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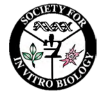

2022 IN VITRO BIOLOGY MEETING ABSTRACT ISSUE

Plant Symposia and Workshops

P-1A

Engineering the "Engineers": Efforts in the Biolistic Gun Improvement and *Agrobacterium* Genome Engineering. KAN WANG. Iowa State University, Ames, IA. Email: kanwang@iastate.edu

The biolistic gun and *Agrobacterium tumefaciens* are two major systems used for the efficient delivery of genetic materials into plant tissues. We developed a methodology to improve the consistency of biolistic delivery results by using a double-barrel device and a cell-counting software. The double-barrel device enables a strategy of incorporating an internal control into each sample, which significantly decreases the variance of the results. The cell-counting software further reduces errors and increases throughput. The utility of this new platform is applied to test the efficacy of multiple gRNAs for CRISPR-Cas9-mediated gene editing. The novel combination of the bombardment device and analysis method allows simultaneous comparison and optimization of parameters in the biolistic delivery. *Agrobacterium tumefaciens* is a soil bacterium that has been employed for plant genetic transformation in the past 40 years. However, our knowledge about this bacterium and our ability to improve the strain is still limited. Here we report the implementation, for the first time, of a CRISPR RNA-guided integrase system for *Agrobacterium* genome engineering. In this work, we demonstrate that INsertion of Transposable Elements by Guide RNA–Assisted TargEting (INTEGRATE) can efficiently generate DNA insertions to generate targeted gene knockouts. In addition, in conjunction with Cre-loxP recombination system, we achieved precise deletions of large DNA fragments. This work provides new genetic engineering strategies for *Agrobacterium* species and their gene functional analyses.

P-2

Developing Highly Efficient Base Editing and Prime Editing Tools in Plants. Simon Sretenovic[1] and YIPING QI[1, 2]. [1]Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD 20742 and [2]Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, MD 20850. Email: yiping@umd.edu

The development of CRISPR-Cas systems has sparked a genome editing revolution in plant genetics and breeding. These sequence specific RNA-guided nucleases can induce DNA double-strand breaks (DSBs), resulting in mutations by imprecise non-homologous end joining (NHEJ) repair or precise DNA sequence replacement by homology-directed repair (HDR). However, HDR is highly inefficient in many plant species, which limits its application for precise genome editing in plants. To fill the vital gap in precision editing, base editing technologies have been recently developed. These base editors, mainly based on Cas9 nickases, can install precise C-to-T and A-to-G transition changes. These cytosine base editors (CBEs) and adenine base editors (ABEs) are highly efficient in plants. However, they can only convert four types of base changes and their editing outcomes cannot be precisely controlled at base precision within the editing window. Prime editors (PEs) largely overcome the limitations of base editors as they can install all 12 possible transition and transversion mutations as well as small indels. Here, I will provide a timely overview of the base editors and prime editors for efficient precise genome editing in plants.

P-3

Gene Editing in Plants Using Plant Viruses. S. P. DINESH-KUMAR[1,2], U. Nagalakshmi[2], N. Meier[1,2], J-Y. Liu[1,2], and D. F. Voytas[3,4,5]. [1]Department of Plant Biology, College of Biological Sciences, University of California, Davis, CA 95616; [2]The Genome Center, College of Biological Sciences, University of California, Davis, CA 95616; [3]Department of Genetics, Cell Biology and Development, University of Minnesota, St. Paul, MN 55455; [4]Center for Precision Plant Genomics, University of Minnesota, St. Paul, MN 55455; and [5]Center for Genome Engineering, University of Minnesota, St. Paul, MN 55455. Email: spdineshkumar@ucdavis.edu

The CRISPR/Cas9 has been used for efficient targeted genome editing in various organisms including plants. However, most published reports require generation of transgenic plants to deliver Cas9 and synthetic guide RNA (gRNA). Production of transgenic plants is time consuming, labor-intensive, and some plants are recalcitrant to transformation. Therefore, efficient delivery of Cas9 and gRNA into plant cells is required for rapid discovery of gene function that circumvents the requirement of transformation or tissue culture-based methods. I will discuss our efforts towards engineering RNA viruses to deliver gene editing reagents in plants.

