP, homolog of *Arabidopsis* TFL1) and its homolog SP5G. Related to fruit characteristics, we have recovered groundcherry fruit with a 24% increase in weight by editing the *CLAVATA1* (CLV1) gene as compared to the wild type, non-edited control. As our research has progressed, we have identified additional traits to improve in *Physalis* that would be considered undesirable from an agricultural productivity perspective. Through this work we intend to establish editing strategies for key genes that most affect traits such as growth habit, productivity, harvestability and others that if improved would increase the likelihood of underutilized plant species being part of a solution to strengthen food and agricultural security.

## **THE SIGNAL AND THE NOISE: UNDERSTANDING AND ENGINEERING PLANT SIGNALING WITH SYNTHETIC BIOLOGY AND NATURAL VARIATIONS**

**Clay Wright**  
Virginia Tech

We now have the ability to precisely engineer plant genomes. However, in most cases we do not know which genetic changes will yield a desired trait. My group aims to fill in this knowledge gap by combining bottom-up approaches from engineering and top-down approaches from biology to gain a quantitative understanding of plant signaling. Particularly, we use synthetic biology to measure the functional effects of natural and non-natural genetic variation in signaling networks and use this measure of functional variation to predict plant phenotype and identify “engineerable” nodes of signaling networks. In the past we have used this approach to identify a natural genetic variant in an auxin receptor which predictably alters root architecture. To allow other researchers to similarly mine natural variation, we have developed an accessible web tool for visualizing variation. We are currently expanding this approach to explore jasmonate signaling as well as the connections between growth signaling and nutrient availability.

## ASSESSING SEED QUALITY TRAITS IN GENOME ALTERED SOYBEAN BY PROTEOMICS

**Nazrul Islam**<sup>1</sup>, Stupar Robert<sup>2</sup>, Philip D. Bates<sup>3</sup>, K.M. Maria John<sup>4</sup>, Hari B. Krishnan<sup>5</sup>, Luthria Devanand<sup>4</sup>, Garrett Wesley<sup>6</sup>, Zhanyuan J. Zhang<sup>7</sup>, Natarajan Savithiry<sup>1</sup>.

<sup>1</sup>USDA-ARS, NEA, BARC, SGIL, MD, <sup>2</sup>Department of Agronomy and Plant Genetics, University of Minnesota, MN, <sup>3</sup>Institute of Biological Chemistry, Washington State University, Pullman, WA, <sup>4</sup>USDA-ARS, NEA, BARC, BHNRC, MD, <sup>5</sup>USDA-ARS, Columbia, MO, <sup>6</sup>USDA-ARS, NEA, ABBL, MD, <sup>7</sup>Division of Plant Genetics, University of Missouri, Columbia, MO.

Email of corresponding author: [Nazrul.Islam@usda.gov](mailto:Nazrul.Islam@usda.gov)

The quality seed traits such as oil and protein contents were analyzed in genome altered soybean seeds. The genome alteration was performed by gene silencing and radiation mediated mutagenesis. Protein changes were quantitatively measured using tandem mass tag (TMT) based mass spectrometry. Silencing of the fatty acid desaturase (FAD-3) gene, which reduces linolenic acid content, resulted in an increased expression of 703 proteins and a reduced expression of 616 out of a total of 6079 proteins identified. Changes in several metabolic pathways including key enzymes of fatty acid metabolism specifically  $\alpha$ -linolenic acids pathways were observed. Fast neutron (FN) bombardment produced soybeans with significant changes in protein/oil content in ten lines (L01-L10). One mutant line (L03) that exhibited a 15% increase in seed protein content was selected for detailed proteomic analysis. Protein profiling of L03 revealed a total of 3,502 proteins. Changes in several proteins were observed specifically basic 7S globulin followed by vacuolar-sorting receptor in addition to protein transporters. The differentially expressed proteins were mapped to global metabolic pathways. A high level of enrichment in ribosomal, endoplasmic reticulum and protein export metabolic pathways was observed. Complementary genome hybridization showed deletions of 24 genes in the L03 mutant located at chromosome 5, 10, and 15. Among the 24 genes, a gene specific DNA binding transcription factor along with other 23 genes might have a cascading effect on protein synthesis, resulting in an increased amount of total protein content in the soybean seeds. This information will be useful to develop new varieties of value-added soybeans.

## **HOW CONTROLLED ENVIRONMENT AGRICULTURE TECHNOLOGY IS GOING TO CHANGE THE FUTURE OF FOOD PRODUCTION**

**Chris Higgins**  
HortAmericas

Controlled environment agriculture (CEA) is anything but new, it has been around for at least 100 years. Mr. Higgins's presentation will take a quick look at the history of CEA industry before diving into its future. He will discuss current industry technology trends and then explain how those trends can be used to solve problems fresh produce farmers face not only today, but over the next 5 to 10 years.

## IMPROVING PLANT GENOME EDITING WITH ENGINEERED CAS9 VARIANTS

Zhaohui Zhong<sup>1</sup>, **Simon Sretenovic**<sup>2</sup>, Qiurong Ren<sup>1</sup>, Lijia Yang<sup>1</sup>, Yu Bao<sup>3,4</sup>, Caiyan Qi<sup>1</sup>, Mingzhu Yuan<sup>1</sup>, Yao He<sup>1</sup>, Shishi Liu<sup>1</sup>, Xiaopei Liu<sup>1</sup>, Jiaheng Wang<sup>1</sup>, Lan Huang<sup>1</sup>, Yan Wang<sup>1</sup>, Dibin Baby<sup>2,5</sup>, David Wang<sup>2,6</sup>, Tao Zhang<sup>3,4</sup>, Yiping Qi<sup>2,7</sup> and Yong Zhang<sup>1</sup>

1 Department of Biotechnology, School of Life Sciences and Technology, Center for Informational Biology, University of Electronic Science and Technology of China, Chengdu, China

2 Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD, USA

3 Jiangsu Key Laboratory of Crop Genetics and Physiology, Co-Innovation Center for Modern Production Technology of Grain Crops, Key Laboratory of Plant Functional Genomics of the Ministry of Education, Yangzhou University, Yangzhou, China

4 Joint International Research Laboratory of Agriculture and Agri-Product Safety of Ministry of Education of China, Yangzhou University, Yangzhou, China

5 Indian Institute of Science Education and Research, Tirupati, Andhra Pradesh, India

6 Seven Lakes High School, Katy, TX, USA

7 Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, MD, USA

Email of corresponding author: yiping@umd.edu

Genome editing is the introduction of desired modifications in genomic DNA sequences and is more attainable due to a powerful class of tools based on chimeric nucleases or Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). CRISPR has become the obvious technology of choice owing to its simplicity of targeting DNA. However, not every DNA sequence is targetable because of the strict NGG Protospacer Adjacent Motif (PAM) requirement to begin the process of genome editing of CRISPR associated nuclease isolated from *Streptococcus pyogenes* (SpCas9). Two recently engineered SpCas9 variants, namely xCas9 and Cas9-NG, have shown improved targeting specificity and broadened PAM recognition range in mammalian cells. In this study, we evaluated these Cas9 variants in the crop plant, rice. We first tested xCas9-3.7, the most effective xCas9 variant in mammalian cells, for targeted mutagenesis at 16 possible NGN PAMs. xCas9 exhibited nearly equivalent editing efficiency to wild-type SpCas9 (Cas9-WT) at most canonical NGG PAM sites tested. With mismatched sgRNAs, we found that xCas9 had improved targeting specificity over Cas9-WT. Furthermore, we tested two Cas9-NG variants, Cas9-NGv1 and Cas9-NG, for targeting NGN PAMs. Both Cas9-NG variants showed higher editing efficiency at most non-canonical NG PAM sites tested. In stable transgenic rice lines, we demonstrated that Cas9-NG had much higher editing efficiency than Cas9-NGv1 and xCas9 at NG PAM sites. To expand the base-editing scope, we developed an efficient C to T base-editing system based on Cas9-NG nickase, PmCDA1, and UGI. Taken together, our work benchmarked xCas9 as a high-fidelity nuclease for targeting canonical NGG PAMs and Cas9-NG as a preferred variant for targeting relaxed PAMs for plant genome editing.

## **CHARACTERIZATION OF TOMATO ANTHRACNOSE RESISTANCE CAUSED BY *COLLETOTRICHUM* SPECIES**

**Chong Zhang**

USDA-ARS, Genetic Improvement for Fruits & Vegetables Laboratory  
10300 Baltimore Avenue Building, 010A, Room. 224  
Beltsville MD 20705

Tomato is one of the most economically important vegetable crops. Many diseases pose a great threat to tomatoes, and anthracnose caused by *Colletotrichum* sp. is one of them. As a hemibiotrophic pathogen, *Colletotrichum* maintains a quiescent state after it infects an unripe tomato. After the ripening process begins in the fruit, the initial quiescent biotrophic hyphae differentiate into necrotrophic hyphae, resulting in circular sunken lesions. Thus, genetic resistance to anthracnose is highly desirable in tomatoes.

