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Plant Symposia and Workshops

P-1

Use of *OsTPS8* for Molecular Genetic Enhancement of Rice Yield and Resilience Under Salt Stress. Bhushan Vishal, Pannaga Krishnamurthy, Rengasamy Ramamoorthy, and PRAKASH P. KUMAR. Department of Biological Sciences, National University of Singapore, 10 Science Drive 4, Singapore 117543, Singapore. Email: prakash.kumar@nus.edu.sg

Soil salinity is a deleterious abiotic stress that reduces growth and yield of crop plants. Emerging evidence clearly shows a firm genetic basis for different degrees of tolerance to salinity exhibited by various plant species. Therefore, understanding how selected genes contribute to the stress responses of plants will help in crop improvement strategies. We will discuss our attempts to apply findings from *Arabidopsis* and the mangrove *Avicennia officinalis* to improve rice plant. We found that *OsTPS8* of the trehalose-phosphate-synthase (*TPS*) gene family is involved in controlling salinity-stress response along with key agronomic traits such as plant height, panicle length, panicle branching, and seed set in rice. There are two classes of *TPS* genes and *OsTPS8* belongs to class II TPS. We characterized the salt sensitive loss-of-function *ostps8* mutant. By functional analysis, we have shown that ectopic expression of *OsTPS8* led to salt tolerance in rice without any undesirable developmental defects. Data from gene expression analyses, genetic complementation of salt sensitive mutant *ostps8,* and histochemical analysis we were able to identify a novel regulatory step controlled by OsTPS8 in the previously known ABA associated deposition of hydrophobic barrier formation (suberin deposition) in the root endodermis and exodermis layers. This provides a mechanistic model of its role in conferring enhanced salinity tolerance. Collectively, our findings would be helpful in improving the plant tolerance to salinity stress without compromising grain yield in rice.

P-2

H+-PPases Are Versatile Enzymes Involved in Carbohydrates Translocation and Metabolism in Plants. Kamesh C. Regmi, Júlia Gomes Farias, Lin Li, Raju Kandel, and ROBERTO A. GAXIOLA. Arizona State University. Email: roberto.gaxiola@asu.edu

Food insecurity is high in arid and semiarid regions of the planet because of marginal soils, frequent drought, biotic stress, and lack of access to agrichemical inputs, including fertilizers. The strategy of the first Green Revolution was to increase yield through the selection of plants that thrive in the presence of abundant water and large amounts of fertilizers. However, as resources become increasingly limited and environmental damage from agricultural intensification becomes more extensive, yield and quality need to be increased with minimum inputs of water and agrochemicals. A *sine qua non* requirement for a new generation of crops is extended root systems. Our group and others have shown that up-regulation of the proton-pumping pyrophosphatase (H^+-PPase) results in increased root and shoot biomass in arabidopsis, rice, corn, barley, wheat, tomato, lettuce, cotton, finger millet, alfalfa, peanuts, and sweet potatoes, compared to controls (see figure). Larger root systems with enhanced rhizosphere acidification capacity explain the drought tolerance and nutrient use efficiencies displayed by these genetically engineered lines. Our working hypothesis postulates that the up-regulation of H^+-PPases augments phloem sucrose loading and transport capacity. More efficient sucrose transport to sink organs (i.e., root and shoot apical meristems, inflorescence cells, and seedling hypocotyls) in H^+-PPase-overexpressing plants explains in part their larger and more energized root systems with higher water and nutrient-uptake capacities. Furthermore, a strong vacuolar expression of type I H^+-PPases in sink organs has been documented. Our group has evidence consistent with the contribution of H^+-PPases expression to sink strength. We posit that enhanced H^+-PPase activity in heterotrophic tissues contributes to sink strength by scavenging inorganic pyrophosphate (PPi) and reducing feedback inhibition on biosynthetic pathways. H^+-PPase would alleviate competition for ATP between biosynthetic reactions and membrane transport processes. In summary, with this simple genetic manipulation it is possible to optimize carbon partitioning and trigger agriculturally relevant phenotypes (i.e., larger root systems) that

would allow small farmers to grow crops more reliably in marginal soils with minimal expensive inputs. For large producers, it would allow for high production at a fraction of the economic and environmental costs. Our ultimate goal is to develop crops that can use soil nutrients and water much more effectively than current cultivars. These water- and nutrient-efficient crops hold the promise of alleviating hunger in much of the arid and semiarid regions of our planet.

P-3

Translating Transcriptome Resources of a Grass Extremeophile to Engineer Salt and Drought Tolerant Rice. NIRANJAN BAISAKH. School of Plant, Environmental and Soil Sciences, Louisiana State University Agricultural Center, Baton Rouge, LA 70803. Email: nbaisakh@agcenter.lsu.edu

Rice is sensitive to salinity and drought stress at varying degrees depending on the stress severity and the growth/developmental stage of the plant and the genotype. Extermophiles such as halophytes, on the other hand, are able to withstand and complete their life cycle under abiotic stresses. Significant progress in the understanding of the abiotic stress adaptation response of some extremophiles has been made through multi-omics approaches. However, most studies in published literatures have focused on dicot halophytes, which are anatomically different than the monocot halophytes. In addition, there are less reports on the successful translation of dicot halophyte resources toward development of salt/drought resistant cereals that constitute more than 50% of the total food crops. Research in my laboratory in the past one decade has focused on two grass halophytes – *Spartina alterniflora*, a salt marsh grass native to Louisiana and *Porteresia coarctata*, a wild relative of rice of Asian origin. Systems biology approaches involving transcriptomics, bioinformatics, and highthroughput functional screening of their transcriptomes have led to the identification of key candidate genes involved in salt and/or drought tolerance traits. The functionality of several genes has been validated in over- and under-expressing transgenic plants including rice. Current research focuses on the mechanistic understanding of the action of a few selected genes. The results obtained thus far on a gene involved in cellular actin dynamics will be discussed during the presentation.

P-4

Stock Plant Maintenance and Initiation. K. GREGORY. Spring Meadow Nursery, 12601 120th Ave, Grand Haven, MI 49417. Email: kara@springmeadownursery.com

When working with tissue culture plants, the health of the mother plant that you initiate from is vital for strong micropropagation. There are many methods that our nursery utilizes to ensure strong, healthy stock plants. This presentation gives an overview of the steps our company takes to produce the healthiest and cleanest plants possible, and the initiation method used for micropropagation. As a company that resides in Michigan, overwintering is a crucial process that involves a strict timeline for when to prune, when to fertilize, and when to warm back up for spring. Pest and disease management includes the use of biocontrols, and different watering methods depending on the need of the plant. We will also discuss the method of initiation, including taking cuttings of the stock plants and as well as the products used in the sterilization process of initiation.