P-4

Transforming the Food System with the Inari SEEDesign™ Platform. H. BERGES, INARI Agriculture, One Kendall Square, Building 600/700, Suite 7-501, Cambridge, MA 02139. Email: hberges@inari.com

Inari is designing seeds to help address one of the greatest challenges of our times - growing enough nutritious calories for a growing population while reducing the footprint of agricultural production on the environment. Embracing the complexity and diversity of nature, we use our SEEDesign™ platform to overcome these challenges. Our platform integrates Predictive Design and Advanced Multiplex Gene Editing tools to develop resilient seeds that require fewer natural resources and inputs, in a drastically shorter time and lower costs than current approaches. In Predictive Design, we harness the power of Artificial Intelligence and cell-based assays to gain a deep understanding of the sequence polymorphisms that underpin crop performance. Once the target sequences have been identified, we generate new allelic diversity using our comprehensive Multiplex Editing toolbox to deliver multiple types of changes into elite parental lines. We then provide our improved seeds to our customers through a simple and collaborative go-to-market strategy. Results and illustrations of our technical approaches and product concepts will be presented.

P-5

Accelerating Precision Breeding through Double Haploids. LORENA B. MOELLER. Bayer Crop Science, 700 Chesterfield Parkway West, Chesterfield, MO 63017. Email: lorena.moeller@bayer.com

Double Haploid (DH) technology is now foundational in many plant breeding programs around the globe including those for staple crops such as corn, wheat, and canola. Along with progress in tissue culture, improved efficiencies in DH induction and selection have been critical to the adoption and deployment of this technology at scale. Commercial agricultural breeding and biotechnology companies have DH programs to drive genetic gain and preserving genetic diversity. Combination of DH technology with transformation and genome editing applications have gained momentum over the last five years. In this talk, we will discuss progress and challenges of driving efficiencies in Bayer's DH pipelines across crops and geographies. We will share how Bayer is leveraging DH programs to enable Precision Breeding for the modern farm, with the vision of delivering tailored solutions to our customers.

P-6

Breeding Technology & Discovery Progress at Syngenta. WEIGUO LIU, Tim Kelliher, and Qiudeng Que. Syngenta Seeds Research, 9 Davis Drive, RTP, NC 27709. Email: weiguo.liu@syngenta.com

Doubled Haploid (DH) and Genome Editing (GE) are important tools to accelerate genetic gains in crops. In this talk, we will share Syngenta Seed Research status on DH and GE. We will report some progresses on Breeding Technology and Discovery research. Examples will be given on gene discovery of corn haploid induction and GE via haploid inducer. The research results enable haploid induction in other crops, and development of HI-Edit platform to accelerate GE trait introgression in commercial elite lines.

P-7

Deciphering *In Planta* Haploidization in Maize. NATHANAËL JACQUIER[1,2], Andrea Calhau[1], Chloé Plagnard[1], Laurine Gilles[2], Peter M. Rogowsky[1], Jean-Pierre Martinant[2], and Thomas Widiez. [1]Laboratoire Reproduction et Développement des Plantes, Univ Lyon, ENS de Lyon, UCB Lyon 1, CNRS, INRAE, Lyon, FRANCE and [2]Limagrain Europe SAS, Research Centre, Chappes, FRANCE. Email: nathanael.jacquier@ens-lyon.fr, thomas.widiez@ens-lyon.fr.

Doubled haploid (DH) technology is a powerful tool enhancing plant breeding. It requires at the first place haploidization, i. e. the production of individuals in which the chromosome number is reduced to half of the number usually found in a somatic cell. Haploidization is mostly performed *in vitro*, by reprograming isolated male or female germ lines to produce haploid embryos/seedlings. However, this *in vitro* process is costly, requires high technical skills and is genotype dependent. On the other hand, maize provides a unique and powerful system to perform haploidization *in vivo* based on so-called haploid inducer lines that have the remarkable property to induce haploid embryos *in planta*. Simple crosses using pollen from these haploid inducer lines, trigger the development of the egg

cell into a haploid embryo containing only the maternal genome. Two recently identified molecular players behind maize haploid induction are *NOT LIKE DAD (NLD) / MATRILINEAL (MTL) / ZmPHOSPHOLIPASE A1* (*ZmPLA1*), and *DOMAIN OF UNKNOWN FUNCTION 679 membrane proteins* (*ZmDMP*). In order to both increase the haploid induction rate and efficiently translate *in vivo* haploidization to other crops, our aim is to characterize the mechanisms underlying maize *in planta* haploid induction. Following the identification of *NLD/MTL/ZmPLA1* we have now demonstrated that NLD*MTL/ZmPLA1* is not localized in the sperm cell as previously thought, but outside the sperm cells on a poorly characterized membrane that encloses the two sperm cells, the endo-plasma membrane or endo-PM. Using cell biology tools, pharmacological and targeted mutagenesis strategies, we have shown the implication of both lipid anchors and electrostatic interactions to target this non-transmembrane protein specifically to the endo-PM. We are currently deciphering the interplays between *NLD/MTL/ZmPLA1*, *ZmDMP,* and new players to elucidate the mechanism of maize haploid induction.