A small-fruited tomato breeding line (95L-368) developed at the USDA-ARS, GIFVL, provides a high level of resistance to *Colletotrichum* in ripening tomatoes. Previous data suggested that the inhibition could be caused by a heat-stable, pH insensitive, non-protein compound. Using TLC-bioassay, we were able to fractionate fruit constituents and focus our study on bioactive compounds that inhibited fungal growth. The inhibition zone was isolated and resuspended in methanol for LC-MS analysis. LC-MS identified steroidal glycoalkaloids (SGA) as the major components which differentiated the resistant tomato 95L-368 from the susceptible genotype US28. Levels of an anti-fungal compound,  $\alpha$ -tomatine, and its downstream product acetoxytomatine were markedly increased in both immature and mature fruit of 95L-368. MALDI-MS analysis of C18 column extracted samples further documented accumulation of these compounds. Our data suggest that a blockage in the metabolic pathway down-stream of acetoxytomatine may be responsible for accumulation of glycoalkaloids in the resistant line 95L368 and be responsible for the fungal resistance observed in this germplasm.

## ROLE OF BACTERIAL ANTAGONISM IN PLANT HOST COLONIZATION

Hanareia Ehau-Taumaunu<sup>1</sup> and **Kevin L. Hockett**<sup>1,2</sup>

<sup>1</sup>Department of Plant Pathology and Environmental Microbiology, The Pennsylvania State University, University Park, PA, USA

<sup>2</sup>The Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA, USA

Plant-associated microbes engage in multiple forms of competition. Such competition forms the basis of many biological control strategies. In particular, organisms that antagonize a target pathogen through the production of a toxic compound are often sought. Such biocontrols, however, are often inconsistent in their effectiveness. We believe that biocontrols can be improved by developing a deeper understanding of the underlying ecological dynamics surrounding plant-associated microbial competition. In this research, we investigated the ecological benefit conferred by protein toxin production to the plant pathogen, *Pseudomonas syringae*. Our model system relies on two distinct pathogens that have converged on a common host (*Phaseolus vulgaris*, common bean). One of the pathogens, *Pseudomonas syringae* pv. *syringae* (Psy) produces a highly specific protein toxin that inhibits another bean pathogen, *Pseudomonas syringae* pv. *phaseolicola* (Pph). Our results have shown that while it is possible to detect the detrimental effects of the toxin toward Pph within a plant environment at most days post inoculation (dpi), our ability to detect a beneficial effect for Psy is limited only to 6 dpi when Psy is inoculated as a minority strain (1:9 with Pph), but not at other dpi, nor when Psy is co-inoculated 1:1 with Pph. Unexpectedly, we were discovered that co-inoculation of both Psy and Pph into the leaf apoplast resulted in a significant negative effect on the Pph population, even when Psy was unable to produce its toxin. In following up on these results, we found that a type III secretion system (T3SS) mutant of Psy was completely abolished in its ability to suppress Pph, regardless of toxin production. These results hint that plant-associated bacteria, particularly pathogens, are able to compete within the plant environment through manipulation of the plant host. To our knowledge, such plant-mediated bacterial competition has not been demonstrated within the literature.



## GENOME-WIDE ANNOTATION AND ANALYSES OF SMALL RNA LOCI IN 48 PLANT SPECIES

Alice Lunardon<sup>1</sup>, Nathan Johnson<sup>1,2</sup>, Emily Hagerott<sup>3</sup>, Tamia Phifer<sup>3</sup>, Seth Polydore<sup>1,2,4</sup>, Ceyda Coruh<sup>1,2,5</sup>, Matthew Jones-Rhoades<sup>2</sup>, and Michael J. Axtell<sup>1,2</sup>

<sup>1</sup> Department of Biology, The Pennsylvania State University, University Park, PA 16802 USA

<sup>2</sup> Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA 16802 USA

<sup>3</sup> Department of Biology, Knox College, Galesburg, IL 61401 USA

<sup>4</sup> Present address: Donald Danforth Plant Science Center, St. Louis, MO 63132 USA

<sup>5</sup> Present address: Salk Institute for Biological Studies, La Jolla, CA 92037 USA

Corresponding author: Michael J. Axtell (mja18@psu.edu)

Sequencing of plant endogenous small RNAs (sRNAs) has produced so far a very large amount of data. microRNA annotations derived from sRNA-seq experiments are stored in miRBase but we are still lacking a database for storing short interfering sRNAs (siRNAs) annotations for multiple species. Additionally, a lot of information has still not been investigated in sRNA-seq data produced so far, for these reasons: 1) many studies focus on microRNAs only, leaving the majority of the sequenced sRNAs not analyzed, 2) every study annotates sRNAs using a different pipeline, 3) the sRNAs annotation produced is not always available. This ultimately results in the impossibility to easily use the current published information as a resource for further studies, most importantly for comparison analyses of sRNA loci between the genomes of different species. We created the first integrated database where a large dataset of sRNA-seq data from 48 model and crop species is analyzed consistently to produce comprehensive and comparable set of annotations of sRNA loci. In total we used 1,333 sRNA-seq libraries that passed strict quality filters and we annotated >2,7 million high-confidence sRNA loci: MIRNA and siRNA loci, the latter classified based on the predominant sRNA size produced, which is an indicator of their biological role. We observed that 24nt siRNA loci are the dominant type of locus in all flowering plants, 21nt siRNA loci are also quite common and some species have more numerous 22nt siRNA loci than average. We found conserved patterns amongst species: 24nt siRNA loci are enriched within the 1kb upstream region of protein-coding genes, while 21nt siRNA loci are strongly enriched in the gene body regions. Although not enriched, 24nt siRNAs are found in large numbers inside genes, where they tend to map to introns, while 21nt siRNAs are associated with exons. Interestingly, we found that genes targeted by 24nt siRNAs are enriched in cytochromes P450, disease resistance genes, xenobiotic transmembrane transporters and other genes involved in the response to environmental stresses. Disease resistance genes are also known sources of 21nt secondary siRNAs in dicots but only in barley and wheat amongst monocots. We found evidence of 21nt siRNAs production from disease resistance genes in four other monocots, suggesting a broader conservation of this siRNA-mediated regulation. Although rarer than in dicots, we described all the genes producing 21nt siRNAs in monocots, unrevealing conserved and species-specific cases. All annotations are available at [plantsmallrnagenes.science.psu.edu](http://plantsmallrnagenes.science.psu.edu). Our integrated annotation of sRNA loci will be useful for further genome-wide sRNAs studies, in individual or multi-species comparison analyses, and also for loci-specific studies to quickly and easily visualize and retrieve expression and other features of sRNAs in the loci of interest.

## **EVOLVING REGULATORY PATHWAYS FOR PRODUCTS DEVELOPED THROUGH GENE EDITING AS WELL AS THOSE UTILIZING DSRNA / RNAI AMONG OTHER TECHNOLOGIES.**

Chris Wozniak, Robert Merker, Neil Hoffman  
EPA, FDA and USDA-APHIS

The Federal government has a coordinated, risk-based system to ensure that new biotechnology products are safe for the environment and human and animal health. Established as a formal policy in 1986, the Coordinated Framework for Regulation of Biotechnology describes the Federal system for evaluating products developed using modern biotechnology. The Coordinated Framework is based upon existing laws designed to protect public health and the environment. The U.S. government has written regulations, policies, and guidance to apply these laws to biotechnology-derived products.

The U.S. Government agencies responsible for oversight of the products of agricultural modern biotechnology are the USDA's Animal and Plant Health Inspection Service (USDA-APHIS), the U.S. Environmental Protection Agency (EPA), and the Department of Health and Human Services' Food and Drug Administration (FDA). Depending on its characteristics, a product may be subject to the jurisdiction of one or more of these agencies. Regulatory officials from the three agencies regularly communicate and exchange information to ensure that any safety or regulatory issues that may arise are appropriately resolved.

USDA first issued biotech regulations based on its statutory authority in 1986 and has only made modest revisions since. Major revisions were proposed in 2008 and 2017, however APHIS withdrew both rules in response to public comments and to reengage in a fresh dialogue with stakeholders on the regulation of biotechnology. A third proposed rule was published June 2019.

FDA has regulatory authority over plant-based human and animal foods under the Federal Food, Drug, and Cosmetic Act. FDA published its thinking on regulation of food from new plant varieties in a statement of policy in 1992. FDA intends to clarify its policy for the regulation of plant products derived using genome editing techniques. In addition, FDA completed its first consultation on food from a genome edited plant, a high oleic soybean in February 2019.