P-6

Commercial Production of Quality Microcuttings for Acclimatization. MURDOCK "RAY" GILLIS. Oglesby Plants International, Inc. Email: rayg@oglesbytc.com

Commercial-scale production of quality microcuttings that thrive upon acclimatization incorporates research with practical application in the laboratory and greenhouse settings. This presentation will focus on the techniques and processes involved in consistently producing quality microcuttings on a commercial scale under timing constraints. Emphases will be given to efficiency and maximizing output of available resources.

P-7

Engineering Microbes for Agricultural Products. BABU RAMAN. Corteva Agriscience™, Agriculture Division of DowDuPont™. Email: BRaman@dow.com

Corteva Agriscience™, Agriculture Division of DowDuPont, has a long history of advancing natural products derived from actinobacteria for agricultural applications (e.g., pesticides, fungicide). At Corteva, we undertake a multidisciplinary approach to strain development and fermentation process improvement for enabling the advancement of an active compound to a commercially viable product. The presentation will review how an integrated technological approach including mutagenesis, high-throughput screening, fermentation process optimization, and targeted genetic engineering, was applied to achieve rapid gains in productivity and to successfully scale-up the production of a novel secondary metabolite.

P-8

Converting Sugarcane into Oilcane by Metabolic Engineering. FREDY ALTPETER[1,4], Saroj Parajuli[1], Baskaran Kannan[1,4], Guangbin Luo[1,4], Ratna Karan[1], Hui Liu[2,4], Eva Garcia-Ruiz[3], Huimin Zhao[3,4], and John Shanklin[2,4]. [1]Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida, IFAS, Gainesville, FL; [2]Biosciences Dept., Brookhaven National Lab, Upton, NY; [3]Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign, Urbana, IL; and [4]DOE Center for Advanced Bioenergy and Bioproducts Innovation. Email: shanklin@bnl.gov; altpeter@ufl.edu

Metabolic engineering to divert carbon flux from sucrose to oil in a high biomass crop like sugarcane has been proposed as a strategy to boost lipid yields per acre for biodiesel production. The energy content of plant oils in the form of triacylglycerols (TAGs) is two-fold greater compared to carbohydrates. However, vegetative plant tissues do not accumulate oil to a significant amount since fatty acid synthesis in these tissues serves primarily membrane construction, in addition TAGs undergo rapid turnover. Therefore, our objectives include: 1.) increasing fatty acid synthesis by expressing a transcription activator of fatty acid biosynthetic genes, 2.) increasing TAG synthesis from diacyl-glycerol and acyl-CoA by over-expression of rate limiting enzymes, 3.) optimizing TAG storage by limiting the access of lipases to TAG storage compartments. Constitutive single or multiple gene expression/suppression cassettes were generated using Golden Gate assembly or traditional cloning and co-delivered with the selectable *nptII* expression cassette by biolistic gene transfer into sugarcane. Plants were regenerated on geneticin containing culture medium and analyzed for presence and expression of target constructs by PCR and RT-PCR, respectively. Quantitative real PCR was performed to study the level of gene expression in transgenic TAG plants in different vegetative tissues. Plants were analyzed for TAG content by Gas-Chromatography and Mass Spectrometry (GC-MS). The presented research outcome will add value to sugarcane for production of advanced biofuels. Acknowledgements: This material is based upon work supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Number DE-SC0018420.

P-9

Systematic Genetic Engineering Approaches for Improvement of Full Scale Fermentation Economics. P. WESTFALL. Zymergen, Inc. 5980 Horton St #105, Emeryville, CA 94608. Email: pjwestfall@zymergen.com

Identifying, understanding, and engineering the complex set of genetic interactions responsible for any cellular phenotype is extremely difficult, traditionally requiring years of study to generate and test hypotheses. To address this problem, Zymergen has developed a powerful data-driven platform for automated high-throughput microbial strain manipulation. The platform is designed to be flexible, reliable, and host-agnostic to allow Zymergen to build and test thousands of designed variant strains and identify the genetic perturbations improving performance. Using this platform, Zymergen takes a highly data-focused approach to biology by using in-line process data and strain phenotype data to inform our heuristically-guided search algorithms that embrace the natural complexity of biology. We have successfully applied our technologies to deliver value to clients, and will discuss three selected case studies to highlight it's different applications: discovery and development of new products, accelerating the time-to-commercialization of bioproducts, and optimizing the economics of large scale fermentations.

P-10

Applications of Gene Editing to Improve Yield Component Traits in Wheat. W. WANG[1], Q. Pan[1], Y. Chen[1], B. Tian[1], F. He[1], A. Akhunova[2], S. D. Evanega[3], L. Yan[4], H. Trick[1], and E. Akhunov[1]. [1]Department of Plant Pathology, Kansas State University, Manhattan KS; [2]Integrated Genomics Facility, Kansas State University, Manhattan KS; [3]Cornell Alliance for Science, Cornell University, Ithaca, NY; and [4]Department of Plant and Soil Sciences, Oklahoma State University, Stillwater, OK; Email: wwang0604@ksu.edu

Gene editing (GE) is a powerful tool for generating novel variation in the genomic regions affecting major agronomic traits. We use GE technology to improve wheat yield potential. For eighteen genes, the CRISPR/CAS9 constructs were designed and successfully tested for editing efficiency using the protoplast assay and next-generation sequencing. GE plants were obtained for nine and six of these genes in spring and winter wheat, respectively. The effects of GE on yield component traits in wheat were confirmed for several genes. Plants with different combinations of multiple edited genes were created by crossing GE lines with single-gene mutations. Modification of multiple genes and their homoeologs was shown can be achieved using a multiplex GE construct, and facilitated by CRISPR/Cas9 activity maintained across generations. GE of *TaGW2*, the negative regulator of grain size and weight in wheat and rice, showed that the effects of its duplicated homoeologs are dosage-dependent and cultivar-specific with 16–20% increase in grain weight and size in triple-genome mutants. The GE variants of seed size increasing gene transferred to other wheat cultivars showed similar phenotypic

effects. An approach for high-efficiency gene editing and inter-cultivar trait transfer was established using a wheat line expressing Cas9 at the high level. Our results show that GE technology can be effectively used to create novel gene variants positively affecting yield component traits, and has a great potential to accelerate the development of wheat varieties with improved yield potential.

