37th Annual Mid-Atlantic Plant Molecular Biology Society

August 19, 2020

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Organization

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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Jim Saunders
John Hammond
Ken Haymes
Nadim Alkharouf
Savi Natarajan
Chris Clarke
Fred Gouker

Publicity:
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Jim Saunders

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Wednesday, August 19, 2020

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Markita Landry University of California, Berkeley
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4:20 Thanks for "attending" – John Hammond

BRASSINOSTEROIDS: SIXTH GROUP OF PLAT HORMONES

N. Bhushan Mandava
Repar Corporation, 8070 Georgia Avenue, Silver Spring, Maryland 20910, USA

Plant hormones play a crucial role in plant growth and development. There were originally 5 major plant hormones, auxins, gibberellins, cytokinins, ethylene and abscisic acid. All these hormones have independent roles within the plant, but also work in tandem in order to regulate plant growth and development. As such, commercial versions of these plant growth hormones [also referred to as plant growth regulators (PGRs)] were developed for their application to agricultural crops.

The first discovery of new plant hormones, named Brassins, was reported by Mitchell and Mandava in 1970 at USDA Laboratory in Beltsville, Maryland. Over a 9-year period at USDA Laboratories in Beltsville, Philadelphia, Peoria, Dr. N. Bhushan Mandava and his coworkers were involved in identifying the active substance from Brassins. He, along with a team of other chemists, biochemists and chemical engineers developed a chemical process that isolated 10 mg of a pure crystalline product from over 500 lbs of rape (Brassica napus) pollen. The discovery of this substance, later named Brassinolide (present at 200 ppb level), is considered one of the greatest scientific achievements in plant sciences. It is recognized as the SIXTH major plant hormone group. and has major cellular implications in the realm of plant biology. In 1979, Dr. Grove and Dr. Mandava characterized it as brassinolide, which is the first plant hormone in a group of over 70 brassinosteroids found in nature. Since brassinolide is difficult to synthesize, the closely related analogs epi-brassinolide (EBR) and 28-homobrassinolide (HBR) were synthesized and developed for commercial uses. Besides Brassinolide (BL), HBR and EBR are the most active brassinosteroids. In the U.S., Switzerland and India, HBR is registered for commercial uses. Like BL, HBR enhances cell division and elongation, interacts synergistically with other other Plant Hormones, and protects plants from a variety of stress factors such as water, salt, heat, etc. It also elicits profound physiological responses at sub-micromolar concentrations. This is highly significant because farmers will need to use less material overall to reap the application benefits, which leads to more sustainable usage. The yield increases are verified by multiple field studies conducted in the United States and South America (Chile) as well as in Switzerland and India.

Application of HBR shows the disease and climate resistance properties, which can further help growers achieve higher yields. HBR also perpetuates substantial yield increase in nut crops, with similar results in trialing with almonds, and walnuts. Dr. Mandava group applied for the registration of Homobrassinolide (HBR) with the U.S. Environmental Protection Agency (EPA) in order to commercialize HBR. The EPA granted the registration for HBR technical as a biopesticide in 2010 after reviewing the pertinent documentation regarding product chemistry, toxicology and other safety data. The EPA also granted a tolerance exemption for HBR, which means that the residues in HBR in treated food and fiber commodities are not of any health and safety concerns, and that HBR can therefore be used on all crops. To further prepare the product for a global market, the usage of HBR on fruit and nut crops (Grapes, Almonds and Walnuts) was also patented, with additional patents being developed for other crops. As a next generation PGR, HBR and other brassinosteroids represent the latest agrochemical technology, and will be increasingly implemented across the world. Currently, there are ongoing initiatives to bring HBR into the EU (starting with Switzerland), Thailand, Kenya, and more.

DATA FROM OMICS STUDIES INDICATE PHYTOPLASMA IS CAPABLE OF MANIPULATING HOST METABOLISM IN FAVOR OF ITS OWN SURVIVAL

Wei Wei1, Yan Zhao1, Yue Tan2, and Qingzhong Liu2
1Molecular Plant Pathology Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD, USA
2Shandong Institute of Pomology, Taian, China

Phytoplasmas are small, wall-less plant pathogenic bacteria that cause various diseases worldwide and adversely impact agricultural economy. During the course of infection, the metabolic networks between phytoplasmas and their plant hosts are interlinked. Phytoplasmas and hosts compete for similar or identical nutrient substrates within the microenvironment, and as a result, a slight alteration in metabolism could significantly affect the outcome of phytoplasma-host interactions. Genomics studies have disclosed that phytoplasmas are highly host dependent, because phytoplasmas have undergone reductive evolution, and lost many genes that are involved in metabolic pathways essential for free-living organisms. Therefore, phytoplasmas must modulate the metabolism of host plant cells for the supply of nutrients, energy, and metabolites to establish successful replication and infection in plants. By employing metabolomics approach and integrating existing omics data, the present study revealed that phytoplasma infection promoted glycolysis and increased pentose phosphate pathway (PPP) activities in plants. Enhanced glycolysis and PPP activities not only provided energy and nutrient substances, but also facilitated biosynthesis of necessary low molecular metabolites, including amino acids, nucleotides and fatty acids/lipids, which are conducive to phytoplasma replication and infection. Conceivably, the host plant relies on the similar nutrient substrates and low molecular metabolites within the same microenvironment to support host defense response to phytoplasma infection. A prime example is enhanced flavonoid biosynthesis upon phytoplasma infection. Phytoplasma infection also led to the accumulation of a compound that attracts phloem sap-sucking insects, which is beneficial to the survival and transmission of phytoplasma. These findings indicate that phytoplasma can induce metabolic reprogramming in host plants to favor its own growth and infection.

EFFECTIVE USE OF HIGH THROUGHPUT PHENOTYPING FOR PLANT BREEDING

James Y. Kim
Plant Physiology and Genetics Research Unit;USDA-ARS, US Arid-Land Agricultural Research Center

Plant breeding and biotechnology are a game changer of ag industry to meet food demand in next decades. Phenotyping is critical to capture genotypic traits of plants through characterizing the plant responses to abiotic stresses such as heat and drought in Arizona. High throughput phenotyping (HTP) plays a key role in field mapping and data processing for timely advancement decisions. The seminar presents why HTP is important and how the HTP system is designed and implemented, as well as analytics software to visualize and analyze the phenotypic data. Discussion includes scale-up and technology transfer for small\large-scale plant sensing.