EPA has regulatory authority over pesticides, including biopesticides, such as biochemical, microbial and plant-based pesticidal substances. More recently EPA has started to regulate genetically engineered mosquitoes intended for population suppression. As part of the update to the Coordinated Framework for the Regulation of Biotechnology, EPA is developing its National Strategy to set forth a vision for ensuring that the federal regulatory system is

equipped to assess efficiently the risks, if any, associated with future products of biotechnology. The regulatory system will continue to evolve in order to keep pace with technological developments, including those pest control agents developed through gene editing.

EPA is considering undertaking rulemaking to simplify and improve the regulatory process specifically for products that are considered to be low-risk products of agricultural biotechnology. An area we may evaluate is a limited subset of plant-incorporated protectants (PIPs) that could have been developed by natural breeding techniques. In the interim, EPA will also be considering the development of a streamlined review process for registering a limited set of genome-edited PIPs. We will provide more information on this facilitated review in the coming months.

## EFFICIENT PLANT GENOME EDITING BY CRISPR-CPF1 AND ITS VARIANTS IN RICE AND MAIZE

**Yingxiao Zhang**<sup>1</sup>, Zhaohui Zhong<sup>2</sup>, Qi You<sup>3,4</sup>, Keunsub Lee<sup>5,6</sup>, Xu Tang<sup>2</sup>, Qiurong Ren<sup>2</sup>, Shishi Liu<sup>2</sup>, Lijia Yang<sup>2</sup>, Yan Wang<sup>2</sup>, Xiaopei Liu<sup>2</sup>, Binglin Liu<sup>2</sup>, Tao Zhang<sup>3,4</sup>, Xuelian Zheng<sup>2</sup>, Ysa Le<sup>1</sup>, Kan Wang<sup>5,6</sup>, Yong Zhang<sup>2</sup>, Yiping Qi<sup>1,7</sup>

<sup>1</sup>Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD; <sup>2</sup>Department of Biotechnology, School of Life Sciences and Technology, Center for Informational Biology, University of Electronic Science and Technology of China, Chengdu, China; <sup>3</sup>Jiangsu Key Laboratory of Crop Genetics and Physiology, Co-Innovation Center for Modern Production Technology of Grain Crops, Key Laboratory of Plant Functional Genomics of the Ministry of Education, Yangzhou University, Yangzhou, China; <sup>4</sup>Joint International Research Laboratory of Agriculture and Agri-Product Safety of Ministry of Education of China, Yangzhou University, Yangzhou, China; <sup>5</sup>Crop Bioengineering Center, Iowa State University, Ames, IA; <sup>6</sup>Department of Agronomy, Iowa State University, Ames, IA; <sup>7</sup>Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, MD.  
Corresponding authors: Yiping Qi (yiping@umd.edu); Yong Zhang (zhangyong916@uestc.edu.cn)

Cpf1 (CRISPR from *Prevotella* and *Francisella* 1, or Cas12a) is a class II type V endonuclease, which has been used as a genome editing tool to target thymidine-rich regions in different biological systems. The short crRNA (42 nt) requirement and the RNase activity make Cpf1 easy to use and multiplex. Cpf1 cleavage generates a staggered end distal from the PAM (Protospacer Adjacent Motif) site, which may promote continuous cleavage of DNA as well as NHEJ (Non-Homologous End Joining)-based gene insertion. Previous work has shown that efficient genome editing in rice (nearly 100% biallelic mutations) can be achieved using Pol II promoters (RNA polymerase II promoters), driving the expression of both Cpf1 and crRNA, with crRNA flanked by hammerhead and hepatitis delta ribozymes to facilitate precise processing. Using this strategy, mutation rates as high as 60% were obtained when introducing Cpf1/crRNA to maize. To further expand the utility of Cpf1 in plant genome editing, we tested whether its fairly uncommon PAM requirement of TTTV (V=A, C and G) could be loosened. We discovered that FnCpf1 can successfully edit target sites with seven of nine VTTV PAMs. Moreover, we demonstrated that LbCpf1 variants generated by introducing mutations to the PAM-interacting domain can recognize CCCC, TYCV and TATG PAM sites. The redefined PAM sites with FnCpf1 and altered PAM sites with LbCpf1 variants nearly quadruple the number of accessible target sites within the rice genome. The efficient genome editing system we developed for Cpf1 and its variants has dramatically expanded the CRISPR toolkit, providing a powerful platform for basic and applied research in plant model systems and crops.

## TACCALONOLIDES, NOVEL MICROTUBULE-STABILIZING AGENTS FOR THE TREATMENT OF CANCER

**Jiangnan Peng**

Departments of Chemistry and Biology, Morgan State University, Maryland

Microtubule-disrupting agents play an important role in anticancer drug discovery and development. Paclitaxel, a plant-derived microtubule stabilizer, is one of the most successful anticancer drugs currently used.

In the effort to find new microtubule disrupting agents we identified a new class of microtubule stabilizers, the taccalonolides, from the tropical plant *Tacca* spp. With the bioassay-guided isolation and identification, we isolated a number of new taccalonolides. Most of them exhibited moderate microtubule stabilizing activity and antiproliferative actions against cancer cells.

To improve its potency, the structure-activity relationship (SAR) was analyzed and the data showed that the 22,23-epoxy group is critical for the potency. Based on the SAR results, we developed a highly efficient and simple Click Chemistry method and synthesized over 40 epoxide derivatives of taccalonolides. The potency of these analogues increased several hundred to over one thousand times. Some of the compounds showed IC<sub>50</sub> values below 1 nM in vitro, which is more potent than paclitaxel. The esterification of 7- and 14-hydroxyl group was also investigated.

We also found the mechanism of action of taccalonolides is unique. Taccalonolides directly bind to a pocket adjacent to that of paclitaxel and form a covalent bond to promote the polymerization of microtubules. In addition, taccalonolides can circumvent all three mechanisms of paclitaxel drug resistance both in vitro and in vivo. The in vivo anti-tumor activity of taccalonolides A, B, E and new synthesized taccalonolides were evaluated.

## **DISSECTING THE ROLE OF ALTERNATIVE SPLICING IN PLANT ABIOTIC STRESS RESPONSES**

**Jianhua Zhu**

University of Maryland-College Park

Plants are sessile organisms and they have to cope with various ever-changing environmental abiotic stress conditions such as cold, heat, and soil salinity. In an effort to identify and characterize key genes for plant abiotic stress responses, we performed several forward genetic screens for mutants with altered responses to abiotic stress conditions. Three of the genes we identified through the forward genetic analyses encode proteins that are part of the spliceosome for alternative splicing. First, Regulator of CBF Gene Expression 1 (RCF1) is a DEAD box RNA helicase. We showed that RCF1 is essential for pre-mRNA splicing and is important for cold-responsive gene regulation and cold tolerance in plants. Second, Regulator of ABA Response 1 (ROA1) is a close ortholog of the human splicing factor RBM25. Our results indicated that RNA splicing is of particular importance for plant response to the phytohormone ABA and that the splicing factor ROA1/AtRBM25 has a critical role in this response. Third, the U1 small nuclear ribonucleoprotein complex protein AtU1A has a critical role as a regulator of pre-mRNA processing and salt tolerance in plants. In summary, we showed in our genetic analyses that alternative splicing is crucial for plant response to abiotic stress conditions.



**Poster #1****COMPARATIVE ANALYSIS OF PEACH AND APPLE  
TRANSCRIPTOMES FROM EARLY DEVELOPING FRUIT**

Muzi Li<sup>1</sup>, Kelsey D. Galimba<sup>2</sup>, Ann M. Callahan<sup>2</sup>, Chris Dardick<sup>2</sup>, Sridhar Hannenhalli<sup>1</sup>, Stephen M. Mount<sup>1</sup>, Zhongchi Liu<sup>1</sup>

1. Dept. of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742

2. USDA-ARS Appalachian Fruit Research Station, 2217 Wiltshire Road, Kearneysville, WV 25430

zliu@umd.edu

Rosaceae is a plant family with about 3,000 species including many economically important fruits (strawberry, raspberry, peach, apple, pear and others). This plant family provides an ideal system to study molecular mechanisms underlying the evolution of fruit development. Peach and apple are two species from the same subfamily of Rosaceae (Spiraeoideae) with publicly available high-quality genomes. Peach and apple share a basic flower structure of five sepals, five petals, and numerous stamens. However, their fruit flesh is developed from distinct part of the flower post-fertilization. In peach, the mesocarp of the ovary wall develops into the fleshy fruit and the endocarp lignifies to form a hard stone surrounding the seeds, while in apple, the hypanthium enlarges and becomes the fleshy fruit. To understand the fleshy fruit diversity, we dissected three specific floral compartments at four critical early stages of peach and apple fruit development in triplicate. Subsequently, a total of 72 RNA-seq libraries were made and sequenced. We examined differentially expressed genes and developed consensus networks to identify the similarities and differences between gene expression during peach and apple early fruit development. Further, the expression of gene families whose members are known to play important roles in fruit development was examined, including auxin and Gibberellin pathway genes and MADS box transcription factors. Our analyses suggest that differential cell division activities, tissue-specific expression of Gibberellin degradation enzymes and MADS box genes likely contributed to the evolution of hypanthium fleshy fruit in apple. We also developed peach and apple eFP browsers that allow the users to visualize the expression of the genes in distinct tissues at different stages.

