In Vitro Cellular & Developmental Biology - Animal
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Plant Symposia and Workshops

P-1

Plant Info Flow: Lessons Learned in Interdepartmental Communication. J. JASINSKI. Microplant Nurseries, Inc. PO Box 237, Gervais, OR, 97302. Email: Jonathan@microplantnurseries.com

Getting a tissue cultured plant from an initial order to an actual final product in a customer's hands requires extensive planning, attention to detail, and seamless communication between multiple departments including Sales, Research, Scheduling, Media Preparation, Production, and Shipping. If key feedback mechanisms are not in place between these areas, the best laid plans can fall apart rapidly when scaling up plant material for an order. This presentation will focus on what information moves through a commercial-scale tissue-culture facility with emphasis on several tools and techniques used to make sure a high-quality Stage II or Stage III plantlet gets through Production efficiently and shipped in a timely manner.

P-2

Leadership Focus on Applied Research in Plant Tissue Culture: Transitioning Research Employees from Academic to Commercial Labs. A. B. OLIVEIRA and C. Sluis. Tissue-Grown Corporation, 15245 W Telegraph Rd. Santa Paula, CA 93060. Email: alex@tissuegrown.com

Despite some common elements in the way that research proceeds, there are many more areas of significant differentiation. In fact, scientific studies can be done to serve different purposes and their main goal usually varies according to the environment in which they are conducted, i.e., academia vs industry. While the first usually desires to expand knowledge and is curiosity-driven, the latter solves practical problems and has specific commercial objectives: products, procedures, or services. Therefore, scientists making the transition from academic to commercial labs should be aware of what type of research they conduct and adapt themselves accordingly with the new career. In fact, it is essential to align experimentation goals with the company's priorities shaping the direction of work to be done in the most applied way possible. Acquiring the appropriate leadership skills is crucial in this new journey, a characteristic that should be pursuit by these new managers/leaders, as not many scientists start out with aspirations of "managing" people. Such skills are likely to make the difference between a peak performing commercial lab and one that is struggling. This presentation will explore the major challenges and opportunities that come with the transition of research employees from academic to commercial labs, focusing on the leadership characteristics required to be successful in Tissue Culture Labs. We highlight how commercial labs can apply leadership concepts in their day-to-day routine, combining state-of-the-art research and commercial micropropagation to develop new processes and improve mass clonal productivity.

P-4

Overcoming Bottlenecks in Editing Plant Genomes. DANIEL F. VOYTAS. Department of Genetics, Cell Biology & Development, Center for Genome Engineering and Center for Precision Plant Genomics, University of Minnesota, MN. Email: voytas@umn.edu

Plant gene editing is usually carried out by delivering reagents such as Cas9 and sgRNAs to explants in culture. Edited cells are then induced to differentiate into whole plants by exposure to various hormones. Creating edited plants through tissue culture is often inefficient, requires considerable time, only works with limited species and genotypes and causes unintended changes to the genome and epigenome. We have been pursuing alternative approaches for plant gene editing that minimize or obviate the need for tissue culture. In one approach, we generate gene edited dicotyledonous plants through *de novo*meristem induction. Developmental regulators and gene editing reagents are delivered to somatic cells on whole plants. Meristems are induced that produce shoots with targeted DNA modifications, and gene edits are

transmitted to the next generation. In a second approach, we use RNA viruses to deliver sgRNAs through infection to transgenic plants that express Cas9. The sgRNAs are augmented with sequences that promote cell-to-cell mobility and movement into the meristem. Gene edited shoots are thus generated that transmit gene edits to the next generation. Because both approaches minimize the need for tissue culture, they promise to help overcome this bottleneck in plant gene-editing.

P-5

Establishing an Efficient Base Editing System for Tomato. JOYCE VAN ECK[1,2], Linnell Randall[1], Simon Sretenovic[3], and Yiping Qi[3,4]. [1]Boyce Thompson Institute, Ithaca, NY; [2]Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, NY; [3]Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD; and [4]Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, MD. Email: jv27@cornell.edu

Base editing, which makes it possible to convert a single base to another, is one of the most recent approaches being utilized for precise modification of plant genomes. To date, there are two groups of base editors available, cytidine base editors (CBE) for conversion of C – T and adenine base editors (ABE) for conversion of A - G. To identify an efficient CBE approach for tomato, we used the BE3 system, which is the most widely used platform for C – T conversion. We compared five different BE3 designs that all contained the Cas9 nickase, Cas9-D10A, but differed in the type of deaminase. Constructs also contained two different gRNAs under control of two different polymerase promoters, AtU3 and AtU6. BE3 designs were tested in tomato protoplasts before generating stably edited plants. Through the protoplast work we identified a highly efficient system designated A3A/Y130F-BE3 that was subsequently used to generate edited plant lines. Selected edited and GFP control lines were assessed for off-targets at both the whole genome sequence (WGS) and transcriptome levels. On the WGS level, we observed an average of approximately 1000 single nucleotide variations (SNVs) and about 100 insertions and deletions (indels) per GFP control plant. Whereas, based-edited plants on average had approximately 1250 SNVs and 300 indels per plant. In addition, approximately, 200 more C-to-T (G-to-A) mutations were found in a base-edited plant than a GFP control, suggesting these were gRNA-independent off-targets. For transcriptome level assessment, we conducted RNA sequencing of the same base-edited and GFP control plants assessed at the WGS level. An average of roughly 200 RNA SNVs was observed per plant for either base-edited or GFP control plants. Specific enrichment of C to U mutations were found in base-edited plants. We did not see any off-target mutations at the transcriptome level.Similar efforts to identify an efficient ABE approach are in progress and results will be presented.