P-11

Genome Editing: A Powerful Tool for Ideotype Breeding in Polypoid Grasses. SHUI-ZHANG FEI[1], Yang Liu[1], Paul Merrick[1], and Bing Yang[2]. [1]Iowa State University, Department of Horticulture, Ames, IA 50011 and [2]University of Missouri, Division of Plant Sciences, 1201 Rollins Street, Columbia, MO 65201. Email: sfei@iastate.edu

Tillering is an important component trait for biomass yield in switchgrass (*Panicum virgatum L.*), an herbaceous model bioenergy crop. Using CRISPR/Cas9 system, we generated T0 switchgrass mutants for a lowland tetraploid cultivar, Alamo for the *Teosinte Branched 1* (*Pvtb1*) genes whose orthologs in maize and other species have been shown to be a negative regulator in tillering. Deep sequencing of the primary mutants revealed the presence of both homozygous and heterozygous mutants and chimeric mutants are prevalent in T0. In vitro culture of nodal segments, each containing an axillary bud is an effective way of obtaining solid mutants. Sexual transmission of the CRISPR/Cas9-induced mutations was demonstrated and transgene-free switchgrass mutants were obtained. By comparing the tiller numbers of heterozygous mutantsfor *Pvtb1a*, *Pvtb1b*, *Pvtb1a-tb1b* and the wild type plants, we show that *Pvtb1* genes negatively regulate tillering in switchgrass, where *Pvtb1b* has a major effect. Transcriptome analysis showed that 831 genes were differentially expressed in the *Pvtb1*a-*tb1b* knockdown mutant compared to the wild type plant. Gene Ontology (GO) analysis revealed that downregulation of *Pvtb1* genes affects multiple biological processes. We also generated mutants for the brassinosteroid receptor gene (*BRI1*) in a turfgrass species, creeping bentgrass, cv. Penn A4 with CRISPR/Cas9. Some primary mutants exhibited expected phenotypes such as shortened internodes and reduced leaf width and length, however phenotype alterations in the CRISPR/Cas9 mutants are not as pronounced as that in RNAi mutants.

P-12

Improvement of Goldenberry and Groundcherry by CRISPR: Ripening the Potential of Underutilized Fruit Crops. JOYCE VAN ECK[1,2], Nathan Reem[1], Kerry Swartwood[1], Esperanza Shenstone[1], Brandon Williams[1], and Zachary B. Lippman[3,4]. [1]Boyce Thompson Institute, Ithaca, NY; [2]Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, NY; [3]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; and [4]Howard Hughes Medical Institute, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. Email: jv27@cornell.edu

The underutilized *Solanaceae* family members, *Physalis peruviana* (goldenberry) and *P. pruinosa* (groundcherry), produce sweet, highly nutritious fruit surrounded by a papery husk. They are underutilized as a food crop due to their "wild" nature, which precludes adoption into large-scale production. Based on our earlier findings with tomato domestication and improvement genes, we used CRISPR/Cas9 to mutate orthologues in goldenberry and groundcherry to affect plant architecture, flower production and fruit size to fast-track their improvement. Before we applied gene editing, we developed plant regeneration and transformation methodologies for both species. For plant regeneration, a Murashige and Skoog (MS) salts-based medium supplemented with 1 mg/l NAA and 2 mg/l BA was most efficient for goldenberry, whereas 2 mg/l zeatin was best for groundcherry. We used *Agrobacterium tumefaciens*-mediated delivery of gene editing constructs that contained the *nptII* selectable marker gene. Two or more guide RNAs were designed for each gene target. T0 plants were characterized, T1 generations were produced, and only Cas9-free material with the intended edits was moved forward to the next generations. Targeting the *Self-Pruning* (*SP*) gene in groundcherry resulted a significantly compact growth habit compared to the wild type. While targeting the closely related *Self-Pruning 5G* gene resulted in edited lines that were less compact than *SP*-edited lines, there was the added benefit of more flowers that resulted in a 50% higher concentration of fruits along each stem. To increase fruit size in groundcherry, we targeted the *CLAVATA1* (*CLV1*) gene. The *CLV1*-edited lines produced fruit that had a 24% increase in weight. We have already expanded our work to include targeting of additional genes that will improve a number of agronomic and consumer-related characteristics. In parallel with our fundamental research, we have engaged growers and home gardeners for input on traits such as plant growth, harvestability, and fruit flavor of the wild, non-edited germplasm to help guide our efforts.

P-13

Improving Targeted Genome Optimization in Plants. ALLAN WENCK[1] and Anna-Marie Kuijpers[2]. [1]BASF, 3500 Paramount Pkwy, Morrisville, NC 27560 and [2]BASF Agricultural Solutions Belgium NV, Technologiepark-Zwijnaarde 101, 9052 Ghent, BELGIUM. Email: allan.wenck@agro.basf-se.com, anne-marie.kuijpers@agro.basf-se.com

Genome editing – also known as new breeding technologies or targeted genome optimization – provides a new tool for

breeders and researchers to deliver new and exciting traits to the market in a potentially faster and cost-effective manner when compared to previous methods. Many traits can and have been developed using traditional introgression of alleles from exotic germplasm or random mutagenesis. However, these techniques take multiple generations to get to the desired product – which may still contain undesirable characteristics. Further, for many crops and traits, the potential return on investment does not justify the cost of development. While not a means to deliver all solutions, genome editing will contribute to the increasing set of tools required to grow and produce food and feed in a more sustainable manner. Challenges remain. Even with the advent of more readily programable editing tools, there is still a need for improved delivery methods for these tools as well as the knowledge of editable targets contributing to improved traits. This talk will focus on where we have been and where we need to go in this space.

P-14

Improving HDR-mediated Genome Editing Through Plant Protoplast Engineering. FENG ZHANG[1,2,3], Raviraj Banakar[1,2,3], Gunvant Patil[2,3,4], Xiaojun Kang[1,2,3], and Trevor Weiss[1,2,3]. [1]Department of Plant and Microbial Genomics, University of Minnesota, Minneapolis, MN; [2]Center for Precision Plant Genomics, University of Minnesota, Minneapolis, MN; [3]Center for Genome Engineering, University of Minnesota, Minneapolis, MN; and [4]Department of Agronomy and Plant Genetics, University of Minnesota. Email: zhangumn@umn.edu

A critical challenge we face today is to produce sufficient food and plant-derived products for a growing population. This challenge is further complicated by an ever-changing and unstable climate. In the past 20 years, a substantial effort has been made to sequence, assemble and characterize the genomes of more than thousands of plant species and landraces. This effort led to significant advances in understanding the basic mechanisms of plant growth, development, and interaction with the environment. The knowledge gleaned from these studies has paved the way to design better plant varieties by modifying, editing and rewriting their genetic code. My research has been focused on developing highly efficient genome editing and synthetic biology approaches to improve plant productivity and quality. In this talk, new approaches will be presented by combining genome editing and protoplast engineering technologies to improve precise genome editing in plant species.