**Poster #2****EXPLORING NATURALLY EXISTING GENETIC VARIATION IN GRAIN CHALK FORMATION IN RESPONSE TO CHANGING CLIMATE**

Jinyoung Y. Barnaby<sup>1,2,\*</sup>, Woojae Kim<sup>1,2</sup>, Lewis H. Ziska<sup>2</sup>, David H. Fleisher<sup>2</sup>, Vangimalla R. Reddy<sup>2</sup>, and Anna M. McClung<sup>1</sup>

1. U.S. Department of Agriculture, Agricultural Research Service, Dale Bumpers National Rice Research Center, Stuttgart, AR 72160, USA;
  2. U.S. Department of Agriculture, Agricultural Research Service, Beltsville Agriculture Research Center, Adaptive Cropping Systems Laboratory, Beltsville, MD 20705, USA;
- \*Corresponding author: [Jinyoung.barnaby@usda.gov](mailto:Jinyoung.barnaby@usda.gov)

The presence of grain chalk, opaque white areas in the rice grain, can reduce milling and cooking quality as well as grain appearance, thus reducing the value of the crop. Over the last several years, the USA rice industry has been concerned about the increasing prevalence of undesirable chalky rice which is resulting in a loss of some international markets. Heat stress is also known to significantly increase grain chalkiness which reduces milling quality, cooking properties, and grain appearance. To identify genotype (G) x environment (E) impact on chalk formation and to inform future breeding efforts toward low chalk varieties, two parents, Kaybonnet and ZHE733, displaying contrasting % chalk formation and 7 recombinant inbred lines (RIL) possessing 3 major chalk quantitative trait loci (QTL) were grown under ambient CO<sub>2</sub> and an elevated atmospheric level projected for 2050, and using two temperature regimes, one with warmer temperature during grain fill stage. Two experiments, one in growth chambers, and the other in greenhouses, were conducted. Our results showed natural genetic variation in heat-induced chalk formation in response to future anticipated climate scenarios, high temperature with elevated atmospheric CO<sub>2</sub>. These results will assist breeders use marker assisted selection for development of new climate resilient varieties that will minimize chalkiness while maintaining economic value.



**Poster #3****IDENTIFYING GENOMIC REGIONS INFLUENCING VARIATIONS IN INORGANIC ARSENIC ACCUMULATION IN RICE CULTIVARS**

Cristina P. Fernandez-Baca<sup>1,2</sup>, Eton E. Codling<sup>2</sup>, Vangimalla R. Reddy<sup>2</sup>, Shannon R.M. Pinson<sup>2</sup>, Anna M. McClung<sup>1</sup>, and Jinyoung Y. Barnaby<sup>1,2,\*</sup>

1. U.S. Department of Agriculture, Agricultural Research Service, Dale Bumpers National Rice Research Center, Stuttgart, AR 72160, USA;
2. U.S. Department of Agriculture, Agricultural Research Service, Beltsville Agriculture Research Center, Adaptive Cropping Systems Laboratory, Beltsville, MD 20705, USA;

\*Corresponding author: [Jinyoung.barnaby@usda.gov](mailto:Jinyoung.barnaby@usda.gov)

Consumption of rice containing levels of arsenic (As) is linked to adverse health impacts including cancer. Limits have been placed on inorganic As (iAs) content in milled rice. For example, FAO/CODEX limit for iAs is 200 ppb, and FDA limit is 100 ppb in rice-based infant food. Rice accumulates As as a result of being typically grown under flooded field conditions. Management (M) practices such as alternate wetting and drying (AWD) for growing rice are popular as a means to save water and reduce rice grain As concentrations. A chromosome segment substitution line (CSSL) mapping population of TeQing-into-Lemont introgression lines (TILs) displaying contrasting grain inorganic arsenic accumulation was used to identify genomic regions controlling iAs accumulation in rice grain, which can inform future breeding efforts toward low iAs accumulating varieties. 123 TILs were grown under continuously flooded and alternately wet and dried fields in Stuttgart, AR over two years. Grain iAs contents were measured from harvested grain samples of 2 years\*2 irrigation treatments. Measured grain iAs concentrations were used to identify 7 QTLs impacting grain iAs accumulation using sequence-based SNPs. Identification of inorganic As QTLs can guide future breeding efforts to develop low iAs accumulating rice cultivar alternatives for farmers. Ultimately, this research can result in a reduction of iAs exposure from rice consumption.

**Poster #4****PEROXISOMAL UBIQUITINATION MACHINERY IMPACTS PEROXISOME FUNCTION**

Yun-Ting Kao<sup>‡</sup>, Wendell A. Fleming, Meredith Ventura, and Bonnie Bartel<sup>§</sup>

Department of BioSciences, Rice University, Houston TX, USA

<sup>‡</sup>Current address: Department of Cell Biology and Molecular Genetics, University of Maryland, College Park MD, USA

<sup>§</sup>bartel@rice.edu

The sorting of eukaryotic proteins to various organellar destinations requires receptors that recognize cargo protein targeting signals and facilitate transport into the organelle. One such receptor is the peroxin PEX5, which recruits cytosolic cargo for delivery into the peroxisomal matrix and is ubiquitinated to allow its removal from the peroxisome membrane after cargo delivery. Peroxisomes are single membrane-bound organelles housing various metabolic reactions; peroxisomal fatty acid  $\beta$ -oxidation provides germination energy in oilseed plants, and peroxisomes are essential for life in both plants and humans. We found that the decreased growth of *Arabidopsis* mutants with impaired PEX4, a peroxisome-tethered ubiquitin-conjugating enzyme, is rescued by growth at elevated temperature. *pex4* accumulates membrane-bound PEX5, and rescue is associated with increased proteasomal degradation of PEX5, suggesting that the detrimental impact of excess PEX5 in the peroxisomal membrane is relieved by decreased overall PEX5 levels at elevated temperature. Along with PEX4, three peroxisomal ubiquitin-protein ligases (PEX2, PEX10, and PEX12) are implicated in PEX5 ubiquitination. *pex2-1* and *pex12-1* are missense alleles that substitute a lysine residue for an arginine or glutamate residue. We found that *pex4* mutants restore peroxisome functions to *pex2-1* and *pex12-1* mutants, suggesting that these new lysine residues are ubiquitinated and target the mutant ligases for degradation. We conclude that controlling ubiquitination of PEX5 and of the peroxisomal ubiquitination machinery itself is critical for peroxisome function.

**Poster #5****ELUCIDATING THE ROLE OF THE FLOWERING ACTIVATOR FLK IN ROS and DEFENSE REGULATION IN ARABIDOPSIS**

Matthew Fabian<sup>1</sup>, Xiaoning Zhang<sup>2</sup>, and Hua Lu<sup>1</sup>

1. Department of Biological Sciences, University of Maryland Baltimore County, United States
2. Department of Biology, St. Bonaventure University, St. Bonaventure, NY 14778, USA  
matthew.fabian@umbc.edu

Flowering and defense control are metabolically costly processes that compete for the same resources during plant growth and development. Through a large-scale screen for defense mutants in *Arabidopsis*, we identified a new allele (*flk-5*) of *FLK*, a canonical flowering activator encoding a KH domain protein that localizes to the nucleus. The KH domain is highly conserved in both plants and animals, and KH proteins are known to form protein complexes in RNA processing. In addition to the expected late flowering phenotype, *flk-5* and another allele (*flk-1*) convey enhanced bacterial susceptibility and diminished accumulation of salicylic acid in response to infection with a virulent *Pseudomonas syringae* strain, but enhanced resistance to the necrotrophic fungal pathogen *Botrytis cinerea*. The *flk* mutants also display reduced defense responses to *flg22*, a defense elicitor derived from a conserved region of *P. syringae* flagellin proteins, including reactive oxygen species (ROS) production, callose deposition at the cell wall, and seedling root growth inhibition. Interestingly, the *flk* mutants are more resistant to methyl viologen, a chemical inducer of superoxide production, and show altered ROS gene expression and ROS scavenging enzyme activities. We will investigate whether this role of *FLK* in ROS regulation underpins the crosstalk between pathogen defense and development.