P-8

Overcoming Bottlenecks that Prevent Efficient Foliar Uptake and Translocation of Polymer-based Nanoparticles in Crop Plants. GREGORY V. LOWRY, Yilin Zhang, and Robert D. Tilton. Carnegie Mellon University, Pittsburgh, PA. Email: glowry@cmu.edu

Increasing global food demand and extreme climate events are driving the need for more efficient agriculture. Approaches to boost crop production and resilience, e.g. genetic modifications, efficient foliar delivery of nutrients, all require efficient delivery of materials to selected locations in plants such as the cell nucleus or chloroplasts. Foliar spraying, followed by efficient uptake into plants is needed to make this approach scalable for application to food crops. However, there are many biological barriers limiting targeted delivery after foliar spray; cuticle, epidermis, cell walls, cell membrane(s). Moreover, there are swings in pH and ionic strength and composition to manage. Our work is aimed at 1) understanding how the properties of nanomaterials can be engineered to efficiently cross barriers to deliver agrochemicals and plasmid DNA, and 2) developing environmentally responsive carriers that could enable stimuli-triggered release of encapsulated agents. We have developed star and bottle-brush polymers that are taken up by crop plants with near 100% efficiency, and that can release an encapsulated agent upon heating or stressing a plant. Confocal microscopy and synchrotron X-ray spectroscopy is used to precisely track the locations of the particles in the plants to understand which barriers have been crossed and which are not. Design rules for efficient uptake and targeting of nanoparticle carriers in plants are beginning to emerge based on these studies. These rules can inform the design of other nanoparticle-based delivery approaches, and the engineered responsiveness may be leveraged to deliver cargo only after the particles have arrived at the desired location.

P-10

Nanomaterials for Plant Genetic Engineering. GOZDE S. DEMIRER[1,2,3], Huan Zhang[1,4], and Markita P. Landry[1]. [1]Department of Chemical and Biomolecular Engineering, University of California, Berkeley, CA; [2]Department of Plant Biology and Genome Center, University of California, Davis, CA; [3]Department of Chemical Engineering, California Institute of Technology, Pasadena, CA; and [4]Department of Chemistry, Jinan University, CHINA. Email: gdemirer@caltech.edu

Food security is threatened by increasing consumption and decreasing crop yields amid population growth and climate change. To mitigate these threats, genetic engineering of plants can be employed to create crops that have higher yields and nutritional value, and are resistant to biotic and abiotic stresses such as diseases and drought. Despite recent progress in the genome editing field, most plant species remain difficult to genetically engineer due to the rigid plant cell wall with a strict size exclusion limit that challenges efficient biomolecule transport into plant cells. The current workhorse method of DNA delivery to plants limits the range of transformable plant species and results in uncontrolled transgene integration, hence eliciting regulatory review of edited plants as genetically modified organisms (GMOs), which is lengthy and costly. Therefore, the development of a delivery tool that is non-pathogenic, non-integrating, and species-independent will greatly advance agricultural biotechnology. In this talk, I present the development of a nanomaterial platform that can efficiently deliver genes into both model and agriculturally-relevant crop plants, without mechanical aid, in a non-toxic and non-integrating manner; a combination of features that is not attainable with existing plant transformation approaches. I discuss how single-walled carbon nanotubes can be chemically modified to both load and deliver DNA to plant cells for expression of functional proteins in various plant species including tobacco, arugula, wheat, and cotton. Efficient delivery and transient expression of plasmid DNA is achieved in mature plants, notably without transgene integration into the plant genome, a feature that could assuage regulatory oversight of the transformed plant as a GMO. These developments demonstrate the unique abilities of nanomaterials to address the main

bottlenecks of plant genetic engineering for a sustainable future with food security.

P-11

Control Plant Growth by Modulating Auxin Biosynthesis and Degradation. YUNDE ZHAO. Section of Cell and Developmental Biology, University of California San Diego, La Jolla, CA 92093. Email: yundezhao@ucsd.edu

Plants have evolved sophisticated mechanisms to maintain auxin homeostasis. Auxin is mainly synthesized from tryptophan via a two-step pathway. Trp is first converted into indole-3-pyruvate, which subsequently undergoes oxidative decarboxylation catalyzed by YUC flavin monooxygenase. Disruption of *YUC* genes leads to defects in all major developmental processes. We also demonstrated that GH3 amido synthetases play essential roles in auxin homoeostasis and plant development. We deleted the eight *GH3* genes involved in auxin conjugation using CRISPR/Cas9. The *gh3 octuple* mutants accumulate free IAA and fail to produce IAA-Asp and IAA-Glu conjugates. Consequently, *gh3 octuple* mutants have extremely short roots, long and dense root hairs. By analyzing *gh3 septuple* mutants, we revealed that *GH3.17* and *GH3.9* play prominent roles in root elongation and seed production, respectively. We show that *GH3* functions correlate with their expression patterns, suggesting that local deactivation of auxin is also important for plant development. We further demonstrated that local auxin biosynthesis and inactivation are coordinated. Auxin biosynthesis mutants are partially rescued by removing *GH3* genes. In this presentation, I will show how the various auxin biosynthesis and degradation genes are coordinated to form a regulatory network for maintaining auxin homeostasis.