ENGINEERED INSECTS & CO.: APPLICATIONS FOR PLANT PROTECTION

Justin Overcash
USDA-APHIS

There is great interest in developing novel techniques and strategies for pest management. Biotech innovations such as engineered insects and microbes could complement an existing toolbox for plant protection by reducing pesticide use and countering resistant pests and pathogens. The discovery and application of CRISPR/Cas9 and associated technologies has stimulated research into various ways to control pest populations. Furthermore, RNAi-based techniques may also provide solutions to emerging and existing impacts by pests and pathogens. In this talk I will present a sampling of recent developments in genetically manipulating insects and microbes for plant protection.

INSIGHTS INTO FUNDING OPPORTUNITIES AND TIPS FOR GRANT WRITING

Rubella S. Goswami,
Branch Chief USDA-Animal and Plant Health Inspection Service; Former National Program Leader, USDA-National Institute of Food and Agriculture

This presentation will provide an overview about the federal funding programs administered by USDA-National Institute of Food and Agriculture (NIFA), the primary extramural funding arm of USDA that supports both capacity and competitive programs. It will highlight some programs relevant to those working in the area of plant sciences that support applied and basic research as well as pre and post-doctoral fellowships. Tips for accessing information regarding funding opportunities and successful grant writing will also be shared.

COMPLEX FAMILIES, COMPLEX HISTORIES: EVOLUTION AND DIVERSIFICATION OF THE LEUCINE RICH REPEAT - RECEPTOR-LIKE KINASE (LRR-RLK) GENE FAMILY

Jarrett Man, Joseph P. Gallagher, Madelaine Bartlett
Biology Department, UMass Amherst, 611 North Pleasant Street, Amherst, MA
mbartlett@umass.edu

LRR-RLK proteins mediate cell-cell signaling in plant development and defense. For example, LRR-RLKs in the CLAVATA1 (CLV1) clade are essential regulators of meristem homeostasis and, in turn, the development of plant form. The LRR-RLK gene family has diversified to an extreme degree; both through whole genome and smaller-scale duplications. The immense size and complexity of the LRR-RLK gene family has made resolving gene trees challenging. To address this challenge, we developed an iterative search and tree-building method for capturing all the LRR-RLKs and related proteins encoded in a genome, and for resolving the evolutionary history of the gene family. We discovered numerous instances of domain loss; domain gain through duplication and subsequent gene fusion events; and domain swapping between LRR-RLK clades. Moving forward, we are using the tools available in Arabidopsis thaliana to assess the functional evolution of developmental regulators in the LRR-RLK gene family. Our fine-grained history of these important genes sets the stage for understanding the diversification of signaling in the evolution of plant development and defense.

NANOMATERIALS ENABLE DELIVERY OF GENETIC MATERIAL WITHOUT TRANSGENE INTEGRATION IN MATURE PLANTS

Gozde S. Demirer,[a,c] & Markita P. Landry,[a,,b,c,d,e a]
Department of Chemical and Biomolecular Engineering, University of California, Berkeley, Berkeley, California 94720, United States b Molecular Biophysics and Integrated Bioimaging, Lawrence Berkeley National Laboratory, Berkeley, California 94720, United States cCalifornia Institute for Quantitative Biosciences, QB3, University of California, Berkeley, Berkeley, California 94720, United States d Innovative Genomics Institute (IGI), Berkeley, California 94720, United States eChan-Zuckerberg Biohub, San Francisco, California 94158, United States

Genetic engineering of plants is at the core of sustainability efforts, natural product synthesis, and agricultural crop engineering. The plant cell wall is a barrier that limits the ease and throughput with which exogenous biomolecules can be delivered to plants. Current delivery methods either suffer from host range limitations, low transformation efficiencies, tissue regenerability, tissue damage, or unavoidable DNA integration into the host genome. Here, we demonstrate efficient diffusion-based biomolecule delivery into tissues and organs of intact plants of several species with a suite of pristine and chemically-functionalized high aspect ratio nanomaterials. Efficient DNA delivery and strong protein expression without transgene integration is accomplished in mature *Nicotiana benthamiana, Eruca sativa* (arugula), *Triticum aestivum* (wheat) and *Gossypium hirsutum* (cotton) leaves and arugula protoplasts. Notably, we demonstrate that transgene expression is transient and devoid of transgene integration into the plant host genome. We demonstrate that our platform can be applied for CRISPR-based genome editing for transient expression of Cas9 and gRNAs. We also demonstrate a second nanoparticle-based strategy in which small interfering RNA (siRNA) is delivered to mature *Nicotiana benthamiana* leaves and effectively silence a gene with 95% efficiency. We find that nanomaterials both facilitate biomolecule transport into plant cells, while also protecting polynucleotides such as RNA from nuclease degradation. DNA origami and nanostructures further enable siRNA delivery to plants at programmable nanostructure loci, which we use to elucidate force-independent transport phenomena of nanoparticles across the plant cell wall. Our work provides a tool for species-independent, targeted, and passive delivery of genetic material, without transgene integration, into plant cells for diverse plant biotechnology applications.

EVOLUTION OF HOST SUSCEPTIBLITY IN THE GOLOVINOMYCES LATISPORUS-ASTERACEAE SYSTEM

Michael Bradshaw and Patrick Tobin
University of Washington, School of Environmental and Forest Sciences, Seattle, WA 98195, USA. Mjb34@uw.edu

The host range and severity of pathogens are dependent on interactions with their hosts and are hypothesized to have evolved as products of a coevolutionary arms race. An understanding of the factors that affect host range and pathogen severity is especially crucial in introduced pathogens that infect evolutionarily-naïve hosts and cause substantial damage to ecosystems. Powdery mildews are detrimental pathogens found worldwide in managed and natural systems. *Golovinomyces latisporus* is a powdery mildew species that is especially damaging to plants within Asteraceae, and in particular plants within *Helianthus.* In this study, we evaluated 126 species within Asteraceae to measure the role of host plant morpho-physiological traits and evolutionary history on their susceptibility and severity to *G. latisporus*. We observed a phylogenetic signal in both susceptibility and severity between and within major clades of the Asteraceae. In general, there was a major phylogenetic structure of host severity to *G. latisporus*, however, there was some fine-scale phylogenetic variability. Phylogenetic statistical methods showed that chlorophyll density, biomass, stomatal index and trichome density were not associated with disease severity, thus providing evidence that phylogenetic structure, rather than observed plant morpho-physiological traits, is the most reliable predictor of pathogen severity. This work sheds light on the role that evolutionary history plays in plant susceptibility and severity to disease and underscores the relative unimportance of commonly assessed host plant traits in powdery mildew severity.