P-15

Plant Protoplast Automation: Production, Transfection and Screening. C. NEAL STEWART, JR. Department of Plant Sciences and the Center for Agricultural Synthetic Biology, University of Tennessee, Knoxville, TN. Email nealstewart@utk.edu

In the Center of Agricultural Synthetic Biology (CASB) at the University of Tennessee, one of its specific goals is to increase the iterations of design-build-test cycles in plants order to accelerate plant biotechnology discoveries and applications. To date, most of our focus as been on protoplasts, plants' answer to single cell biology. To that end, we have sequntially installed two robotic systems in CASB that focus on the automated production of protoplasts, their transfection, and then characterization of transfections using a 96-well plate format. Our first application is the design-build-test of a library of over 2000 synthetic promoters for use in plants and the screening the function of the promoters in different crop species' protoplasts. These protoplasts were developed to represent relevant tissues of interest and against various inducers. I will discuss what we've learned so far in our attempts to make high throughput methods in plant biotechnology as well as specific findings with regard to synthetic plant promoter design and function.

P-18

LED Lighting for Indoor Plant Propagation. E. RUNKLE. Department of Horticulture, Michigan State University, 1066 Bogue Street, East Lansing, MI 48824. Email: runkleer@msu.edu

Light-emitting diodes (LEDs) are increasingly suitable for plant lighting applications, including indoors for plant propagation. Compared with conventional lighting fixtures (e.g., fluorescent tubes), LEDs are more efficient, emit less heat and thus reduce air conditioning costs, and have a longer lifetime, but they are also more expensive to purchase. The use of LEDs allows one to deliver a light spectrum to elicit desirable plant growth attributes. We have been investigating the roles of blue (B, 400–500 nm), green (500–600 nm), red (600–700 nm) and far red (FR, 700–800 nm) radiation on growth of young specialty plants since 2011. While each radiation waveband elicits general growth responses, they also interact with each other, as well as with the total photon flux density. For example, FR radiation usually promotes extension growth, creating longer internodes and larger leaves while B radiation does the opposite. Generally, B attenuates the effects of FR and thus, these wavebands should be considered simultaneously when developing a lighting spectrum for indoor plant application. This presentation will focus on the effects of light quality on growth of young plants, as well as review important considerations when selecting an indoor LED lighting system.

P-20

Application of Biotechnology to Develop Huanglongbing Resistant/Tolerant Citrus Scions. ED STOVER[1], Goutam Gupta[2], David Hall[1], Guixia Hao[1], Qingchun Shi[1], Robert Shatters, Jr.[1], Shujian Zhang[1], Joseph Krystel[1], John Hartung[3], and William Belknap[4]. [1]USDA/ARS, 2001 S Rock Rd., Ft. Pierce, FL, 34945; [2]New Mexico Consortium, 100 Entrada Dr., Los Alamos, NM, 87544; [3]USDA/ARS, 10300 Baltimore Ave. Bldg. 004, Rm. 118, BARC-West, Beltsville, MD, 20705; and [4]USDA/ARS, 800 Buchanan St., Albany, CA 94710. Email: ed.stover@ars.usda.gov

The disease huanglongbing (HLB) has devastated Florida citrus and threatens production around the world. The associated pathogen *Candidatus* Liberibacter asiaticus (CLas), is phloem-limited in citrus, and is vectored by the Asian citrus psyllid. HLB-resistant or -tolerant cultivars will be essential to sustain the Florida citrus industry, with solutions needed in the short and long term. Potentially useful tolerance to HLB is present in cultivated citrus and greater resistance is apparent in more distant members of the gene pool. However, the Florida industry is based largely on sweet orange: a group of near-isogenic cultivars. Due to the focus of our industry and consumers on specific cultivars, the solutions through biotechnology are particularly desirable. Our program collaborates broadly to leverage methodologies and expertise for more rapid practical solutions. The most successful transgenics to date are expressers of: a modified plant thionin; chimeras derived from the citrus proteome with membrane binding and lytic peptides connected by a linker; and ScFv directed at a CLas membrane protein or a protein secreted by CLas. Several transgenics are showing in-planta reductions of CLas titer by 100–800 fold and are now in field trials with the promise of greater HLB control than is seen in conventional material. Many of our most promising transgenics express constructs optimized using in-silico modeling of peptide association with gram-negative bacterial membranes. Due to the long time-period to assess effects of transgenics on CLas in-planta, we have developed several in-vitro assays for more rapid assessment of potential CLas-killer transgenes, and also methods to assess transgenic suppression of CLas and ACP in detached leaves fed on by CLas+ ACP. A major advance in developing transgenics targeting HLB, was the identification of a phloem-specific promoter from the most highly expressed gene in citrus phloem. Where appropriate targets can be identified, genes are being knocked-down using RNAi (from hairpin expression) and/or CRISPR.

P-21

Recombinase Technology for Transgene Manipulation. Min Shao, Ron Chan, Roger Thilmony, and JAMES THOMSON. USDA-ARS-CIG, 800 Buchanan Street, Albany CA 94710. Email: James.Thomson@ARS.USDA.GOV

Research in this lab addresses the need for publicly available recombinase technology. Data for a practical combinatorial approach for transgene integration and DNA stacking combined with subsequent excision of the selectable marker will be presented. Technology is rapidly expanding the way genetic engineering can be accomplished, the questions asked and the applications that can be attained. With possibilities in genetic manipulation the interest in metabolic engineering requires the addition of many genes into the host cell for proper expression has been renewed. However, the combination of required genes and control elements is often empirically determined and thus requires multiple rounds DNA manipulation. While genetic engineering can be accomplished by nuclease targeting and host cell mediated homologous recombination, the efficiencies for precisely inserting large DNA sequences are low and tend to contain errors. Recombinase-mediated engineering offers a solution for sequential rounds of DNA stacking at high rates of integration and low (to non-existent) levels of error introduction. Another issue is the limitation of available selectable markers for multiple rounds of engineering. Recombinase technology, when properly designed allows the removal of selection marker genes from the system while integrating the next gene(s) of interest. This in turn allows marker gene recycling between successive rounds of DNA insertion. Recombinase-mediated engineering provides a favorable direction for enhancing the precision of biotechnological approaches to genome modification.

P-22

Generating Disease Resistant Citrus Varieties Using the CRISPR Technology. NIAN WANG. Citrus Research and Education Center, Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, University of Florida, Lake Alfred, FL 33850. Email: nianwang@ufl.edu

Citrus is one of the most important fruit crops worldwide. However, citrus production in the USA is facing an unprecedented challenge due to Huanglongbing (HLB, also known as citrus greening). HLB is the most destructive disease of citrus and causes tremendous damage to citrus industry worldwide. HLB, caused by the uncultivated Gram-negative bacterium *Candidatus* Liberibacter asiaticus (Las), is endemic in Florida and is spreading to other citrus producing states of USA including California and Texas. Las resides inside the phloem and is vectored by psyllids. All commercial citrus varieties are susceptible to HLB. Generating disease resistant citrus varieties is an efficient and sustainable long-term solution to HLB. However, the traditional approach of breeding

for resistant plants for citrus is difficult and has not provided a timely solution to control HLB due to the long juvenility, large tree size, and prevalence of polyembryonic seeds in commercial varieties. Here I will present our current progress in understanding the virulence mechanism of Las, identification of putative HLB susceptibility (S) genes, and application of CRISPR in citrus improvement against HLB by editing the HLB S genes.