P-12

ABA Receptors: Agonists, Antagonists, and Scaffolds for Biosensors Development. SEAN R. CUTLER. Department of Botany and Plant Sciences, University of California, Riverside, CA 92521. Email: cutler@ucr.edu

This talk will cover recent advances in developing small molecules that activate or inhibit plant abscisic acid (ABA) receptors for applications in plant signal transduction research and ongoing biotechnological efforts to develop ABA receptors as a new module for constructing biosensors. The ability to generate biosensors for user-defined molecules would substantially accelerate many areas of biotechnology. To address this challenge, we have developed a platform for the rapid isolation of biosensors using the ABA receptor PYR1 (*Pyrabactin Resistance 1*), which has a malleable ligand-binding pocket and uses a ligand-induced dimerization mechanism that facilitates the construction of sense-response functions. We validate PYR1 as a novel and easily engineered biosensor scaffold platform by evolving 21 PYR1-derived sensors with nM to μM sensitivities for a range of small molecules, including structurally diverse natural and synthetic cannabinoids and several organophosphate insecticides. Moreover, we demonstrate that PYR1-derived receptors can be used to construct diverse ligand-responsive outputs, including ELISA-like assays, luminescence by protein-fragment complementation, and transcriptional circuits, all with pM to nM sensitivity. Thus, PYR1 provides an effective scaffold for rapidly engineering new biosensors that are portable to diverse sense-response platforms.

P-13

Agrobacterium-mediated Transformation of the Plastid Genome. PAL MALIGA, Julia Ferranti, and Aki Matsuoka. Waksman Institute of Microbiology, Rutgers University, 190 Frelinghuysen Road, Piscataway, NJ, 08854. Email: maliga@waksman.rutgers.edu

Efficient plastid transformation in *Arabidopsis thaliana* has been achieved using a recipient plant lacking a duplicated ACCase enzyme. Obtaining a fertile transplastomic plant from the cell line is still a problem caused by somaclonal variation in tissue culture cells. Tissue culture limitations in case of nuclear gene transformation have been overcome by direct transformation of the female gametocyte using the floral dip protocol, and identification of transgenic events in the seed progeny. To enable floral dip transformation for plastid engineering, we decided to re-engineer *Agrobacterium* for T-DNA delivery to chloroplasts. During *Agrobacterium* transformation the VirD2 protein guides the T-complex consisting of single stranded T-DNA coated with VirE2 proteins to the plant nucleus. We report here successful retargeting VirD2 to plastids, an essential step to enable delivery of the T-complex to plastids. Reengineering VirD2 will enable organellar genome engineering, extending the utility of *Agrobacterium* beyond the nucleus.

P-14

Modification of the Plastid Genome Using Base Editing. SHIN-ICHI ARIMURA. Graduate School of Agricultural and Life Sciences, the University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo, JAPAN, Email: arimura@g.ecc.u-tokyo.ac.jp

Plastid genomes, which encode key genes for photosynthetic processes, including both light reactions and carbon assimilation, are potential targets of plant breeding. Plastid genetic transformation can now be used for a limited number of species and is difficult even in the model plant Arabidopsis. In addition, it requires the insertion of a marker gene into plastid genomes, so the created plants are regarded as genetically modified organisms (GMOs). Recently, bacterial

cytidine deaminase (CD) fused to DNA binding domains of TALENs, which converts C to U to change G:C pairs to A:T pairs in double-stranded DNA, was successfully used for in vitro targeted-base editing of mitochondrial DNAs in mammalian cultured cells. Here, we applied the technology to edit a targeted base in the plastid genome in Arabidopsis plantlets, without leaving any foreign genes in either the plastid or nuclear genomes. The targeted Cs were homoplasmically substituted to Ts in some plantlets of the T1 generation, and the mutations were inherited by their offspring independently of their nuclear-introduced vectors. Targeted single nucleotide substitutions are expected to be the best way to make desired SNPs without disturbing any other genes or regulatory regions in plastid genomes of accepted crops or elite lines.