CLONING ospA FOR PLANT (ARABIDOPSIS) EXPRESSION

Eleanor Browne, David Puthoff
Department of Biology. Frostburg State University, 101 Braddock Rd, Frostburg, MD
ebrowne0@frostburg.edu, dpputhoff@frostburg.edu

Borrelia burgdorferi (Lyme disease) is a vector-borne disease that is both very common and capable of causing long term negative health effects. Because it is spread by deer ticks, which also bite and transfer infection through mice, our goal is to design an oral vaccine capable of preventing mice from becoming infected. This strategy will use ospA from *B. burgdorferi*, produced by altered Arabidopsis thaliana which will, when eaten, deliver the ospA to mice who will begin to produce antibodies effective against B. burgdorferi. Primer pairs for both control and ospA amplification were designed Both primer pairs amplified the respective fragments which have been kept refrigerated due to a laboratory shutdown due to COVID-19. The ospA coding sequence will be expressed in Arabidopsis using pCambia 1201 The cloned ospA/ pCAMBIA plasmid will be transferred into Agrobacterium tumefaciens to be transferred to A. thaliana by floral dip. The offspring of these dipped A. thaliana will be grown, tested for expression of ospA and crossed for homozygosity of the transgene. Those which express ospA at the highest level will be used in tests with mice which will be further tested to determine whether the mice have developed the necessary antibodies to have protection against *B. burgdorferi*.

FUNCTIONAL CHANGES OF DNA METHYLATION PATTERNS OVER MICRORNA GENES DURING SOYBEAN NODULE FORMATION AND DEVELOPMENT

Valeria S. Lopes-Caitar1, ‡, Sarbottam Piya1,‡, Won-Seok Kim2, Vince Pantalone1, Hari B. Krishnan2,3, Tarek Hewezi1,*
1 Department of Plant Sciences, University of Tennessee, Knoxville, TN, 37996, USA
2 Plant Science Division, University of Missouri, Columbia, MI, 65211, USA,
3 Plant Genetics Research, USDA-Agricultural Research Service, Columbia, MI, 65211, USA
‡ Contributed equally Corresponding author, Tarek Hewezi (thewezi@utk.edu)

DNA methylation is a widely spread epigenetic mark that contributes to gene regulation in various developmental programs, including nodulation. Nevertheless, it is currently unknown whether microRNA (miRNA) genes are subjected to DNA methylation changes that may impact their expression, and hence their function during nodule development in soybean (Glycine max). In the current study, we examined DNA methylation levels and patterns of miRNA genes at three distinct nodule developmental stages (formation, development and senescence) using whole-genome bisulfite sequencing (BS-seq) and RNA-seq data. Bulk DNA methylation levels over miRNA genes (promoter and primary transcript regions) in the CG, CHH and CHH sequence contexts were significantly higher in the nodules than the corresponding control root tissues. A significant number of differentially methylated miRNAs were identified mainly in the promoter region and to a much lesser extent in the primary transcript region. Quantification of miRNA expression levels revealed a link between DNA methylation in the promoter and primary transcript regions, and miRNA expression and biogenesis, respectively. RNA-seq data analysis revealed that the expression patterns of miRNA target genes were associated with the methylation status of their negative regulators, particularly during development and senescence stages. More specifically, target genes of differentially hypomethylated miRNAs were down-regulated, whereas target genes of differentially hypermethylated miRNAs were up-regulated. Taken together, our data demonstrate that DNA methylation of miRNA genes is of functional significance and may contribute nodule transcriptome reprograming during development and senescence.

DNA METHYLATION PATTERNS DURING EMBRYOGENESIS AND SEED DEVELOPMENT IN ARABIDOPSIS

Morgan Bennett1, Kailyn Cleaves1, Tarek Hewezi1
1University of Tennessee, 2431 Joe Johnson Drive, Knoxville, TN 37996 thewezi@utk.edu

DNA methylation and demethylation precisely and effectively modulate gene expression during plant growth and development and in response to fluctuating environments. Recent experiment evidence indicates that patterns and levels of DNA methylation are dynamically reprogramed during embryogenesis and seed development. However, expression profiles of genes involved in DNA methylation and demethylation during embryogenesis remain partially known. Thus, we have characterized the spatiotemporal expression patterns of genes involved in DNA methylation and active demethylation in developing seeds of Arabidopsis using GUS reporter lines. Our data shows the expression of several genes throughout seed development, indicating that the methylomes of developing seeds are established through coordinated interactions between DNA methylation and demethylation pathways. Transcriptional activity of the CG methyltransferase MET1 and its homologs MET2b, MET3 was detected in all embryogenesis stages assessed. Our expression analysis also revealed that the non-CG methyltransferases CMT1 and CMT2 were expressed in a sequential manner during embryogenesis and seem to support the RdDM pathway methyltransferase DRM2 in establishing de novo CHH methylation sites. In addition to confirming the key role of DME in mediating extensive hypomethylation in the endosperm, our suggests DML2 and DML3 aid in this process. Taken together, our results demonstrate that DNA methylation pathways act in an overlapping and specific manner in various seed and embryonic tissues, highlighting the dynamic configuration in the patterns and levels of DNA methylation during seed development.

EVALUATION OF A NEW METHOD FOR DNA EXTRACTION OF "LEGAL HIGH" PLANT SPECIES

Cassandra P. O'Hern, B.S., Angelique L. Ryan, B.S., and Kelly M. Elkins, Ph.D.
Towson University, Chemistry Department, Forensic Science Program, 8000 York Rd, Towson, MD 21252 kmelkins@towson.edu

As of late, the investigation of plant species that produce "legal highs", or chemicals, plants, or fungi accessible without legal restrictions that mimic the psychoactive effects of structurally similar controlled substances, have become increasingly relevant within the field of forensic science. Consequently, it is important to develop a straightforward, high yield, and practical nucleic acid isolation process to integrate into a forensic laboratory's operating procedures. The focus of this study was placed on the capability of the MicroGEM PDQeX phytoGEM system, which utilizes enzymatic reactions and an innovative cartridge structure that degrades the cell wall and purifies DNA, to extract amplifiable DNA from several known legal high plant species: Artemisia absinthium, Datura stramonium, Ipomoea purpurea, Mitragyna speciosa, and Papaver somniferum. The pulverized plant material DNA was extracted using the PDQeX Nucleic Acid Extractor plant protocol, quantified using the Invitrogen Qubit 2.0 Fluorometer, and evaluated for amplifiability by PCR-HRM using the Qiagen Rotor-Gene Q. The melt curves produced from this method of extraction were concordant with those examined in a previous study that utilized the Qiagen DNeasy Plant Mini Kit for extraction. The PDQeX phytoGEM system proved to be simple, rapid, affordable, and compatible with downstream processes.