P-23

Deciphering the Genetic Mechanisms Underlying Somatic Embryogenesis and Plant Regeneration in Maize. HEIDI F. KAEPPLER[1,2], Frank McFarland[1,2], Stella Salvo[3,] Alvar Carlson[4], Jason Cook[5], Shawn Kaeppler[1,2], and Candice Hirsch[6]. [1]Department of Agronomy, University of Wisconsin, Madison, WI; [2]Wisconsin Crop Innovation Center, University of Wisconsin, Middleton, WI; [3]Bayer Inc., St. Louis, MO; [4]Vestaron Inc., Kalamazoo, MI; [5]Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT; and [6]Department of Agronomy and Plant Genetics, University of Minnesota, St Paul, MN. Email: hfkaeppl@wisc.edu

Tissue cultures that produce somatic embryos (embryogenic cultures) capable of regenerating into plants are critical components in crop biological and genomic research, clonal propagation systems, and transformation/editing-based crop improvement applications. Germplasm utilized in those applications are significantly limited, however, due to genotype-dependent culture response in most species. Deciphering the genetic mechanisms controlling embryogenic, regenerable culture response will enhance basic understanding of processes involved in somatic embryogenesis, and aid in the development of improved clonal propagation and crop bioengineering systems. Research has been conducted by our group and others to determine the genetic and epigenetic factors underlying embryogenic, regenerable culture response in the globally important crop, maize. In our studies, we utilized RNA based sequencing (RNA-seq) to characterize the transcriptome of immature embryo explants of the embryogenic, regenerable maize genotype, A188, at various time points after culture initiation. We also conducted genetic fine mapping and candidate gene/element identification based on molecular and phenotypic analysis of inbred backcross lines derived from the cross of maize lines A188 and B73. Following transcriptome analysis a model was proposed for the coordinated expression of somatic embryogenesis-related genes in embryogenic culture response. Fine mapping analysis led to the identification of a 3053 kb region on maize chromosome 3 (containing nearly 90 gene models) significantly associated with embryogenic, regenerable culture response. Comparative sequence and expression analysis between A188 and B73 sequences within the mapped region aided in identification of a small subset of candidate genes for investigation. Additional expression analysis and high resolution fine mapping is underway to further dissect the genomic segment to identify genomic sequences for characterization and confirmation of effect on embryogenic culture response and plant regeneration.

P-25

A Rapid Sunflower Transformation Method: Applying New Tools to an Old Method. GEORGE J. HOERSTER[1], Keith Lowe[1], Hyeon-Je Cho[1], Jeff Berringer[2], Bill Gordon-Kamm[1], and Todd Jones[1]. [1]Corteva Agriscience, 8305 NW 62nd Ave, Johnston, IA 50131 and [2]Inari Agriculture, Inc. West Lafayette, IN. Email: george.hoerster@pioneer.com

Since the generation of the first sunflower transgenics using hypocotyl derived callus in 1989, many other transformation methods have been tried using explants such as cotyledons, immature embryos, and meristems. Meristems are an ideal target tissue for reducing genotype dependence because the regeneration steps require minimal tissue culture and de novo organogenesis or embryogenesis is not required. Most meristem transformation methods are based off the 1990 Schrammeijer protocol. However, sunflower meristem transformation frequencies using variations of this method have historically been low and germline transmission to progeny rare, due to the chimeric nature of the T0 plants. By applying new agro strains and selection technology to this old method we can obtain non-chimeric plants that can be transferred to soil in as little as 3 weeks. The role of morphogenic genes to improve transformation will also be discussed.

P-27

Why Transgenics Are Important in Understanding Meristem Regulation. DAVID JACKSON. Cold Spring Harbor Laboratory. Email: jacksond@cshl.edu

Plants grow and develop in remarkably challenging and changing conditions, and are critical for our food supply and all life on earth. Plant growth depends upon meristems, pools of stem cells that are maintained in a number of ways, including a negative feedback loop between the *CLAVATA* pathway and a homeodomain transcription factor, *WUSCHEL*. We are developing tools to understand this and other developmental pathways, to allow analysis of maize at a systems level. We have generated over 100 stable, natively expressed, fluorescent protein (FP) fusion lines that mark most of the common subcellular compartments in maize. These lines are publicly available via our project website (http://maize.jcvi.org/cellgenomics) and have been used by the maize research community for developmental, physiological and functional

studies. We also developed an LhG4 two-component transactivation system to drive cell, tissue and organ-specific expression. Selected promoters activate expression of the LhG4 transcription factor, which in turn will transactivate genes of interest driven by the pOp promoter in responder lines. Driver constructs have been produced to drive expression in shoot and inflorescence meristems, leaves, embryo or roots, using tissue-specific promoters. Responder constructs have been made to test the role of specific genes in maize meristem development, including FLOWERING LOCUS T like *Zea mays* CENTRORADIALIS 8 (ZCN8), *Zea mays* FON2-LIKE CLE PROTEIN1 (ZmFCP1), *Zea mays* CLV3/ESR-related7 (*Zm*CLE7) and MALE STERILE CONVERTED ANTHER 1 (MSCA1), a maize glutaredoxin involved in the establishment of phyllotaxy. Transactivation has been successfully tested using this system: for example, the ZCN8 responder driven by the constitutive promoter driver from maize ELONGATION FACTOR 1 alpha phenocopies the early flowering phenotype of ZCN8 overexpression. This system has been used to test new hypotheses in meristem regulation by differentiated cells; expressing *Zm*FCP1 using a leaf primordia-specific driver line strongly inhibited meristem growth, confirming this feedback regulation. Additionally, we have developed root and shoot tissue-specific marker lines and are using them to profile gene expression in specific developmental domains using Fluorescence Assisted Cell Sorting (FACS) protocols, in collaboration with the Birnbaum Lab (NYU). These resources, and their use in understanding meristem development in maize, will be discussed.