**Poster #6****IMPROVING PLANT GENOME EDITING WITH ENGINEERED CAS9 VARIANTS**

Adam Levav - presenter

Zhaohui Zhong<sup>1</sup>, Simon Sretenovic<sup>2</sup>, Qiurong Ren<sup>1</sup>, Lijia Yang<sup>1</sup>, Yu Bao<sup>3,4</sup>, Caiyan Qi<sup>1</sup>, Mingzhu Yuan<sup>1</sup>, Yao He<sup>1</sup>, Shishi Liu<sup>1</sup>, Xiaopei Liu<sup>1</sup>, Jiaheng Wang<sup>1</sup>, Lan Huang<sup>1</sup>, Yan Wang<sup>1</sup>, Dibin Baby<sup>2,5</sup>, David Wang<sup>2,6</sup>, Tao Zhang<sup>3,4</sup>, Yiping Qi<sup>2,7</sup> and Yong Zhang<sup>1</sup>

1 Department of Biotechnology, School of Life Sciences and Technology, Center for Informational Biology, University of Electronic Science and Technology of China, Chengdu, China

2 Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD, USA

3 Jiangsu Key Laboratory of Crop Genetics and Physiology, Co-Innovation Center for Modern Production Technology of Grain Crops, Key Laboratory of Plant Functional Genomics of the Ministry of Education, Yangzhou University, Yangzhou, China

4 Joint International Research Laboratory of Agriculture and Agri-Product Safety of Ministry of Education of China, Yangzhou University, Yangzhou, China

5 Indian Institute of Science Education and Research, Tirupati, Andhra Pradesh, India

6 Seven Lakes High School, Katy, TX, USA

7 Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, MD, USA

Email of corresponding author: yiping@umd.edu

Genome editing is the introduction of desired modifications in genomic DNA sequences and is more attainable due to a powerful class of tools based on chimeric nucleases or Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). CRISPR has become the obvious technology of choice owing to its simplicity of targeting DNA. However, not every DNA sequence is targetable because of the strict NGG Protospacer Adjacent Motif (PAM) requirement to begin the process of genome editing of CRISPR associated nuclease isolated from *Streptococcus pyogenes* (SpCas9). Two recently engineered SpCas9 variants, namely xCas9 and Cas9-NG, have shown improved targeting specificity and broadened PAM recognition range in mammalian cells. In this study, we evaluated these Cas9 variants in the crop plant, rice. We first tested xCas9-3.7, the most effective xCas9 variant in mammalian cells, for targeted mutagenesis at 16 possible NGN PAMs. xCas9 exhibited nearly equivalent editing efficiency to wild-type SpCas9 (Cas9-WT) at most canonical NGG PAM sites tested. With mismatched sgRNAs, we found that xCas9 had improved targeting specificity over Cas9-WT. Furthermore, we tested two Cas9-NG variants, Cas9-NGv1 and Cas9-NG, for targeting NGN PAMs. Both Cas9-NG variants showed higher editing efficiency at most non-canonical NG PAM sites tested. In stable transgenic rice lines, we demonstrated that Cas9-NG had much higher editing efficiency than Cas9-NGv1 and xCas9 at NG PAM sites. To expand the base-editing scope, we developed an efficient C to T base-editing system based on Cas9-NG nickase, PmCDA1, and UGI. Taken together, our work benchmarked xCas9 as a high-fidelity nuclease for targeting canonical NGG PAMs and Cas9-NG as a preferred variant for targeting relaxed PAMs for plant genome editing.

**Poster #7****ABCB AUXIN TRANSPORTER CONTRIBUTIONS TO VEGETATIVE LEAF DEVELOPMENT AND LIGHT-MEDIATED RESPONSES**

Gabrielle A. Bate\*, Mark K. Jenness and Angus S. Murphy  
University of Maryland, Department of Plant Science and Landscape Architecture, 4291  
Fieldhouse Drive 2102 Plant Sciences Building College Park, MD, 20742  
bategabrielle@gmail.com

The phytohormone auxin is involved in several aspects of plant growth and development including organogenesis, cell expansion/elongation, and tropic growth. Auxin is primarily synthesized at the shoot, root, and leaf tips. Polar cell-to-cell movement of auxin from sites of synthesis to the rest of the plant utilizes a subset of ATP-binding cassette subfamily B (ABCB) transporters. In *Arabidopsis*, single *abcb19* mutants exhibit upright but downward curled leaves. Additionally *ABCB1*, *ABCB6*, *ABCB20* and *ABCB21* have been shown to contribute to auxin transport in leaves. Presented here are phenotypic analyses of single and multiple mutants that suggest that *ABCB1*, *ABCB19*, *ABCB6*, and *ABCB20* are the primary ABCB transporters involved in vegetative leaf growth. Previous studies have shown that the blue-light photoreceptor phototropin1 (*phot1*) directly phosphorylates to inactivate *ABCB19* during phototropic bending. *ABCB6*, *ABCB20*, and *ABCB14* have been identified in guard cell proteomic and stomatal conductance assays suggesting that other ABCBs may be regulated by blue-light and *phot1*. Analysis of these single and double ABCB mutants during blue-light regulation of hypocotyl elongation, hook opening, and stomatal conductance will be presented. A better understanding of the role ABCB transporters play in leaf development and light-mediated processes will contribute to crop productivity efforts by optimizing photosynthetic and water-use efficiency.

**Poster #8****EVIDENCE FOR ENDORIBONUCLEOLYTIC CLEAVAGE OF NONSENSE-MEDIATED DECAY (NMD) TARGETS IN PLANTS**

Anna DiBattista\*, Vinay K. Nagarajan, Monica Accerbi and Pamela J. Green

\* Summer Undergraduate Researcher, Department of Biological Sciences,  
Delaware Biotechnology Institute, Newark, DE 19711

The degradation of mRNA plays an important role in the post-transcriptional control of gene expression in eukaryotic cells. Endoribonucleases are key to mRNA degradation and cleave mRNA molecules internally to create fragments that can be further degraded by exoribonucleases. A critical cellular process in which endoribonucleases play a major role is nonsense-mediated decay (NMD). During NMD, incorrectly processed mRNA molecules such as those with a premature stop codon, are degraded before they can be translated into truncated proteins, which are toxic to cells. The endoribonuclease that initiates NMD in metazoans, SMG6, has been identified and characterized, however it is absent in plants. Our global analysis of *Arabidopsis* XRN4 (major cytoplasmic 5' to 3' exoribonuclease) substrates showed an over-representation of 3' RNA decay fragments among NMD-sensitive transcripts. This strongly indicated that cleavage of NMD substrates reminiscent of SMG6 activity occurs in plants. Our goal is to functionally characterize two endoribonucleases, PS1 (Parallel Spindle) and MFL1 (MARF1 Like) in *Arabidopsis thaliana*, which have catalytic domain sequences similar to that of SMG6. To do so, we isolated mutant plants deficient in PS1 or MFL1 and later introduced an XRN4 mutation to stabilize 3' mRNA fragments. Identification of endoribonucleases and their substrates, the contributions of endoribonucleases to RNA turnover, and impacts on plant growth and development will be discussed.

**Poster #9****AtABCB19 REGULATES LEAF MORPHOLOGY AND POSITION DURING PHOT1-MEDIATED BLUE LIGHT RESPONSES**

Mark K Jenness, Reuben Tayengwa, and Angus S. Murphy  
Department of Plant Science and Landscape Architecture, University of Maryland, 4291  
Fieldhouse Drive, 2104 Plant Science Building, College Park, MD 20740  
mjenness@umd.edu

Blue light regulates multiple processes that optimize light capture and gas exchange in plants. These include chloroplast movement, changes in stomatal conductance, and altered organ positioning. In *Arabidopsis* these processes are primarily modulated by the blue light phototropin photoreceptors (phot1 and phot2). Changes in leaf positioning and shape involve several downstream signaling components that include NON-PHOTOTROPIC HYPOCOTYL 3 (NPH3), PHYTOCHROME KINASE SUBSTRATE (PKS), ROOT PHOTOTROPISM 2 (RPT2), and alterations in localized auxin streams. Direct phosphorylation of the auxin transporter ATP-BINDING CASSETTE subfamily B 19 (ABCB19) by phot1 in phototropic seedlings suggests that phot1 may directly regulate ABCB19 to adjust auxin-dependent leaf responses. Here, *abcb19* mutants were analyzed for fluence and blue light-dependent changes in leaf morphology and positioning. *abcb19* mutants develop irregularly wavy rosette leaves that are insensitive to blue light-mediated leaf flattening. Similarly, *abcb19* is also unresponsive to changes in leaf petiole angle in response to blue light. Visualization of auxin distribution, measurement of auxin transport in protoplasts, and direct quantification of free auxin levels suggest these irregularities are due to mis-regulation of ABCB19 mediated auxin distribution in addition to light-dependent auxin biosynthesis.