P-15

Targeted Delivery of Plasmid DNA to Chloroplasts by Nanomaterials. Israel Santana[1], Christopher Castillo[1], Gail F. Garcia[1], Gregory M. Newkirk[2], Su-Ji Jeon[1], and JUAN PABLO GIRALDO[1]. [1]Department of Botany and Plant Sciences, University of California-Riverside, Riverside, CA 92521 and [2]Department of Microbiology and Plant Pathology, University of California-Riverside, Riverside, CA 92521. Email: juanpablo.giraldo@ucr.edu

Chloroplasts are key targets for genetic engineering due to their multiple roles in plant photosynthesis, metabolic pathways, and signaling. These semi-autonomous organelles with a small prokaryotic-like genome, their own transcription and translation machinery, and lacking gene silencing mechanisms enable high expression of transgenes while isolating genetic markers in parental lines. Current chloroplast transformation techniques, including gene gun bombardment, require costly instrumentation and materials that are limited to specialized core facilities, and are unable to target the delivery of genes to these organelles. Herein, we demonstrate a novel and facile nanotechnology-based tool that can deliver transgenes to chloroplasts in *Arabidopsis thaliana* plants without instrumentation aid. Single-walled carbon nanotubes (SWCNT) coated with plasmid DNA (pATV1) and suspended in surfactant are shown to passively enter leaf tissues and deliver to chloroplasts a transgene encoding a green fluorescent protein (GFP). GFP gene mediated delivery by SWCNT and transient expression in chloroplasts was confirmed by confocal fluorescence microscopy and RT-qPCR analysis. This topical delivery method for plasmid DNA delivery by nanomaterials has the potential to extend to other plant tissues including roots and meristems. A biorecognition approach of coating the nanomaterials with a rationally designed chloroplast targeting peptide improved the delivery of plasmid DNA-SWCNT complexes (TP-pATV1-SWCNT) to 56.8 ± 4.6 % chloroplasts *in planta*. We also investigated the impact of TP-pATV1-SWCNT on plant molecular and cell biology for engineering biocompatible targeted nanomaterials. There was no significant difference in the percentage of dead cells in plants treated with TP-pATV1-SWCNT (2 mg/L) nor in chloroplast intactness relative to controls without nanoparticles. However, targeted nanomaterials transiently increased leaf H_2O_2 levels relative to controls without nanoparticles inducing temporary oxidative DNA damage in chloroplast genomes, and impacting carbon assimilation rates but not photosystem II quantum yields. A comprehensive understanding of the effect of targeted nanomaterials on plant cell and molecular biology will lead to engineering biocompatible and efficient plasmid DNA delivery tools. This work provides novel targeted delivery approaches for delivering transgenes to plastid genomes that can passively bypass biological barriers in plants such as cell walls, plasma membranes, and organelle envelopes.

P-18

Applied Cannabis Tissue Culture: Innovations in Cleaning, Screening, and Germplasm Maintenance. C. LEAVITT, L. Rincon Mautner, E. Hsu, R. Saephan, and S. Healy. Node Labs Inc, 205 Deer Creek Lane, Petaluma, CA 94952. Email: chris@nodelabsca.com

The conventional paradigm for tissue culture micropropagation is difficult to justify for cannabis because of its ease of propagation ex-vitro, and because it presents unique challenges in-vitro. However, with the phenomenon of bioaccumulation in clone mothers, tissue culture remains an essential tool for cannabis propagators. Node Labs has created a hybrid technique, using both in-vitro and ex-vitro propagation phases to maximize efficiency, cleanliness, consistency, and cost. We will challenge the effectiveness of the Initiate-Multiply-Root-Acclimatize micropropagation model for cannabis as well as other crops, and suggest that the role of tissue culture for these crops may be more focused on Cleaning, Screening and Germplasm. Finally, we will discuss Node Labs innovative tissue culture techniques that optimize high-resolution screening and long-term germplasm storage.

P-20

Application of Plant Rapid Expression Systems in Accelerating Gene Expression and Gene Editing Discovery. P. YANG. Bayer Crop Science, 700 Chesterfield Pkwy W, Chesterfield, MO 63017. Email: peizhen.yang@bayer.com

In plant biotechnology, it typically takes months to produce transgenic plants, before assessing the efficacy of elements

and gene of interests. Rapid Expression Systems (RES) present an opportunity to accelerate the screening of different designs for expression and editing, with early detection systems and therefore enable a faster test and learn cycle. In this presentation, we will give an overview of an array of transient assay systems and explore the suitability and efficacy to address questions around expression and editing in the discovery pipeline. The applications of using RES to accelerate research in these areas will be shared.