P-28

miRNA Regulation of Early Embryogenesis in *Arabidopsis thaliana.* STEWART GILLMOR. Langebio-CINVESTAV, Irapuato, MEXICO. Email: stewart.gillmor@cinvestav.mx

Early embryogenesis is one of the most amazing processes in biology. Fertilization of the egg and sperm produces the zygote, a single cell capable of generating all the different tissue types of the adult organism. In *Arabidopsis thaliana*, all major tissue types of the adult plant are generated in the first 5 days after fertilization. By studying embryogenesis, we can learn about fundamental processes that determine cell identity, with the advantage that the small number of cells in early embryos makes tracking cell fate and morphology relatively simple. miRNAs are small (20-24 bp) regulatory RNAs that target mRNAs for destruction or translational repression. Using next generation RNA sequencing, we have discovered about 40 miRNAs that are expressed in the first few days of embryogenesis in Arabidopsis. These include highly conserved miRNAs, and well as novel miRNAs whose function has never been studied. We have analyzed the functional importance of miRNAs using mutants in miRNA biogenesis pathways, as well as loss of function of individual miRNA genes. These experiments have demonstrated that miRNAs are required for determining the polarity and first asymmetric of the zygote. Current work in my laboratory is focused on miR156, one of the most highly conserved miRNAs in plants. In my talk, I will present recent results on the function of miR156, as well as previously unstudied miRNAs, in early embryogenesis of Arabidopsis.

P-29

Improved Maize Leaf Transformation Using Babyboom and Wuschel. N. WANG, E. Wu, W. Zhu, W. Hua, M. Arling, N. Sardesai, A, Anand, L. Ryan, K. Lowe, and B. Gordon-Kamm. CORTEVA Agriculture Division of DowDuPont, 8305 NW 62nd Ave, Johnston IA 50131. Email: ning.wang@pioneer.com

For maize and a number of other crops, immature embryos have been the primary transformation target for almost 20 years. However, producing a consistent, continuous supply of immature embryos for many cereal crops is both labor- and space-intensive. As an alternative, we have developed an Agrobacterium-based transformation protocol for leaf-base tissue from maize seedlings. When tissue from wild-type maize leaf-base segments were transformed using a T-DNA containing a constitutively-expressed ZsGREEN + HRA selectable marker, no transgenic sectors were typically recovered. However, when a strongly-expressed maize Babyboom (BBM) gene plus a weakly-expressed maize Wuschel (WUS2) gene were included in the T-DNA, growing transgenic calli were obtained and vigorous, fertile T0 plants were readily produced. By using this combination of morphogenic gene expression cassettes (BBM under the control of the NOS promoter, WUS2 under the control of the maize Ubiquitin-1 promoter), we report successful transformation of several Pioneer maize inbreds from different heterotic groups. We produced hundreds of regenerated transgenic plants from PHH5G, PHI-Flint and PH85E (each plant an independent transgenic event) that were grown to maturity in the greenhouse. Further improvements to the method have increased the abundance and growth rate of somatic embryos that develop from transformed leaf segments, with somatic embryo maturation and regeneration starting as early as 14–16 days after Agrobacterium infection. Using this method, leaf base transformation has been successfully demonstrated in numerous cereals including pearl millet, sorghum, switchgrass, rice, and the maize public inbred W22.

P-30

Gene Editing Through *De Novo* Induction of Meristems on Seedlings. RYAN A. NASTI, Michael F. Maher, Colby G. Starker, and Daniel F. Voytas. Department of Genetics, Cell Biology and Development; Center for Genome Engineering; and Center for Precison Plant Genomics, University of Minnesota, St. Paul, MN 55108. Email: nasti002@umn.edu

Plant gene editing begins by delivering gene editing (GE) reagents to somatic plant cells in culture, using either the gene-transferring bacterium, *Agrobacterium tumefaciens,* or physical means such as particle bombardment. Edited cells are then induced to differentiate into whole plants by exposure to various combinations of plant hormones, namely auxin and cytokinin. Regeneration of plants through tissue culture is not ideal for large-scale, high-throughput production of gene edited plants. The process is often inefficient, requires considerable time, works with limited genotypes, and causes unintended changes to the genome and epigenome. Methods that circumvent these limitations would greatly enhance the ability to create edited plant lines. By editing the stem cells within plant meristems, all tissues derived from the meristem would be expected to contain GE events of interest, leading to vertical transmission. However, direct modification of existing meristematic tissue has proven challenging since it is a highly regulated tissue type that has been historically recalcitrant to genetic modification. Combinations of developmental regulators like WUSCHEL (WUS) and SHOOT MERISTEMLESS (STM), among others, have been implicated in the patterning and formation of shoot meristems. Co-opting these types of patterning regulators, a new meristem can be generated from transformed somatic tissues. Using our method of fast treated *Agrobacterium* co-culture (Fast-TrACC), various combinations of WUS, STM and ISOPENTENYL TRANSFERASE (IPT) were found to facilitate *de novo* meristem generation in the model species *Nicotiana benthamiana*. Combining these developmental regulators with GE reagents provides the potential to establish an edited shoot directly from somatic tissue in order to avoid tissue culture. In this regard, *de novo* meristem induction promises to alleviate the tissue culture bottleneck, allowing for larger collections of genetically engineered germplasm to be assembled, analyzed and applied to solve agricultural problems.

P-31

Symbiotic Seed Germination of Orchids: Techniques and Perspectives. L. W. ZETTLER. Department of Biology, Illinois College, 1101 W College Avenue, Jacksonville, IL 62650. Email: lwzettle@ic.edu

Current estimates project that by 2050 ca. 40% of all plant species will become extinct. Orchids have the unparalleled distinction of being the most diverse plant family on earth (25,000+ species), but also the most vulnerable. Part of their vulnerability stems from the family's susceptibility to acute environmental changes exacerbated by climate change, and their extreme dependence on other organisms (e.g., insect pollinators, mycorrhizal fungi) to complete their life cycle. About two-thirds of the world's orchids live as epiphytes and lithophytes in tropical latitudes where deforestation continues at a rapid pace further adding to their demise. Taken together, orchids face a survival crisis of epic proportions, and to conserve these remarkable plants this century will require scientists, enthusiasts, land managers, and growers to coordinate their efforts. Since the 1920s, several effective techniques have been developed to propagate orchids from seed *in vitro* leading to seedling reintroduction and establishment in natural areas. For tropical epiphytes, especially the appealing species that cater to horticulturalists and hobbyists, growing orchids from seed on carbon-based media (= asymbiotic germination) is typically practiced. In recent years, however, the use of naturally-occurring mycorrhizal fungi (= symbiotic germination) to propagate these plants has received considerable interest on a global level for tropical epiphytes, not just hardy (temperate terrestrial) species. Compared to asymbiotic techniques, symbiotic germination often results in rapid germination and development in vitro leading to high seedling survival *ex vitro*, especially when an effective fungus is utilized. These fungi often consist of free-living saprophytic basidiomycetes in the *Rhizoctonia* complex (*Tulasnella*, *Ceratobasidium*) that are easy to work with. This presentation will highlight some of the symbiotic techniques that were successfully applied during the past three decades for epiphytes and terrestrials native to North America and abroad (e.g., Madagascar). Among the topics presented will include fungal isolation, provisional identification, seed pre-treatment (e.g., photoperiod, cold-moist stratification), media, and seedling reintroduction. Ethical concerns (e.g., using fungi from distant geographical regions) will also be discussed.