**Poster # 10****POLYAMINE ACCUMULATION DURING ABIOTIC STRESSES IN TOMATO (SOLANUM LYCOPERSICUM L.) IS DEPENDENT UPON TRANSCRIPTIONAL MANEUVERING OF ITS BIOSYNTHESIS VERSUS CATABOLISM GENES**

Rakesh K. Upadhyay<sup>1,2</sup>, Tahira Fatima<sup>2</sup>, Avtar K. Handa<sup>2</sup>, Autar K. Mattoo<sup>1\*</sup>

1. Sustainable Agricultural Systems Laboratory, USDA-ARS, Henry A. Wallace Beltsville Agricultural Research Center, Beltsville, MD 20705-2350, USA
2. Department of Horticulture and Landscape Architecture, Purdue University, W. Lafayette, IN, USA

Address for correspondence: \*autar.mattoo@usda.gov; rakesh.upadhyay@usda.gov

Polyamines (PAs) are ubiquitous biogenic amines that regulate plant life cycle. The main PAs studied include the diamine putrescine (PUT), triamine spermidine (SPD) and tetraamine spermine (SPM)/thermo- (T- SPM). Their involvement in plant responses to different abiotic stresses was highlighted when SPM and T-SPM were shown to protect Arabidopsis from heat stress. Our laboratory is involved in studying regulation of plant processes by PAs in tomato. Although PA biosynthesis has received much attention in literature, very little is known about PA homeostasis due to catabolism involving back pathway from SPM to SPD to PUT. If and how PA catabolism is involved in responses to abiotic stresses has received lesser attention. Here we present a comparative analysis of PA biosynthesis and catabolism genes and polyamine estimation in tomato in response to five abiotic stresses (drought, salt, cold, heat, wounding). Genome-wide transcript accumulation patterns and quantitative PCR of 18 genes encoding for PA metabolic pathway enzymes in tomato were analyzed in the absence and presence of a given abiotic stress, including arginase (SIARG1, 2); arginine decarboxylase (SIADC1,2); agmatine iminohydrolase/deiminase (SIAIH); N-carbamoyl putrescine amidase (SICPA), ornithine decarboxylase (SIODC1, 2), S-adenosylmethionine decarboxylase (SISAMDC1,2,3); spermidine synthase (SISPDS1, 2); spermine synthase (SPMS); flavin-dependent polyamine oxidase (SIPAO4-like, SIPAO2) and copper-dependent amine oxidase (SICuAO, SICuAO-like). A principal component analysis (PCA) was carried out to find out correlations between expression of specific gene members versus polyamine levels. This study will highlight PA homeostasis during each given stress involving distinct combination of genes as related to PA biosynthesis or catabolic genes.



**Poster #11****Evasion of plant innate defense response by *Salmonella* on Lettuce.**

Nick Johnson<sup>1,2\*</sup>, Harsh Bais<sup>1,2</sup>

<sup>1</sup>Department of Plant and Soil Sciences, <sup>2</sup>Delaware Biotechnology Institute, University of Delaware, Newark, Delaware.

\*Corresponding author: Email: Njohnso@udel.edu

Interactions between bacteria and higher organisms cause numerous physiological responses, such as disease and autonomous stress responses. To establish disease the pathogen must first circumvent the hosts immune system for effective internalization. Plants encounter a plethora of foreign organisms throughout their life cycle, these will no doubt include some enteric foodborne pathogens, notably *Escherichia coli* and *Salmonella enterica*. It is possible for these interactions to result in outbreaks, recalls, and litigation. Recurring outbreaks suggest that some bacteria can circumvent the plant immune system and ingress through regions such as the stomata, roots, and hydathodes. The purpose of this study was to determine if application of *Salmonella enterica* Typhimurium can impede *Lactuca sativa*'s stomata closure and more broadly aspects of the innate immune system. We found that *S. enterica* Typhimurium caused a pronounced reduction in stomatal closure. SPI1 and SPI2 mutants of *S. enterica* Typhimurium had a higher rate of closure than that of wild type *S. enterica* Typhimurium, suggesting that a T3SS was required for effective immune system subversion. Microscopy and genetic assays also show that the ABA pathway were perturbed by *Salmonella* causing reduced stomatal closure. These findings impact issues of contamination related to plant performance and innate defense responses for plants grown hydroponically and in soil.

**Poster #12****INVESTIGATING THE INTERACTIONS OF AUXIN WITH PLANT MEMBRANES**

Anna Reachmack\*, Mark K. Jenness and Angus S. Murphy  
University of Maryland, Department of Plant Science and Landscape Architecture, 4291  
Fieldhouse Drive 2102 Plant Sciences Building College Park, MD, 20742  
annarecap2016@gmail.com

The phytohormone auxin (indole-3-acetic acid, IAA) plays key roles in plant development, including organ development and cell elongation/expansion. Auxin is synthesized in the shoot apex, the tips of young leaves, and root tips and is transported in a cell-to-cell manner to sites of response. Textbook examples of auxin transport depict auxin freely floating in the cytoplasm but experimental evidence suggests that auxin may actually be imbedded in the membrane and favors lipophilic conditions. Since these previous studies were primarily conducted in non-plant membrane systems, we designed systems to analyze and quantify the partitioning of auxin using *Arabidopsis* microsomal membranes. When microsomes were incubated in solution containing auxin at room temperature and pH 5.0, a 19% loss of auxin in solution was observed after pelleting the membranes. Assays conducted on ice show a 10% loss of auxin levels at pH 5. No significant change was observed at pH 7.0 under either condition. This suggests that ~10% of the auxin enters membranes through diffusion and ~9% enters via AUX1/LAX uptake proteins. We hypothesized that the membrane environment would have an impact on auxin partitioning. However, no partitioning was detected in mutants deficient in sterol and sphingolipid biosynthesis. Additionally, assays conducted with auxin transport inhibitors and direct quantification of auxin in membrane and cytosolic fractions will be presented. These results will inform our fundamental understanding of the mechanisms of auxin transport and the role auxin plays in plant growth and development.

**Poster #13****ARABIDOPSIS SCAFFOLD PROTEIN RACK1A REGULATES AUXIN MEDIATED LATERAL ROOT DEVELOPMENT PATHWAY.**

Shifaa Alshammari<sup>1</sup>, Sivanesan Dakshanamurthy<sup>3</sup>, Hemayet Ullah<sup>2</sup>

1, 2. Biology department, Howard University, Washington, DC, USA;

3 Georgetown University Medical Center, Washington, DC, USA.

RACK1 (Receptor for Activated C Kinase 1) is a WD-40 type scaffold protein family, conserved in single cell eukaryote yeast to human and plays regulatory roles in diverse signal transduction and stress response pathways. Loss of function mutant in the predominant isoform-RACK1A in Arabidopsis, indicates that it regulates diverse environmental stress resistance through negative regulation of stress hormone ABA and positively regulates auxin mediated diverse developmental pathways. It is hypothesized that chemical knock-out, as opposed to genetic knock-out, of RACK1A will provide a functional advantage in protecting plants from diverse stress and a small compound stabilizing RACK1 will be useful to promote auxin regulated developmental pathways. Dozens of small compounds based on our lab derived crystal structure of Arabidopsis RACK1A are isolated and functionally tested as their ability modulate the auxin signaling pathways. These functional modulators of RACK1A appear to regulate the auxin induced lateral root development process. In this pathway, the small compound inhibiting stable RACK1A expression appears to produce hyposensitivity to auxin. On the other hand, a RACK1A stabilizing compound provided hypersensitivity to the auxin induced lateral root development. The compound augmenting auxin mediated lateral root development has been found to promote diverse auxin responsive gene expression as well. Taken together, these results suggested that RACK1A may act as a modulator in the auxin signal pathways. This work may lead to understand the molecular interaction between RACK1A and auxin and the possible application of novel RACK1A modulating small compounds as fertilizers to promote auxin mediated developmental pathways safely in non- genetically modified crops.