P-21

Pre-selection of Guide RNAs Enables Efficient Genome Editing in Planta. YAN LIANG. Joint Genome Institute, B91R225, Lawrence Berkeley National Laboratory, One Cyclotron Road, Berkeley, CA 94720. Email: yliang@lbl.gov

Up to date, studies on the impacts of single guide RNA (sgRNA) efficiency on Crispr/CAS9-mediated genome editing in plants are very limited. We developed an assay to evaluate sgRNA efficiency within the genomic context for plant genome editing. Efficient sgRNAs selected from the assay were shown to enable highly efficient genome editing in two case studies with Arabidopsis: 1. sgRNAs with high efficiency were used to generate heritable chimeric plants with an essential gene manipulated in a tissue specific manner. 2. sgRNAs with high efficiency were utilized to obtain biallelic mutant plants in the first transgenic generation. Our results emphasize the importance of pre-selection of sgRNAs for efficient editing *in vivo*. The transient assay is widely applicable and ready to be modified to assess different elements in genome editing systems.

P-22

CoverCress – A Novel Oilseed Winter Crop with Canola-like Composition That Helps Sequester Carbon and Prevent Soil Erosion. TIM ULMASOV. CoverCress Inc, St. Louis, MO 63132. Email: tulmasov@covercress.com

There is an urgent need for reducing greenhouse gas emissions and other detrimental impacts of civilization on the environment. One of the solutions proposed in agriculture are cover crops that are generally grown between regular cropping seasons, providing significant benefits such as enhanced soil health and increased carbon sequestration. The main problem with lack of wide-spread cover crops adoption is in their economics, as most farmers avoid them due to guaranteed costs and uncertain returns from the benefits to the following crop. This results in misplaced economic incentive where the society greatly benefits from increased cover crop use, but most farmers are not prepared to pay for that. To address this dilemma, we developed a novel crop that can be used as a feedstock for bioenergy, as well as for human and animal consumption. The main advantage is that it doesn't compete for land with established crops, resulting in very low Carbon Intensity (CI) score of the oil and meal. It is based on the domesticated version of weed field pennycress (*Thlaspi arvense*) and can be used to produce oil with attractive properties for renewable diesel, jet fuel or food. CoverCress seeds are also a good source of proteinaceous meal that can be used for animal feed or plant-based protein for human consumption. Pennycress seeds have high (~32%) oil content with lowest saturated fat content among commercially available plant-based oils. As pennycress is genetically very close to Arabidopsis, it can be transformed using floral dip and improved faster than any other crop. CRISPR-Cas9 system works very efficiently in pennycress in part because of our optimized expression and crop's small diploid genome. Using a combination of conventional breeding and multiplex genome editing we were able to rapidly domesticate wild pennycress into CoverCress, a low CI, canola-like crop that is planned for launch in central Midwest in fall of 2022.

P-23

Transforming Blackberry from Niche Crop to High Throughput Platform for Precision Breeding Technologies. R. D. CHAUHAN, E. Dean, P. Vogel, J. Calero, W. Lowry, D. Heckart, J. McAdams, F. Lai, N. Wada, A. Taylor, H. Kim, N. Graham, M. R. Willmann, and A. Hummel. Pairwise, 110 TW Alexander Dr., Durham, NC. Email: dchauhan@pairwise.com

Pairwise (www.pairwise.com) is exploring and deploying gene editing tools to generate genetic diversity and create better fruits and vegetables. Flavor, nutrition, seedlessness, texture and shelf life are some of the highly desirable consumer traits in caneberries, but traditional breeding approaches are constrained by long generation of genotypes. Precision editing provides a great alternative to these bottlenecks, but successful implementation of gene editing tools requires a robust transformation system, effective gene editing tools and a process to assess their performance. The efficiency of stable transformation systems is typically genotype dependent and can take a long time to recover plants. For this reason, we have established rapid assays for multiple caneberry genotypes to test the efficacy of editing tools, and to enable continuous research and innovation. These rapid systems have facilitated optimization of editing tools in market-ready genotypes. We

have also established high throughput transformation capabilities in several caneberry genotypes to enable product development. We will share how we have turned blackberry from niche to state-of-the-art biotechnology system to enable our mission of building a healthier world through better fruits and vegetables.