FLOWERING LOCUS T CHIMERIC PROTEIN INDUCES FLORAL PRECOCITY IN EDIBLE CITRUS

Judith P. Sinn1, Jeremy Held1,2,#, Chad Vosburg1, Sara M. Klee1,3, Vladimir Orbovic4, Earl Taylor5, Tim Gottwald5, Ed Stover5, Gloria Moore6, and Timothy W. McNellis1, *
1Department of Plant Pathology and Environmental Microbiology, The Pennsylvania State University, University Park, PA, USA
2The Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA, USA
3Present address: Department of Microbiology, University of Washington, Seattle, WA, USA
4Citrus Research and Education Center, University of Florida, Lake Alfred, FL, USA
5United States Horticultural Research Laboratory, United States Department of Agriculture, Agricultural Research Service, Fort Pierce, FL, USA
6Institute of Food and Agricultural Sciences, Department of Horticultural Sciences, University of Florida, Gainesville, FL, USA
#Poster presenter: jbh249@psu.edu
*Correspondence: twm4@psu.edu

Breeding of perennial tree crops is challenging due to a multi-year juvenility period. Rapid cycle breeding represents a solution by inducing early flowering through constitutive expression of flower-promotion genes. This approach has so far failed in edible citrus varieties due to difficulty regenerating plants overexpressing floral regulatory genes, possibly due to excessive responsiveness to the floral signal. In the present study, we expressed a translational fusion of the Poncirus trifoliata (trifoliate orange) Flowering Locus T (PtFT1) floral regulator to a single-chain variable fragment antibody (scFv) in the edible grapefruit (Citrus x paradisi) cultivar ‘Duncan’. We hypothesized that fusion of PtFT to the scFv might attenuate PtFT activity and allow regeneration of precocious plants. Fifteen successful transformants were obtained and grafted onto Carrizo citrange rootstocks. Western blotting revealed variability in the expression levels of PtFT1-scFv protein among the 15 lines. Of the seven lines closely monitored for a flowering phenotype, four displayed precocious flowering within the first year after transfer to a growth chamber. Flowers and inflorescence architecture were morphologically normal in all precocious lines. Fruit developed normally on the PtFT1-scFv plants, but hand pollination was necessary for seed set. A reduction in thorn size was also associated with PtFT1-scFv expression, a trait corresponding to maturity. The highest expressing PtFT1-scFv lines exhibited dwarfism and a weeping growth form. These results document successful constitutive FT expression in edible citrus for the purpose of reducing juvenility and provide a tool for advancing rapid cycle breeding in citrus.

EXPANDING THE SCOPE OF PLANT GENOME ENGINEERING WITH NOVEL CAS12A ORTHOLOGS AND HIGHLY MULTIPLEXABLE EDITING SYSTEMS

Yingxiao Zhang1, Qiurong Ren2, Xu Tang2, Shishi Liu2, Aimee A. Malzahn1,Jiaheng Wang2, Desuo Yin1,3, Changtian Pan1, Mingzhu Yuan2, Lan Huang2, Han Yang2, Yuxin Zhao2, Qing Fang2, Xuelian Zheng2, Li Tian2, Yanhao Cheng1,4, Ysa Le1, Bailey McCoy1, Lidiya Franklin1, Jeremy D. Selengut5, Stephen M. Mount6, Qiudeng Que7, Yong Zhang2, Yiping Qi1,8
1Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD, USA; 2University of Electronic Science and Technology of China, Chengdu, China; 3Food Crop Institute, Hubei Academy of Agricultural Sciences, Wuhan, Hubei, China; 4College of Agriculture, Nanjing Agricultural University, Nanjing, Jiangsu, China; 5Center for Bioinformatics and Computational Biology, University of Maryland, College Park, MD, USA; 6Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD, USA; 7Syngenta, Research Triangle Park, NC, USA; 8Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, MD, USA
Email of corresponding authors: Yiping Qi: yiping@umd.edu; Yong Zhang: zhangyong916@uestc.edu.cn

CRISPR-Cas12a is a promising genome editing system for targeting AT-rich genomic regions. Compared to Cas9, Cas12a has shown higher targeting specificity in plants. Unlike Cas9 that usually generates small deletions, Cas12a cleavage generates staggered ends resulting in larger deletions, making it a suitable nuclease for gene knockout. Moreover, Cas12a only requires a short CRISPR RNA (crRNA) for each target and possesses RNase activity for crRNA array processing, making it an ideal platform for multiplexed editing. Cas12a has been widely applied in plants and achieved high editing efficiencies for single gene targets. However, comprehensive genome engineering using CRISPR usually requires simultaneous targeting of multiple genes at defined locations, which cannot be achieved easily and efficiently with current Cas12a systems, due to their strict PAM (protospacer adjacent motif) requirements and limited multiplex capacity. To expand the targeting scope of Cas12a, we screened nine new Cas12a orthologs and identified six that possess high editing activity and specificity in rice. Among them, Mb2Cas12a stands out with high editing efficiency, relaxed PAM requirements and tolerance to low temperatures. Engineered Mb2Cas12a can also target altered PAMs. These new Cas12a systems have greatly expanded the targeting scope of Cas12a in major crops. To further enable large-scale genome engineering, we compared 12 multiplexed Cas12a systems and identified a potent system that exhibited nearly 100% biallelic editing efficiency with the ability to target as many as 14 sites in rice. This is the highest level of multiplex edits in plants to date using Cas12a. This study has greatly expanded the targeting scope of Cas12a for crop genome engineering.