| Name                    | Affiliation & address  | Phone & Email                               |
|-------------------------|--|---|
| Abdelkreem, Reham       | Plant Biotechnology LLC, 61 Matthews Place, Harpers Ferry, WV 25425                            | 240 704-2259, Reham.Youssef@usda.gov        |
| Abrahamian, Peter       | USDA ARS, 10300 Baltimore Ave. , Beltsville, MD 20705  | 3015046209, peter.abrahamian@ars.usda.gov   |
| Alkharouf, Nadim        | Towson University, 8000 York Road, Dept of Computer and Information Sciences, Towson, MD 21252 | 410-704-3149, nalkharouf@towson.edu         |
| Allen, Carol            | University of Maryland, 2104 Plant Science Bldg., College Park, MD 20742                       | 2403345043, cxallen12@und.edu               |
| Alshammari, Shifaa      | Howard University, 1505 28th St South apt #2, Arlington, VA 22206                              | 3302898970, shifaa8988@hotmail.com          |
| Anderson, Tom           | AAAS, 401 Holland Lane apt 616, Alexandria, VA 22314   | 2157761222, tom@entoniche.com               |
| Bare, Gabrielle         | University of Maryland, 2104 Plant Science Bldg., College Park, MD 20740                       | 3014056937, gbare@umd.edu                   |
| Barnaby, Jinyoung       | USDA, ARS, Dale Bumpers National Rice Res Unit, 2890 Highwa 130E, Stuttgart, AR 72160          | 3015048436, jinyoung.barnaby@USDA.GOV       |
| Bates, Philip           | Washington State University, 100 Dairy Road, PO Box 646340, Pullman, WA 99164                  | 5093350553, phil_bates@WSU.edu              |
| Beetham, Patricia       | USDA Aphis BRS, 4700 River Road, Unit 147, Riverdale, MD 20737                                 | 3018513889, patricia.k.beetham@usda.gov     |
| Boulais, Virginia       | USDA Aphis BRAP, 4700 River Road, Unit 147, Riverdale, MD 20737                                | 3018513888, virginia.l.boulais@usda.gov     |
| Burgos, Angie           | USDA APHIS, 9901 Powdermill Rd, Bldg 580 Barc East, Beltsville, MD 20705                       | 301-313 9306, robert.p.jones@usda.gov       |
| Campbell, Kimberly      | USDA, ARS, BARC West, 10300 Baltimore Ave, Beltsville, MD 20705                                | 3015045316, Kimberly.Campbell@ARS.USDA.GOV  |
| Carvalho Costa, Larissa | USDA APHIS, 9901 Powdermill Rd, Bldg 580 Barc East, Beltsville, MD 20705                       | 301-313 9306, robert.p.jones@usda.gov       |
| Casteel, Clare          | University of California Davis, 1 Shields Ave., Davis, CA 95616                                | 5307526897, ccasteel@ucdavis.edu            |
| Christensen, Rial       | FDA/CVM/Division of Animal Feeds, 7519 Standish Place, Derwood, MD 20855                       | 2404026200, rial.christensen@FDA.HHS.gov    |
| Clarke, Christopher     | USDA ARS GIFVL, 10300 Baltimore Ave, Bldg 010A, Rm 226, BARC west, Beltsville, MD 20705        | 3015045953, christopher.clarke@ars.usda.gov |
| Collins, Ron            | USDA, ARS, SPCL, 10300 Baltimore Ave, Bldg 001, Rm 226, BARC-West, Beltsville, MD 20705        | 301-504-6135, Ron.Collins@usda.gov          |
| Collum, Tami            | USDA, 1301 Ditto Ave, Fort Detrick, MD 21702   | 3016193745, tami.collum@usda.gov            |

| Name                      | Affiliation & address   | Phone & Email                           |
|---------------------------|---|---|
| Dibattista, Anna          | University of Delaware, Delaware Biotechnology Institute, 15 Innovation Way , Newark, DE 19711                    | 4102066128, annadiba@udel.edu           |
| Eadie, Kahmilah           | USDA Aphis BRS, 4700 River Road, Riverdale, MD 20737  | 3018513886, Kahmilah.eadie@usda.gov     |
| Fabian, Matt              | UMBC, 1000 Hilltop Circle, Baltimore, MD 21250  | 4104552263, ae65298@umbc.edu            |
| Fahey, Andrew             | USDA Aphis BRS, 4700 River Road, Riverdale, MD 20737  | 3018513886, Andrew.fahey@usda.gov       |
| Farrell, Bob              | Pennsylvania State University, 1031 Edgecomb Ave., York. PA 17403   | 7177714052, jrf10@psu.edu               |
| Feijo, Jose               | University of Maryland, 4066 Campus Drive, College Park, MD 20742   | 3014059746, jfeijo@umd.edu              |
| Fernandez Baca, Cristina  | USDA, ARS, Dale Bumpers National Rice Res Unit, 2890 Highway 130E, Stuttgart, AR 72160                            | 3015048436, cristina.fernandez@USDA.GOV |
| Galvez-Gargurevich, Marco | USDA/ Aphis PPQ CPHST, 9901 Powdermill Rd, Bldg 580 Barc East, Beltsville, MD 20705                               | 3013139237, marco.e.galvez@usda.gov     |
| Gichuki, Samson           | Morgan State University, 1700 E. Coldspring Lane., Baltimore, MD 21251  | 443-885-1361, sagic1@morgan.edu         |
| Grant, Doug               | USDA Aphis BRS, 4700 River Road, Riverdale, MD 20737  | 3018513886, douglas.w.grant@usda.gov    |
| Hammond, John             | USDA ARS USNA FNPRU, 10300 Baltimore Ave. Bldg. 010A , Beltsville, MD 20705                                       | 3015045313, john.hammond@ars.usda.gov   |
| Haymes, Kenneth           | USDA Aphis BRS, 4700 River Road, Riverdale, MD 20737  | 3018513879, kenneth.m.haymes@usda.gov   |
| Higgins, Christopher      | Hort Americas LLC, 2801 Renee St., Bedford, TX 76021  | 4695322261, chiggins@hortamericas.com   |
| Hockett, Kevin            | Pennsylvania State University, Dept. of Plant Pathology and Environmental Microbiology, University Park, PA 16802 | 8148654472, klh450@psu.edu              |
| Hoffman, Neil             | USDA Aphis BRS, 4700 River Road, Riverdale, MD 20737  | 3018513947, neil.e.hoffman@usda.gov     |
| Howe, Natalie             | USDA Aphis BRS, 4700 River Road, Riverdale, MD 20737  | 3018513865, Natalie.m.howe@usda.gov     |
| Islam, Nazrul             | USDA-ARS Soybean Genomics and Improvement Laboratory, 10300 Baltimore, Ave. Bldg. 006, Beltsville, MD 20705       | 3015045258, nazrul.islam@ars.usda.gov   |
| Jenness, Mark             | University of Maryland, 2104 Plant Science Bldg., College Park, MD 20740  | 3014056937, mjenness@umd.edu            |
|                           |   |   |
|                           |   |   |

| Name                 | Affiliation & address  | Phone & Email                                  |
|----------------------|--|--|
| Johnson, Nicholas    | University of Delaware, Dept of Plant and Soil Science, 531 South College Av, Newark, DE 19716             | 8363813087,<br>njohnso@udel.edu                |
| Jones, Richard       | USDA ARS/ GIFVL, 10300 Baltimore Ave, Bldg 010A, Rm 311 Barc West, Beltsville, MD 20705                    | 301-504-8395,<br>richard.jones@ars.usda.gov    |
| Jones, Bob           | USDA APHIS, 9901 Powdermill Rd, Bldg 580 Barc East, Beltsville, MD 20705                                   | 301-313 9306,<br>robert.p.jones@usda.gov       |
| Jordan, Ramon        | US National Arboretum, USDA ARS, 10300 Baltimore Ave, Bldg 010A, Rm 238, Beltsville, MD 20705              | 3015045646,<br>ramon.jordan@ars.usda.gov       |
| Kao, Yun-Ting        | University of Maryland, Bldg 413, Rm 0209, College Park, MD 20742  | 8325898996,<br>chinhonor@gmail.com             |
| Kepner, Cody         | USDA APHIS, 9901 Powdermill Rd, Bldg 580 Barc East, Beltsville, MD 20705                                   | 301-313 9306,<br>robert.p.jones@usda.gov       |
| Kovalskaya, Natalia  | US National Arboretum, USDA ARS, 10300 Baltimore Ave, Bldg 004, Rm 211, Beltsville, MD 20705               | 3015045203,<br>natalia.kovalskaya@ars.usda.gov |
| Kreger, Nancy        | USDA ARS Molecular Plant Pathology Lab, 10300 Baltimore Ave. Bldg. 004 Rm 211, Beltsville, MD 20705        | 3015045203,<br>nancy.kreger@ars.usda.gov       |
| Kundagram, Neil      | University of Maryland, 5118 Plant science bldg, 4921 Fieldhouse Rd, College Park, MD 20742                | 3018253598,<br>neilkund@gmail.com              |
| Lawrence, Susan      | USDA-ARS IIBBL, 10300 Baltimore Ave., Bldg 007, Rm 301, Beltsville, MD 20705                               | 3015046144,<br>susan.lawrence@usda.gov         |
| Levav, Adam          | University of Maryland, 5118 Plant science bldg, 4921 Fieldhouse Rd, College Park, MD 20742                | 3014057682,<br>adam.z.levav@gmail.com          |
| Li, Muzi             | University of Maryland, 0225 Bioscience Res Bldg, Dept of Cell & Molecular biology, College Park, MD 20742 | 5409982223,<br>limuzi92@terpmail.und.edu       |
| Lunardon, Alice      | Pennsylvania State University, , University Park, PA 16802   | 8147777370,<br>aul24@psu.edu                   |
| Matthews, Ben        | Plant Biotechnology, 61 Matthews Place, Harpers Ferry, WV 25425  | (443) 280-2492,<br>benfmatthews@gmail.com      |
| McAllister, Jennifer | USDA APHIS, 9901 Powdermill Rd, Bldg 580 Barc East, Beltsville, MD 20705                                   | 301-313 9306,<br>robert.p.jones@usda.gov       |
| McGonigle, Brian     | FMC Stine Research Center, 1090 Elkton Rd., Newark, DE 19711   | 3023189445,<br>brian.mcgonigle@fmc.com         |
| Mendoza, Joshua      | USDA APHIS, 9901 Powdermill Rd, Bldg 580 Barc East, Beltsville, MD 20705                                   | 301-313 9306,<br>robert.p.jones@usda.gov       |
| Merker, Robert       | US FDA, 5001 Campus Drive, College Park, MD 20740  | 2404021226,<br>robert.merker@fda.hhs.gov       |