P-32

A Multi-species Evaluation of Factors Affecting Growth and Phenotype *In Vitro.* VALERIE C. PENCE[1], Randall P. Niedz[2], Linda R. Finke[1], Jessica M. Wedig[1,3], Allan R. Pinhas[3], and Robert T. Voorhees[3]. [1]Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220; [2]USDA-ARS-U.S. Horticultural Research Laboratory, 2001 South Rock Road, Ft. Pierce, FL 34945; and [3]Department of Chemistry, University of Cincinnati, PO Box 210172, Cincinnati, OH 45221–0172. Email: valerie.pence@cincinnatizoo.org

Tissue cultures are miniature ecological systems, which can be used to study the responses of tissues to environmental factors. Both the quantity and quality of growth of tissues *in vitro* are influenced by the multi-component medium, as well as the physical properties of the medium and the environment. To examine the response of different species to common *in vitro* environments, a DOE screening experiment was run with multiple species from the CREW In Vitro Collection. Factors tested were chosen from those that appear to affect the physiological disorder of hyperhydricity *in vitro*, while responses measured were those that often differ in hyperhydric and more normal tissues. Venting, which can reduce humidity, had significant effects on increasing dry weight, reducing hyperhydricity, and reducing overall growth and the height of shoots in most species. Measurements of the humidity in culture tubes revealed marked differences in the humidity of vented and nonvented tubes, e.g. 71% vs. 100%, respectively. Gelling agent was also a strong driver, with gellan gum, in contrast to agar, decreasing dry weight, increasing hyperhydricity, and increasing growth and the height of shoots in many species. A test using cellulose plugs showed 24 hr water uptake from agar was 57% of that from gel, suggesting greater availability of water from gel at the concentrations used. Two of the DOE treatments (9 and 10) were compared across species. Treatment 10 contained lower levels of NH_4^+ and BAP, was vented and exposed to higher light than Treatment 9. Species fell into three groups: 1) those that grew well on 9 but not on 10 (8 species); 2) those that grew on both (5 species); and 3) those that grew well on 10 and not on 9 (3 species). Those in group 3 were all desert species, while those in group 1 were all adapted to humid environments. Natural adaptations of species to moisture and water availability appear to play a strong role in their responses *in vitro*. *In vitro* systems hold the potential for providing insights into the physiology of species differences, rarity, and potential responses to a changing climate.

P-34

Development of Tissue Culture Methods for Marijuana (*Cannabis sativa* L.) Strains to Achieve *Agrobacterium*-mediated Transformation to Enhance Disease Resistance. Z. K. PUNJA, J. Holmes, D. Collyer, and S. Lung. Department of Biological Sciences, Simon Fraser University, Burnaby, BC, CANADA. Email: punja@sfu.ca

Meristems and nodal segments with axillary buds of five strains of *Cannabis sativa* L. were surface-sterilized and placed on Murashige & Skoog (MS) medium containing Gamborg B5 vitamins, sucrose (20 g/L), activated charcoal (1 g/L) and phytagel (3 g/L) (MS-C) supplemented with different growth regulators to promote shoot development. Addition of 1 uM thidiazuron (TDZ) and 0.5 uM napthaleneacetic acid (NAA) were found to induce greater shoot development from meristems and nodal segments compared to gibberellic acid and 6-benzylaminopurine, or TDZ alone. The mean height of shoots after 4–6 weeks at 25 ± 2°C and at 102 umoles m^{-2} s^{-1} light intensity was significantly ($P = 0.05$) different between strains. Elongated shoots were transferred to rooting medium (MS-C containing indole-3-butyric acid), resulting in 76% rooting. Plantlets were acclimatized in rockwool or peat plugs, with a survival rate of >50%. Callus formation from petiole and leaf segments was achieved on MS medium with TDZ and NAA at 1.0 and 0.5 uM. Further callus growth was achieved on MS with 2,4-D at 1.0 uM. Transformation of *C. sativa* was conducted with *Agrobacterium tumefaciens* strain GV3101 containing a construct with the Arabidopsis NPR1 gene (non-expressor of pathogenesis-related genes) under control of the CaMV 35S promoter and with phosphinothricin as a selection agent (through expression of the phospinothricin acetyltransferase (PAT) gene). Meristems, axillary buds, and leaf and petiole explants were incubated with *Agrobacterium* for 5–10 min, co-cultivated in the dark for 2 days, and placed on selection medium containing PPT at 1 mg/L and 200 mg/L timentin. Surviving tissues were transferred to MS medium with 2,4-D at 1.0 uM and 5 mg/l PPT to allow putatively transformed cells to continue growth. Results obtained to date will be discussed.

P-35

Cataloging Existing Variation and Rebuilding Better Cannabis Genomes for New Markets. J. K. MCKAY and R. S. Fletcher, New West Genetics, Inc. Email: jkmckay@newwestgenetics.com

With the end of prohibition, Cannabis cultivation is coming out of the closet. 2018 saw major changes in Federal law in many large countries, allowing for large scale production and marketing of Cannabis. In the US, the 2018 Farm Bill/Hemp Farming Act removed legal barriers for hemp — defined as <0.3% THC — launching a fast-growing supply chain for grain, fiber and flower. The new bottleneck is genetics, due to a lack of cultivars bred to optimize sustainable production and meet the demands of new and emerging markets. In addition, production agronomy has yet to be standardized for hemp, which has important implications to defining selection targets in a breeding program. I will discuss the breeding program of New West Genetics, a company that is producing novel, proprietary, certified hemp cultivars, optimized for large scale, mechanized production. The current state of production agronomy and proposed improvements will also be addressed.