OVER-EXPRESSION OF CELLULASE ENZYMES IN STREPTOMYCES TO PROBE THE TRANSITION FROM SAPROPHYTE TO PHYTOPATHOGEN

Kara LeClair, Dr. Christopher Clarke, and Chad Kramer USDA, GIFVL, 10300 Baltimore Ave, Beltsville, MD 20705 karaleclair97@gmail.com

Common scab is a disease caused by multiple species of Streptomyces bacteria, resulting in raised or pitted lesions on the surface of potato tubers. Production of the phytotoxin thaxtomin, the main virulence factor produced by pathogenic Streptomyces, is elicited by the disaccharide cellobiose. Therefore, the environmental presence of cellobiose is considered the primary trigger for the transition of pathogenic Streptomyces from saprophyte to pathogen. We are testing the hypothesis that Streptomyces controls its own access to cellobiose through expression of cellulase enzymes that can degrade environmentally ubiquitous cellulose into cellobiose. We identified 35 Streptomyces cellulases and cloned 11 genes into an overexpression vector. We will grow Streptomyces overexpressing cellulase enzymes in the presence of cellulose and observe for elicitation of thaxtomin production. We hypothesize a subset of enzymes in a secreted complex will elicit thaxtomin production. We aim to identify the necessary genes for cellulase production to identify the initial steps of Streptomyces transition from saprophyte to pathogen.

IDENTIFICATION OF PROTEIN KINASE HUB GENES THAT REGULATE SOYBEAN RESISTANCE TO SOYBEAN-CYST NEMATODE

Sarbottam Piya, Bhoomi Patel, Tracy Hawk, and Tarek Hewezi* Department of Plant Sciences, University of Tennessee, Knoxville, TN, 37996 * thewezi@utk.edu

Soybean cyst nematode (SCN, Heterodera glycines) is one of the most devastating pests affecting soybean production worldwide. SCN induces specialized multinucleate feeding site, termed syncytium, in the root vascular tissues. The development of a functional syncytium involves massive induction of signal transduction pathways that orchestrate various cellular functions and activities required for correct cell fate differentiation of this unique cell type. The goal of this study was to investigate the role of protein kinases, which constitute the core components of signal transduction pathways, in soybean–SCN interaction. We generated two independent co-expression networks of soybean genes using control and stress response gene expression data from publicly available RNA-seq datasets. We identified 145 protein kinase hub genes that were highly interconnected in control condition network compared to the stress condition network. Similarly, we identified 247 kinase hub genes that were highly interconnected in stress condition network compared to the control condition network. Out of these 392 protein kinase hub genes, 90 genes were previously identified as differentially expressed in the SCNinduced syncytium, suggesting that these protein kinases may play role in regulating signal transduction pathway during SCN infection. To test the biological significance of the syncytiumkinase hub genes, we assayed SCN susceptibility of soybean plants overexpressing a set of the identified protein kinase hubs using transgenic soybean hairy root system. Overexpression of inactive variants of a set of the kinase hub genes significantly altered soybean susceptibility to SCN. These results suggest that protein kinases are promising targets for developing robust resistance against SCN.

HEMP (CANNABIS SATIVA L.) STRAINS GENETIC COMPOSITION AND CHEMICAL PRODUCTION

Janai Heise, Abigail Hunker and David Puthoff
Frostburg State University, 101 Braddock Road, Frostburg, MD jaheise97@gmail.com

In the United States, the Hemp Farming Act of 2018 nationally legalized hemp (Cannabis sativa L.) for research and cultivation. The Cannabis sativa species yields strains such as marijuana, industrial hemp, and medical hemp; medical hemp is the focus of this study. Hemp's chemical composition varies throughout the plant's tissue. The desired chemicals for medicinal hemp, CBD (cannabidiol) and terpene compounds, are found in high concentrations in the female flowers. In 2019, the USDA set out regulations for medicinal hemp to have no more than 0.3% total THC (tetrahydrocannabinol) concentration. This study is being completed to learn more about hemp's requirements for high CBD production in Maryland. Our research question is: What combination of variety and location results in the best production of chemical compounds in hemp? The study will address variety, location, planting date, and genetic diversity to determine what combinations of variables lead to the best productions of chemical compounds in hemp. Hemp growers located across western Maryland provided the field sites and hemp was sampled when harvestable. Hemp flowers were collected to determine chemical composition-including THC, CBD, and terpene content, while the leaves were collected for DNA analysis. Several standard Cannabis sativa microsatellites, along with novel microsatellites, will be used. Analysis is still being completed. With this research, medical hemp growers in Maryland will be able to determine the strains with the best combination of CBD, THC, and terpenes.

NAME	ADDRESS	PHONE/EMAIL
Abdelkreem, Reham	Plant biotechnology LLC, 19 Hickory tree court, Charles town, WV 25414	240-704-0259, Reeyoussef@gmail.com
Abrahamian, Peter	USDA-ARS, 10300 Baltimore Ave, Beltsville, Maryland 20705	352-514-8852, peter.abrahamian@usda.gov
Alkharouf, Nadim	TU, 8000 York rd, Towson, Md 21252	nalkharouf@towson.edu
Atha, Benjamin	USDA - APHIS, , Beltsville, Maryland 20705	301-313-9316, Benjamin.Atha@usda.gov
Bailey, Bryan	Sustainable Perennial Crops Laboratory, USDA/ARS, Rm 302, Building 001, Northeast Area, BARC-West, Beltsville, Maryland 20705	301-504-7985, bryan.bailey@usda.gov
Baker, Jacyn	USDA MPPL, 10300 WASHINGTON BLVD B-004 R-012, BELTSVILLE, MD 20705	410-245-2187, jacyn.baker@usda.gov
Baranova, Natalya	EPA, 2419 Zion Rd, Halethorpe, MD 21227	773-603-5855, baranova.natalya@epa.gov
Barnaby, Jinyoung	USDA-ARS, 10300 Baltimore Avenue, Beltsville, Virginia 22705	301-310-8681, jinyoung.barnaby@usda.gov
Bartlett, Madelaine	University of Massachusetts Amherst, 611 North Pleasant St, 374 Morrill 4 South, Amherst, Massachusetts 01003	mbartlett@umass.edu
Bate, Gabrielle	University of Maryland	gbate@umd.edu
Beetham, Patricia	USDA-APHIS-BRS, 4700 River Road Unit 147, Riverdale, Maryland 20737	301-851-3889, patricia.k.beetham@usda.gov
Bennett, Dennis	USDA- Appalachian Fruit Research Station, 2217 Wiltshire Road, Kearneysville, West Virginia 25430	304-261-6235, Dennis.Bennett@usda.gov
Bennett, Morgan	University of Tennessee, Knoxville, 131 Faber Street, Knoxville, TN 37918	mmbennet@vols.utk.edu
Bolus, Stephen	USDA-ARS, Beltsville, MD 20705	Stephen.Bolus@usda.gov
Bottner-Parker, Kristi	USDA-ARS, 10300 Baltimore Ave Bldg 004, Rm 225, Beltsville, MD20705	301-504-6024, kristi.bottner@usda.gov
Bradshaw, Michael	USDA	mjb34@uw.edu
Brenya, Eric	University of Tennessee, 343 Hesler Biology Building, knoxville, TENNESSEE 37996	865-348-4099, ebrenya@utk.edu
Browne, Eleanor	Frostburg State University, 101 Braddock Rd, Frostburg, MD 21532	3013384723, eqbrowne0@frostburg.edu
Burgos, Angie	USDA APHIS PQ, 9901 Powder Mill Road Bldg 580 BARC-East Beltsville, MD 20705, Beltsville, Maryland 20705	Angie.Burgos@usda.gov
Campbell, Kimberly	USDA, ARS, 10300 Baltimore Ave., Beltsville, MD 20705	301 828 0330, kimberly.campbell@usda.gov
Carter, Melissa	USDA ARS FDWSRU, 1301 Ditto Avenue, Ft. Detrick, MD 21702	3016197211, melissa.carter2@usda.gov