| Name                    | Affiliation & address   | Phone & Email                                     |
|-------------------------|---|---|
| Ming, Meiling           | University of Maryland, 5118 Plant science bldg, 4921 Fieldhouse Rd, College Park, MD 20742                     | 2404136171,<br>meilingm@umd.edu                   |
| Moore, Kevin            | USDA APHIS, 9901 Powdermill Rd, Bldg 580 Barc East, Beltsville, MD 20705  | 301-313 9306,<br>robert.p.jones@usda.gov          |
| Mount, Stephen          | University of Maryland, 0225 Bioscience Res Bldg, Dept of Cell & Molecular biology, College Park, MD 20742      | 3014056934,<br>smount@umd.edu                     |
| Natarajan, Savithiry    | USDA-ARS Soybean Genomics and Improvement Laboratory, 10300 Baltimore, Ave. Bldg. 006, Beltsville, MD 20705     | 301-504-5258,<br>savithiry.natarajan@ars.usda.gov |
| Padmanabhan, Chellappan | USDA/ Aphis PPQ, 9901 Powdermill Rd, Bldg 580 Barc East, Beltsville, MD 20705                                   | 3013150108,<br>chellappan.padmanabhan@usda.gov    |
| Pedley, Kerry           | Foreign Disease Weed Science Research Unit, 1301 Ditto Ave, Fort Detrick, MD 21702                              | 301-619-1668,<br>kerry.pedley@ars.usda.gov        |
| Peng, Jiangnan          | Morgan State University, 1700 E. Cold Spring Lane, Baltimore, MD 21251  | 4438853955,<br>jiangnan.peng@morgan.edu           |
| Pie, Hannah             | Howard Community College, 10901 Little Patuxent Pkwy, Columbia, MD 21044  | 4485183111,<br>hpie@howardcc.edu                  |
| Puthoff, David          | Frostburg State University, 101 Braddock Road, Frostburg, MD 21532  | 301-687-4172,<br>dpputhoff@frostburg.edu          |
| Qi, Yiping              | University of Maryland, 5118 Plant science bldg, 4921 Fieldhouse Rd, College Park, MD 20742                     | 3014057682,<br>yiping@umd.edu                     |
| Qian, Bilian            | University of Maryland, 6125 Plant science bldg, 4921 Fieldhouse Rd, College Park, MD 20742                     | 3016425935,<br>bqian@umd.edu                      |
| Reachmack, Anna         | University of Maryland, 2104 Plant Science Bldg., College Park, MD 20740  | 2404783654,<br>areachmark@terpmail.umd.edu        |
| Rivera, Yazmin          | USDA Aphis PPQ Center for Plant Health Science and Technology, Bldg 589, Powder Mill Road, Beltsville, MD 20705 | 3133019273,<br>yazmin.rivera@usda.gov             |
| Ruck, Amy               | USDA ARS FbWSRU, 1301 Ditto Ave, Frederick, MD 21702  | 301-619-0517,<br>Amy.ruck@usda.gov                |
| Saunders, James         | Towson University, 14590 Triadelphia Mill Road, Dayton, MD 21036  | 443-386-4695,<br>jsaunders@towson.edu             |
| Schulden, Taylor        | USDA APHIS, 9901 Powdermill Rd, Bldg 580 Barc East, Beltsville, MD 20705  | 301-313 9306,<br>robert.p.jones@usda.gov          |
| Sechler, Aaron          | USDA, 1301 Ditto Ave, Fort Detrick, MD 21702  | 3016192232,<br>aaron.sechler@usda.gov             |
| Shen, Zhengxing         | USDA/ APHIS, 4700 River Road, Unit 147, Riverdale, MD 20737   | 3018513922,<br>zhengxing.shen@usda.gov            |
|                         |   |   |



| Name              | Affiliation & address   | Phone & Email                              |
|-------------------|---|--|
| Slocum, Richard   | USDA APHIS, 9901 Powdermill Rd, Bldg 580 Barc East, Beltsville, MD 20705                    | 301-313 9306, robert.p.jones@usda.gov      |
| Sretenovic, Simon | University of Maryland, 5118 Plant science bldg, 4921 Fieldhouse Rd, College Park, MD 20742 | 2022366797, simonsre@umd.edu               |
| Stone, Christine  | Foreign Disease Weed Science Research Unit, 1301 Ditto Ave, Fort Detrick, MD 21771          | 301-619-2862, Christine.stone@ars.usda.gov |
| Strachan, Janice  | USDA Aphis BRAP, 4700 River Road, Unit 147, Riverdale, MD 20737                             | 3018513878, Janice.strachan@usda.gov       |
| Sweeney, Emma     | USDA APHIS, 9901 Powdermill Rd, Bldg 580 Barc East, Beltsville, MD 20705                    | 301-313 9306, robert.p.jones@usda.gov      |
| Turner, Roy       | USDA APHIS, 9901 Powdermill Rd, Bldg 580 Barc East, Beltsville, MD 20705                    | 301-313 9308, roy.s.turner@usda.gov        |
| Upadhyay, Rakesh  | SASL, USDA ARS, 10300 Baltimore Ave, Bldg 001, Rm 120, Beltsville, MD 20705                 | 3016426932, rakesh.upadhyay@ars.usda.gov   |
| Van Eck, Joyce    | Boyce Thompson Institute, 533 Tower Rd., Ithaca, New York 14850                             | 6072541686, jv27@cornell.edu               |
| Wang, Fuxi        | University of Maryland, 3413 Tulane Dr, Apt 34, Hyattsville, MD 20783                       | 2407010669, fxwang@umd.edu                 |
| Wozniak, Chris    | US EPA BPPD, 1200 Pennsylvania Ave, NW, MC: 7511P, Washington, DC 20460                     | 703-308-4043, wozniak.chris@epa.gov        |
| Wright, Clay      | Virginia Tech, 1230 Washington St. SW, Blacksburg, VA 24060                                 | 7045177517, wrightrc@vt.edu                |
| Wunsch, Anna      | USDA APHIS, 9901 Powdermill Rd, Bldg 580 Barc East, Beltsville, MD 20705                    | 301-313 9306, anna.o.wunsch@aphis.usda.gov |
| Yin, Desuo        | University of Maryland, 5118 Plant science bldg, 4921 Fieldhouse Rd, College Park, MD 20742 | 2408257213, yindesuo@163.com               |
| Zale, Brooke      | USDA/ Aphis PPQ CPHST, 9901 Powdermill Rd, Bldg 580 Barc East, Beltsville, MD 20705         | 3013139274, brooke.zale@usda.gov           |
| Zhang, Yingxiao   | University of Maryland, 5118 Plant science bldg, 4921 Fieldhouse Rd, College Park, MD 20742 | zhangyx@umd.edu                            |
| Zhang, Deshui     | USDA Aphis BRS, 4700 River Road, Unit 147, Riverdale, MD 20737                              | 3018513913, deshui.zhang@usda.gov          |
| Zhang, Chong      | USDA/ ARS, 10300 Baltimore Ave, Genetic Imp. Fruits & Veg Lab, Beltsville, MD 20705         | 3015040140, chong.zhang@ars.usda.gov       |
| Zhou, Jing        | USDA/ Aphis PPQ CPHST, 9901 Powdermill Rd, Bldg 580 Barc East, Beltsville, MD 20705         | 3013139244, jing.zhou@usda.gov             |
| Zhu, Jianhua      | University of Maryland, 2121 Plant Sciences Bldg, College Park, MD 20742                    | 3014050920, jhahu@umd.edu                  |