P-24

Of Media and Miracles: Successes and Frustrations in the Search for Efficient Regeneration and Transformation Methods for Trees and Crops. STEVEN H. STRAUSS, Michael F. Nagle, Nathaniel Ryan, Cathleen Ma, Greg Goralogia, Ekaterina Peremyslova, Alexa Niño de Rivera, Megan McEldowney, Chris Willig, and Michelle Wiseman. Departments of Forest Ecosystems and Society and Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331. Email: Steve.Strauss@OregonState.Edu

The capacity for genetic engineering and gene editing methods is limited by the ability to efficiently transform and regenerate most plant species and genotypes. Trees and clonal crops are among the most recalcitrant and genetically variable in this respect, with many important species and genotypes being nearly impossible to transform without costly and extensive research and development. Based on successes in the model plant Arabidopsis and some other dicot crops, and with prominent success in several monocot crops, it appears that the overexpression of morphogenetic regulatory genes (MRGs) as regeneration stimulating "reagents" may be the basis of a more generalized approach to transformation of trees and diverse crops. In our laboratory, we have been exploring the potential for MRGs to promote transformation and regeneration in diverse genotypes of Populus, Eucalyptus, and Humulus. To date we have tested 8 different types of MRGs *in vitro* or *in planta* in one species or another. These include many variations of *GRF-GIF, WUS, BBM, LEC1, EBB1, IPT*, and the native gall- and hairy-root inducing gene regulons from Agrobacterium. So far, these experiments have shown either marginal positive effects or strong inhibitory effects on transgenic shoot regeneration. Overexpressing *WUS* consistently impeded regeneration, with variable degrees of inhibition depending on the source species. In contrast the gall regulon from C58 of Agrobacterium was effective in producing transgenic callus in a wide variety of Populus and Eucalyptus genotypes *in planta*, but these galls could not be induced to produce shoots. GRF-GIF effects were highly variable depending on the promoter used to drive expression, the degree of miRNA sensitivity, and the genotype being transformed. For one construct, its effect completely suppressed transgenic shoot regeneration from a Populus genotype that is otherwise highly regenerable. We will discuss our most recent results, including attempts to use multiple MRGs and obtain excision of the MRGs to enable normal plant development.

P-25

A CRISPR-combo Approach for Speed Breeding and Regeneration of Genome-edited Plants. Changtian Pan[1], Gen Li[1], Aimee A. Malzahn[1], Benjamin Leyson[1], Simon Sretenovic[1], Yanhao Cheng[1], Filiz Gurel[1], Gary D. Coleman[1,2], and YIPING QI[1,2]. [1]Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD and [2]Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, MD. Email: yiping@umd.edu

CRISPR-Cas9, its derived base editors and CRISPR activation systems have greatly aided genome engineering in plants. However, these systems are mostly used separately, leaving their combinational potential largely untapped. Here, we develop a versatile CRISPR-Combo platform, based on a single Cas9 protein, for simultaneous genome editing (targeted mutagenesis or base editing) and gene activation in plants. We showcase CRISPR-Combo's powerful applications for boosting plant genome editing. First, CRISPR-Combo is used to shorten the plant breeding cycle and reduce the efforts in screening transgene-free genome-edited plants by activation of a florigen gene in *Arabidopsis*. Next, we demonstrate accelerated regeneration and propagation of genome-edited plants by activation of morphogenic genes in poplar. Furthermore, we apply CRISPR-Combo to achieve rice regeneration without exogenous plant hormones, which is established as a novel method to enrich high-efficiency genome-edited plants. Our work establishes CRISPR-Combo as a versatile genome engineering tool with promising applications in crop breeding.

P-26

Efficient Plant Regeneration from Protoplasts Isolated from Multiple Grape Genotypes and Demonstration of PEG-mediated Gene Editing Using CRISPR/Cas9. DAVID M. TRICOLI[1] and J. Debernardi[2]. [1]University of California, Plant Transformation Facility, Davis, CA and [2] University of California, Department of Plant Sciences, Davis, CA. Email: dmtricoli@ucdavis.edu

We have developed a protocol for isolating protoplasts from embryogenic cultures of the wine grape genotypes Chardonnay, Columbard, Merlot, the table grape genotype Thompson Seedless and the grape rootstock

genotype, 101-14. By encapsulating the protoplasts in calcium alginate beads and co-culturing them with grape cell suspension feeder cultures conditioned to grow on medium containing 0.4 M mannitol, we were able to stimulate the protoplasts to divide and form callus colonies, regenerate into embryos and germinate into whole plants. The ability to regenerate plants from protoplasts offers an avenue to employ gene editing techniques to grapevines using either plasmid DNA or Ribonucleoprotein (RNP)-Based Genome Editing. We have successfully transfected protoplasts of Thompson Seedless using plasmid DNA encoding for the scorable marker gene mCherry which allowed us to monitor the effect of various parameters on transfection efficiency and protoplast viability. Next, we transfected protoplasts with a CRISPR-Cas9 vector and guide RNAs targeting the conserved DELLA domain of the VvGAI1 gene of grape. We were able to regenerate hundreds of plants from transfected protoplasts. Sequence data on a subset of these plants demonstrated successful recovery of plants with edits in the DELLA domain. In addition to generating edited lines, using protoplast-mediated transfection also allows us to efficiently recover stably transformed grape plants.