Chaluvadi, Srinivasa	USDA-APHIS-BRS, Riverdale, MD 30677	7062558097, srinivasa.chaluvadi@usda.gov
Chen, Maddy	USDA, 3759 Chateau Ridge Drive, ELLICOTT CITY, MD 21042	443-695-5480, madeleine.k.chen@gmail.com
Clarke, Christopher	USDA-ARS, Building 010A, Rm 226, Beltsville, Maryland 20705	540-385-9362, christopher.clarke@usda.gov
Collins, Ronald	USDA, 13208 Ithan Ln, Bowie, United States 20715	13016482710, ron.collins@usda.gov
Collum, Tami	USDA ARS FDWSRU, 1301 Ditto Avenue, Frederick, MD 21702	3149432385, tami.collum@usda.gov
Cournoyer, Patrick	FDA	patrick.cournoyer@fda.hhs.gov
Dumm, Judith	USDA-ARS-BARC-GIFVL, 10300 Baltimore Ave, Bldg 010A, BARC-West, Beltsville, MD 20705	301-504-6242, Judith.Dumm@usda.gov
Eichenseer, Herb	USDA-Animal Plant Health Inspection Service Biotechnology Regulatory Services, 4700 Riverside Drive, Riverdale, MD 20737	Herbert.Eichenseer@usda.gov
Elkins, Kelly	Towson University , 8000 York Rd, Chemistry Dept , Towson , Maryland 21042	410-704-6217, kmelkins@towson.edu
Farrell, Robert	Penn State, 1031 Edgecomb Avenue, York, PA 17403	717-771-4052, jrf10@psu.edu
Fletcher, Rebecca	USDA-APHIS, Riverdale, MD	rebecca.fletcher2@usda.gov
Frederick, Reid	USDA-ARS Foreign Disease-Weed Science Research Unit, 1301 Ditto Avenue, Ft. Detrick, MD 21702	301-619-7339, reid.frederick@usda.gov
Fuentes-Bueno, Irazema	USDA, 10300 Baltimore Ave, Beltsville, MD 20705	301-504-5458, irazema.fuentes@usda.gov
Gaskins, Verneta	USDA- Food Quality Laboratory, 10300 Baltimore Ave., Beltsville, Maryland 20705	301-504-6510, verneta.gaskins@usda.gov
Gichuki, Samson	Morgan State University	sagic1@morgan.edu
Goswami, Rubella	USDA-Animal and Plant Health Inspection Service, 4700 River Road, Riverdale, MD 20737	240-534-9435, rubella.goswami@usda.gov
Grinstead, Sam	USDA, 10300 Baltimore Ave, Beltsville, MD 20705	301-504-8573, sam.grinstead@usda.gov
Hammond, John	USDA-ARS, USNA, Floral and Nursery Plants Research Unit, 10300 Baltimore Avenue, B-010A , Beltsville, MD 20705	301-504-5313, john.hammond@usda.gov
Haymes, Kenneth	USDA/APHIS, 4700 River Road, Riverdale, MD 20737	301-851-3879, kenneth.m.haymes@usda.gov
Heck, Michelle	USDA ARS, 538 Tower Road, Ithaca, NY 14853	607-351-5016, michelle.cilia@usda.gov
Heise, Janai	Frostburg State University, 101 Braddock Rd, Frostburg, Maryland 21740	301-988-0038, jaheise97@gmail.com
Held, Jeremy	The Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA 16801	614-787-1921, jbh249@psu.edu