P-36

Polyploidization for the Genetic Improvement of *Cannabis sativa*. JESSICA L. PARSONS [1,2], Katya Boudko[1], Shelley Hepworth[2], Sara Martin[3], and Tracey James[3]. [1]Canopy Growth Corporation, Smiths Falls, CANADA; [2]Biology Department, Carleton University, Ottawa, CANADA; and [3]Department of Agriculture and Agri-Food, Government of Canada, Ottawa, CANADA. Email: jessica.parsons@tweed.com

In light of increasing demand for Cannabis products, polyploidization is under investigation as a method for developing improved Cannabis varieties. Although this method has previously been used in hemp-type Cannabis, it has never been applied to drug-type strains. Here, we describe the production of tetraploid drug-type Cannabis lines using the mitotic spindle inhibitor oryzalin, and test whether this transformation alters yield or the profile of important secondary metabolites. Optimal oryzalin treatment varies between genotypes but is generally within the range of 20–40 μM oryzalin for 24–30 hours. Tetraploid clones of a single strain were assessed for changes in morphology and chemical profile compared to diploid control plants. Tetraploid fan leaves were larger, with stomata about 30% larger and about half as dense compared to diploids. Glandular trichome density was increased by approximately 40% on tetraploid sugar leaves, which was coupled with significant changes in the terpene profile. There was no significant increase in dried bud yield or THC content, however a modest 9% increase in CBD was noted in the buds. Further investigations are underway to confirm the effect of polyploidization across multiple genotypes. This research lays important groundwork for the breeding and development of new Cannabis strains with diverse chemical profiles, of benefit to medical and recreational users.

P-37

The Role of Genetics in Large-scale Hemp Production. JONATHAN VAUGHT. Front Range Biosciences, Boulder, CO. Email: frontrange@n6a.com

Modern-day large-scale agronomic crops have benefitted from decades of professional breeding programs, where years of research and refinement have resulted in streamlined production systems. The success of high-value crop cultivation, like industrial hemp, hinges on similar systems to allow for year-over-year improvements in disease- and pest-resistance, increased yields, and other desired agronomic traits. As demand for industrial hemp-derived ingredients and products increases, breeders are looking to target these desirable traits to increase efficiency throughout the production process, with consideration toward the environment and sustainable agriculture practices. Genomics research and proper plant breeding practices for a nascent industry like hemp is especially critical to the development of plant varieties with commercially valuable traits that support large-scale production of consistent crops. Despite the newly minted Farm Bill, only a few certified hemp varieties are currently available, indicating that industrial hemp is an emergent industry with room for improvement. As CEO and Co-Founder of agricultural biotech company Front Range Biosciences, Dr. Jonathan Vaught can discuss how embracing genomics-driven breeding technologies in agriculture will allow hemp growers and beyond to produce reliable, consistent, and profitable crops at scale. Dr. Vaught will draw on knowledge gained from his company's industry-leading breeding platform, FRB's support and collaboration of hemp genome research at UC Davis, and its recently opened breeding program branch at CRAG-IRTA.

P-38

Simultaneously Genome Editing with Haploid Induction in Crops. Timothy Kelliher[1], Dakota Starr[1], Xiujuan Su[1], Guozhu Tang[1], Zhongying Chen[1], Julie Green[1], Erin Burch[1], Jamie McCuiston[1], Weining Gu[1], Yuejin Sun[1], Tim Strebe[1], James Roberts[1], JIANPING XU[2], and Qiudeng Que[1]. [1]Syngenta Plant Protection LLC, 9 Davis Drive, Research Triangle Park, NC 27709 and [2]Syngenta Biotechnology (China), 25 Life Science Park Rd., Changping District, Beijing, CHINA 102206. Email: jianping.xu@syngenta.com

The sequence-specific CRISPR/CAS system enables efficient and targeted genome editing in crops. Precise genome editing is revolutionizing the plant breeding process and is regarded as the new generation of breeding tool enabling faster deployment of desired traits in commercially important varieties. Currently, genome editing is typically achieved by delivering editing reagents either in protein/mRNA or DNA format to plant cell mediated by protoplast, biolistic or *Agrobacterium* transformation techniques. However, many important germplasms are recalcitrant to plant transformation, fast deployment of important traits to broad range of commercially important varieties is very challenging. Here we present a novel method of Simultaneous Haploid Induction and Editing (Hi-Edit) for editing plant genome which can greatly shorten timeline to deploy traits in commercially important varieties without germplasm barrier. In plant breeding, the doubled haploids generated by haploid induction line are highly desirable, resulting in homozygous pure inbred line in one generation. We have demonstrated in corn and wheat, the haploid inducer line pollen carrying the editing machinery, i.e. Cas9/gRNA, can deliver the editing machinery into the egg cells of target female donor lines, and simultaneously edit the target gene and induce haploid formation. We have also shown that Hi-Edit is highly efficient in Arabidopsis which

has a different haploid induction mechanism based on CenH3 mutation resulting in elimination of the female nuclear genome and the derived haploids contain genome from the male donor line. The method demonstrated using the pollen from the haploid inducer can deploy the editing reagents to many elite varieties same time, and pure edited elite lines can be obtained by doubled haploid edited by the donor pollen.

P-39

Advances in CRISPR Cas12a and Cas12f Portfolio of Nucleases and Optimization Strategies for High Editing Efficiencies in Plant and Microbial Genomes. M. OUFATTOLE. Benson Hill Biosystems, 1100 Corporate Square Drive, St. Louis, MO 63132. Email: moufattole@bensonhillbio.com

The advent of precision genome editing technology has ushered an era of hope that promises to help solve centuries-long challenges in agriculture, which have constrained the progress in crop improvement. Despite an unprecedent progress in the discovery and application of Genome editing tools over the last decade, many hurdles continue to stand in the way of broadening its applicability to a large number of crops and traits of commercial value; chief among them are the inefficiencies in editing activity of the existing technology in a vast range of commercially relevant crops; thus the need for continued effort to uncover novel, potentially more efficient tools, while driving the optimization process to improve efficiency of existing ones. At Benson Hill Biosystems, we have uncovered a large portfolio of Type V CRISPR nucleases and validated their editing activity in a broad range of living systems. This portfolio includes members of CRISPR-Cas12a (or Cpf1) as well as a novel group of nucleases classified as CRISPR-Cas12f, also known as Cms1. Multiple plant and microbial-based assays have been developed and have helped drive step-change improvements in editing efficiencies. A review of how these systems are used to predict editing performance in a commercial product development pipeline will be discussed.

P-40

Gene Editing Innovation at Pairwise for Consumer and Row Crops. MIKE MANN and AARON HUMMEL. Pairwise Plants, 110 TW Alexander Dr., Durham, NC. Email: ahummel@pairwise.com

Pairwise (www.pairwise.com) is harnessing plant diversity to make healthy food more available, affordable, convenient and sustainable. To fulfill this mission, since March 2018 (https://www.businesswire.com/news/home/20180320005790/en/Pairwise-Raises-25M-Series-Financing-Accelerate-Crop) we have assembled a world class portfolio of CRISPR technology licenses, and a state-of-the-art R&D effort focused on delivering differentiated products to the produce aisle as well as productivity traits to the farm. We have established high efficiency gene editing and transformation capabilities in several species, and a platform for enzyme technology improvements to drive continuous innovation in editing options. We will describe our current activities and collaborations, our vision for the future of gene editing for the consumer, as well as important research capabilities Pairwise has established.