P-28

The Creation of *In Vitro* Collections to Conserve Forest Genetic Resources: The Magnolia and Avocado Cases. R. FOLGADO, T. Thibault, J. Tin, S. Lahmeyer, and N. Cavender. The Huntington Library, Art Museum, and Botanical Gardens, 1151 Oxford Rd., San Marino, CA 91108. Email: rfolgado@huntington.org

In vitro conservation can be used for plants that produce non-orthodox or low viability seeds or vegetatively propagated genotypes. Furthermore, many woody plants show sensitivity to tissue culture, making the optimization of different stages essential. The methodology includes collection from ex-vitro plants, micropropagation (initiation, establishment, multiplication), rooting, and hardening to ex-vitro. The in vitro repositories reduce the maintenance cost of the field collections and facilitate international distribution. A robust method is needed to ensure long-term storage since the plant regeneration after cryopreservation (storage at ultra-low temperatures) depends greatly on the tissue culture. Magnolias are appreciated as ornamental and medicinal plants worldwide, and avocado is a fruit crop with increased demand. However, both face threats to their conservation, such as new pests and changing environmental conditions. Indeed, half of the magnolia species are endangered, and many do not have an *in vitro* system. The avocado culture is clonal, and the conservation of its diversity is crucial for its sustainability. Since both plant groups share some of the main issues for micropropagation (i.e., oxidation), the acquired knowledge in one can be applied to the other and vice versa. Therefore they may be model plants to implement protocols in other woody species. Budwoods from different magnolia and avocado trees were collected and introduced in tissue culture. After initiation, we studied the effect of media composition on the micropropagation of magnolias and avocados. The hardening method was optimized to reduce loss during the acclimation from in vitro to ex vitro conditions. As a result, we could create in vitro repositories of wild magnolias and wild and cultivated avocados at the Huntington Library, Art Museum, and Botanical Gardens (San Marino, CA). Accessions of the collections have been distributed to other institutions to contribute to the ex-situ conservation of these plants.

P-29

Ex-Situ Conservation of Critically Endangered Species at the Hawaiian Rare Plant Program. D. GORDON. University of Hawaii, Lyon Arboretum, 3860 Manoa Road, Honolulu, HI 96822. Email: devong@hawaii.edu

Hawaii is home to over 400 threatened and endangered plant species, with more than half of those species having fewer than 50 individuals remaining in the wild. Ongoing threats from invasive species and habitat loss make ex-situ conservation a necessary safeguard against plant extinction. The Hawaiian Rare Plant Program (HRPP) utilizes ex-situ conservation technologies to aid in the prevention of further extinction of Hawaiian plant species by initiating and maintaining an in-vitro plant collection and seed bank. In addition to long-term storage, germplasm from these collections is also made available for use in approved restoration and reintroduction projects. In 2020, The Hawaiian Rare Plant Program received a United States Fish and Wildlife Service Recovery Challenge Grant to initiate a cryopreservation program at the HRPP Micropropagation Lab. Cryopreservation protocol development for several Hawaiian species has begun, with the end goal of a permanent cryogenic storage facility for Hawaiian plants located at the HRPP Micropropagation Lab. The total number of propagules in tissue culture at the HRPP Micropropagation Lab is currently over 33,000 and requires a large amount of labor and material resources to maintain. Cryogenic storage of a portion of the in-vitro collection will help reduce maintenance costs, allowing for expansion of the HRPP to focus on further ex-situ conservation research and initiation of underrepresented species both in-vitro and in seed storage.

P-30

DoE Methodology vs. “Standard Stats” – Software for DoE Methodology – DOE Principles and Types of Experiments, RANDALL P. NIEDZ. USDA – ARS, U.S. Horticultural Research Laboratory, 2001 S. Rock Rd., Fort Pierce, FL 34945-3030. Email: randall.niedz@usda.gov

DOE methodology includes an experimental framework as well as specific experimental designs that are deployed within the DOE framework. The DOE framework will be presented and the types of problems where DOE is the best approach explained by contrasting the DOE approach to “standard” statistics. Further, because DOE requires specialized statistical software, software options and considerations will be discussed.

P-31

DoE Experiment Walkthrough. UYEN CAO CHU.Corteva Agriscience, 8305 NW 62nd Ave., PO Box 7060, Johnston, IA 50131-7060.Email: uyen.chu@corteva.com

To illustrate the use of DOE, a step-wise walkthrough will be presentedaddressing a complex in vitro problem that involves many factors. You willlearn how to initiate DOE by first screening factors to identify those factorsthat strongly affect the response(s) being measured and then move to responsesurface methodology to optimize factor settings/levels to achieve the bestpossible overall response. Each step will be described and will include why itis included and possible considerations and alternative approaches.