Henderson, Ashley	University of Delaware, 119 Canyon Ridge Drive, Morgantown, WV 26508	484-725-5519, ahende@udel.edu
Howe, Natalie	USDA, 4834 7th St NW, Washington, DC 20011	408-838-5242, natalie.m.howe@usda.gov
Hu, Xiaojun	USDA, APHIS, PPQ, PGQP, 9901 Powder Mill Rd., Laurel, MD 20708	240-425-9545, xiaojun.hu@usda.gov
Huang, Qi	FNPRU, USDA/ARS, Bldg 004, room 116, 10300 Baltimore Ave, Beltsville, MD 20705	301-504-9159, qi.huang@usda.gov
Islam, Nazrul	ARS, Beltstville,USDA, Bldg. 006, Room 203, BARC-West 10300 Baltimore Ave., Beltsville, MARYLAND 20705	3013269561, nazrul.islam@usda.gov
Ivanauskas, Algirdas	Nature Researche Centre	algirdas.ivanauskas@gamtc.lt
Jones, Christian	Morgan State University, PO Box 23940, L'Enfant Plaza, Washington DC 20026	301-433-1396, chjon45@morgan.edu
Jordan, Ramon	USDA-ARS, US National Arboretum, Floral & Nursery Plants Research, 10300- Baltimore Ave, Bldg-010A, Rm-238, Beltsville, MD 20705	301-504-5646, Ramon.Jordan@usda.gov
Julkowska, Magdalena	Boyce Thompson Institute, 533 Tower Rd, Ithaca, NY 14853	607-279-6002, mmj55@cornell.edu
K, A	Macherey-Nagel, Raleigh, NC	akarimi@mn-net.com
Kang, IL-Ho	FMC Agricultural Solutions, 1090 Elkton Road S300/476B, Newark, DE 19711	302-318-9506, IL-Ho.Kang@FMC.com
Kao, Yun-Ting	UMD	chinhonor@gmail.com
Kelman, Lori	Montgomery College, 20200 Observation Drive, Germantown, MD 20876	240-567-6929, Lori.Kelman@montgomerycollege.edu
KianiFariz, Mahnaz	Thegreencell, Inc	mahnazk@thegreencell.com
Kim, James	USDA-ARS U.S. Arid-Land Agricultural Research Center, 21881 N. Cardon Lane, Maricopa, AZ 85138	480-689-9580, james.kim2@usda.gov
Kokkaliari, Sofia	University of South Florida	skokkaliari@usf.edu
Kovalskaya, Natalia	ORISE-USDA-ARS-FNPRU, 10300 Baltimore Ave, Bldg 004, Rm 211, Beltsville, MD 20705	301-504-8785, Natalia.Kovalskaya@usda.gov
Kreger, Nancy	USDA,ARS, MPPL, 910 WESLEY RD, ROCKVILLE, Maryland 20850	301-251-5544, vetnancy@verizon.net
Landry, Markita	UC Berkeley, 1424 Lincoln Street, Berkeley, CA 94702	919-349-4877, landry@berkeley.edu
LeClair, Kara	Genetic Improvement of Fruits and Vegetables Laboratory, USDA, 2005 Treetop Lane, Silver Spring, MD 20904	8564269400, karaleclair97@gmail.com
Li, Zhijian	USDA, Appalachian Fruit Research Station, 2217 Wiltshire Road, Kearneysville, WV 25430	304-725-3451, Zhijian.li@usda.gov
Liu, Zongrang	USDA-ARS, 2217 Wiltshire Road, Kearneysville, WV 25430	304-725-3451 X239, Zongrang.liu@uads.gov

Lopes-Caitar, Valeria	University of Tennessee, 2505 E J Chapman Dr., Knoxville, TN 37996	8659747559, vcaitar@utk.edu
Lutton, Elizabeth	USDA Appalachian Fruit Research , 2217 Wiltshire Rd, Kearneysville, WV 25430	3047253451x220, elizabeth.lutton@usda.gov
Maliga, Pal	Waksman Institute, Rutgers University, 190 Frelinghuysen Road, Piscataway, NJ 08854	732-763-1333, maliga@waksman.rutgers.edu
Mandava, Bhushan	Repar Corporation, 8070 Georgia Avenue, Silver Spring, MD 20910	12022231424, bhushan@mandava.com
Mathioni, Sandra Marisa	Syngenta Crop Protection _ Brazil, Rodovia BR-452, Km 142, UberlÃ¢ndia, Minas Gerais 38407049	553432334581, mathioni@gmail.com
Matthews, Benjamin	Towson University & Plant Biotechnology, 8000 York Rd, Towson, Md 21252	4432802492, bmatthews.pbt@gmail.com
Mazo, Diana	Post-Doc, , Ithaca, New York	dcm286@cornell.edu
McGonigle, Brian	FMC, 1090 Elkton Rd, Newark, DE 19711	3027771107, brian.mcgonigle@fmc.com
Mendoza, Joshua	USDA-APHIS, 7612 Mathis Lane , Mount Airy , MD 21771	4439562897, joshua.mendoza@usda.gov
Mischke, Sue	USDA, Retired, 4203 Wicomico Avenue, Beltsville, Maryland 20705	3019372817, smischke1@hotmail.com
Mount, Steve	Univ. of Maryland, College Park, 0122 Bioscience Research Bldg., College Park, Maryland 20742	3014056934, smount@umd.edu
Mowery, Joseph	USDA ARS, 10300 Baltimore Ave, Beltsville, Maryland 20705	3015049027, joseph.mowery@usda.gov
Muhle, Anthony	USDA-ARS, Maryland	anthony.muhle@usda.gov
Natarajan, Savithiry	USDA-ARS, 10800 BALTIMORE AVENUE, BELTSVILLE, 20705	301-512-9518, savi.natarajan@ars.usda.gov
Nguyen, Hien	USDA-ARS, 10300 Baltimore Avenue, Beltsville, MARYLAND 20705	3017681549, nguyenphuochien92@gmail.com
Nien, Yachi	Penn State University	yxn5072@psu.edu
Nieto, Thomas	Hood college	thopumanieto@gmail.com
Nunes, Custodio	UMD, 4302 Pennsylvania street, Hyattsville, MD 20873	2402809962, conunes@umd.edu
Obae, Samuel	Stevenson University , 11200 Ted Herget Way, Owings Mills , MD 21117	4103146021, sobae@stevenson.edu
O'Connell, Mary	USDA-APHIS	mary.oconnell@usda.gov
Ogden, Elizabeth	USDA-ARS-GIFVL, 10300 Baltimore Ave Bldg., 010A, Rm. 247 BARC-West, Beltsville, MD 20705	301-504-7088, elizabeth.ogden@usda.gov
O'Hern, Cassandra	Towson University, Department of Chemistry, Forensic Science, 8000 York Road, Towson, MD 21252	862-354-1519, cohern1@students.towson.edu
Olsson, Abraham	USDA ARS FDWSRU, 2905 Kling Ct , Frederick, MD 21703	3014017740, abraham.olsson@usda.gov
Overcash, Justin	USDA-BRS, 4700 River Road, Riverdale, MD 20737	3018513907, justin.overcash@usda.gov
Pathologist, Ruhui	USDA-ARS, Bldg. 004/Rm 015, Beltsville, Maryland 20705	3015047656, Ruhui.Li@ars.usda.gov

Pedley, Kerry	USDA ARS FDWSRU, 1301 Ditto Ave., Ft. Detrick, MD 21702	301-619-1668, kerry.pedley@usda.gov
Peng, Jiangnan	Morgan State Unicersity, 1700 East Cold Spring Lan, Baltimore, MD 21251	4438853955, jiangnan.peng@morgan.edu
Perez, Frances	USDA-ARS/GIFVL, 10300 Baltimore Ave., B010A, BARC-West, Beltsville, MD 20705	301-504-7507, frances.perez@usda.gov
Piya, Sarbottam	University of Tennessee, 2431 Joe Johnson Drive, Knoxville, Tennessee 37996	2523059399, spiya@utk.edu
Podeti, Dr. Srinivas	Kakatiya University, Department of Biotechnology,Kakatiya University, Warangal. Telangana State.India, Warangal, Telangana State.India 506009	9849303560, srinivas7586@gmail.com
Puthoff, David	Frostburg State University, 101 Braddock Rd, Frostburg, MD 21532	301-687-4172, dpputhoff@frostburg.edu
Qian, Bilian	University of Maryland	bqian@umd.edu
Quresh , Alifiya	Dr pal Maligas lab, 102 south Adelaide Ave apt 1B, Highland park , NJ 08904	6094015250, aq91@scarletmail.rutgers.edu
RAHMAN, MDMAHBUBUR	Virginia Tech, Department of Biological Systems Engineering, Blacksburg, VA 24060	4237377384, rahmanm@vt.edu
Randall, Linnell	Boyce Thompson Institute, 533 Tower Rd, Ithaca, NY 14853	lbr65@cornell.edu
Routray, Pratyush	Boyce Thompson Institute, 533 Tower Rd, Ithaca, NY 14850	5093395825, pr474@cornell.edu
Rowland, Jeannie	USDA-ARS, USDA-ARS, GIFVL, Bldg 010A, BARC-West, Beltsville, MD 20705	3015046654, Jeannine.Rowland@usda.gov
Salazar, Beatrice	ACS Maryland , 1204 Roundhill Rd. , Baltimore, Maryland 21218	papa51196@verizon.net
Saunders, James	Towson University, 14590 Triadelphia MIll Road, Dayton, MD 21036	4433864695, jsaunders@towson.edu
Schachterle, Jeffrey	USDA	jeffrey.schachterle@usda.gov
Sechler, Aaron	USDA, 1301 Ditto Ave, Fort Detrick, MD 21702	Aaron.Sechler@usda.gov
Sherman, Diana	USDA-ARS-FDWSRU, 1301 Ditto Avenue, Fort Detrick, MD 21702	240-7722757, diana.sherman@usda.gov
Shih, Justin	The Pennsylvania State University, 445 Waupelani Dr B05, STATE COLLEGE, PA 16801	8326830399, jws52@psu.edu
Singh, Dharmendra	AgriCureTech	drdsingh@ucdavis.edu
Sitther, Viji	Associate Professor, Morgan State University, 1700 EColdspring Lane, Maryland 21251	4438854688, viji.sitther@morgan.edu
Sparks, Erin	University of Delaware, 590 Avenue 1743, Newark, DE 19713	3028313428, esparks@udel.edu
Starker, Colby	University of Minnesota, 1500 Gortner Avenue, Saint Paul, MN 55108	612-624-5196, stark224@umn.edu
Stommel, John	USDA, ARS, Genetic Improvement of Fruits and Vegetables Laboratory, B-010A, BARC-	301-504-5583, john.stommel@usda.gov

	West, 10300 Baltimore Ave., Beltsville, MD 20705	
Taylor, Chris	USDA/APHIS	christopher.taylor2@usda.gov
Teng, Chong	Donald Danforth Plant Science Center, N 975 Warson Road, St Louis, MO 63132	3022601554, CTeng@danforthcenter.org
Van Eck, Joyce	Boyce Thompson Institute, 533 Tower Road, Ithaca, NY 14853	607-254-1686, jv27@cornell.edu
Wang, Jinbo	USDA-APHIS-BRS, 4700 River Road, Unit 147, Riverdale, MD20737	3018513884, Jinbo.Wang@usda.gov
Wang, Chunfang	University of Minnesota-Twin cities	wang9519@umn.edu
Webb, Kevin	USDA-ARS-AFRS, 2217 Wiltshire road, Kearneysville, West Virginia 25430	3046719791, kevin.webb@usda.gov
WEI, WEI	USDA-ARS-Molecular Plant Pathology Laboratory, Rm219, BLDG004, 10300 Baltimore Ave, Beltsville, Maryland 20705	3015040786, wei.wei@usda.gov
Welch, Kara	EPA, 2777 Crystal Dr., Arlington, VA 22202	7033088150, welch.kara@epa.gov
welser, phil	usda ars, 2217 wiltshire rd, kearneysville, west virginia	3155210211, philipp.welser@usda.gov
Wilcox, Robin	USDA-APHIS-Biotechnology Regulatory Services(BRS), 4700 River Road, Unit # 147, Riverdale, Maryland 20737	301-851-3886, Robin.J.Wilcox@aphis.usda.gov
Williams, Phoebe	William and mary, 2901 robert Hunt south, Williamsburg, VA 23185	5402904823, phoebe.williams@gmail.com
Wingeart, Jennifer	U.S. EPA, 2803 Erics Ct, Crofton, MD 21114	7033470100, wingeart.jennifer@epa.gov
Wozniak, Chris	US EPA, 1200 Pennsylvania Ave, NW, 7511P, Washington, DC 20460	7035818554, wozniak.chris@epa.gov
Wright, Clay	Biological Systems Engineering, 1230 Washington ST SW, Biological Systems Engineering, Blacksburg, Virginia 24061	5402314546, wrightrc@vt.edu
Wyatt, LaDonna	Morgan State University, 3209 Granite rd, Woodstock, MD 21163	4449003733, lawya2@morgan.edu
yalcin, yavuz	Morgan State University- Dr. Viji Sitther's Lab., 3205 E Market St. H7-Apt, York, PA 17402	6677703195, yavuz.yalcin@morgan.edu
Yang, Yinong	Penn State University, 405C Life Sciences Bldg, University Park, Pennsylvania 16802	914-867-0324, yuy3@psu.edu
Yin, Weixiao	The Pennsylvania State University, Life Sciences Building University Park, STATE COLLEGE, Pennsylvania State 16801	wuy69@psu.edu
Yu, Jingyi	penn state university, , ,	8148529460, juy323@psu.edu
Zhang, Qian	Pennsylvania State University, State College, Pennsylvania16801	qqz5137@psu.edu
Zhang, Yingxiao	University of Maryland, 4291 Fieldhouse Dr, College Park, MD20742	zhangyx@umd.edu

Zhao, Yan	Molecular Plant Pathology Laboratory, ARS-USDA	yan.zhao@usda.gov