P-6

Optimizing CRISPR-Cas Systems for Efficient Multiplexed Genome Engineering in Plants. YIPING QI. Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD 20742. Email: yiping@umd.edu

CRISPR based genome engineering tools have unleased unlimited potential in plant genetics, engineering, and breeding. To enable large-scale genome editing by Cas12a, we compare 12 multiplexed Cas12a systems and identify a potent system that exhibits nearly 100% biallelic editing efficiency with the ability to target as many as 16 sites in rice. This is the highest level of multiplex edits in plants to date using Cas12a. Two compact single transcript unit CRISPR-Cas12a interference systems are also developed for multi-gene repression in rice and *Arabidopsis*. To enable multiplexed gene activation, we developed CRISPR-Act3.0 which is based on the Cas9 system and allows for robust activation of multiple genes in rice and *Arabidopsis*. Collectively, we have developed multiple new CRISPR tools for multiplexed genome editing, gene repression, and gene activation.

P-7

Genome Editing in Soybean (G. max) with Cas12a. R. GAETA. Bayer Crop Science Division. 700 Chesterfield Parkway West, Chesterfield, MO 63017. Email: robert.gaeta@bayer.com

Plant breeders have been selecting upon naturally occurring genetic variation since the domestication of plants from wild populations. Genetic variation for traits like yield, flavor, nutrient composition, disease resistance, and drought tolerance has enabled improvements in plants resulting in the better performing crops humans are familiar with today. Gene Editing techniques are a new, diverse, and expanding set of tools that can be used to generate genetic variation, accelerate genetic gain, and further improve crops to accommodate the growing human population in the face of climate change. These technologies can generate desirable variation in a specific manner that is similar or identical to naturally occurring genetic variation. In this talk we outline the need for these technologies and review various techniques and applications. We summarize data and observations on the DNA editing efficiency of 180 Cas12a gRNAs targeting intergenic locations spanning the soybean genome, and speculate on the implications and considerations for gene targeting applications. Finally, we demonstrate that DNAs can be tethered to

Cas12a, facilitating their insertion efficiency at targeted DNA breaks.

P-8

Overview on Micropropagation of Horticultural Crops. YONGJIAN CHANG. North American Plants, Inc., 9375 SE Warmington Road, McMinnville, OR 97128. Email: ychang@naplants.com

The micropropagation of tree fruit, nut and berry crops have been greatly developed in the last decade. It has been widely used to all orchard crops and become an essential technique for Horticulture industry. Updates on faced challenges in micropropagation worldwide will be discussed.

P-9

Micropropagation of Woody Plants and the Challenges of Maintaining True-to-type Commercial Scale Production of Trees. JAVIER CATILLON. Duarte Nursery, 1618 Baldwin Road, Hughson, CA 95326–9699. Email: javier@duartenursery.com

Micropropagation of woody plants has long been a challenge compared to herbaceous or other non-woody types of plants. Because woody plants are primarily perennials, often with long life spans, it is important to ensure that clonal propagation techniques maintain genetic stability and avoid the production of off-types. This is especially important when producing rootstock varieties that have been selected for their genetic attributes as they relate to field performance in orchards that may be in commercial production for 20 to 30 years. This presentation will review factors that can affect genetic/phenotypic stability of plants produced from in vitro cultures of commercial fruit and nut tree rootstock varieties.

P-10

From *Populus* Molecular Networks to Transgenic Field Phenotypes. A. M. BRUNNER[1], E. P. Beers[2], X. Sheng[1], H. E. Petzold[2], and R. Teixeira[1]. [1]Virginia Tech, Forest Resources and Environmental Conservation, Blacksburg, VA 24061 and [2] Virginia Tech, School of Plant and Environmental Sciences, Blacksburg, VA 24061. Email: abrunner@vt.edu

The continued development of genomic resources and biotechnology such as CRISPR gene editing are opening new opportunities for genetic modification of woody crops. Concurrent with advances in *in vitro* methods we need to identify genes with functions relevant to genetic improvement goals that for *Populus* and other forest trees includes simultaneous improvements to wood yield, biomass quality and environmental adaptation. In between the genotype and phenotype are macromolecule interaction networks. We have taken two approaches to to identifying *Populus* networks: 1) experimentally determined wood protein-protein interactions and protein-DNA interactions involving promoters of lignin biosynthesis genes; and 2) gene regulatory network inference from time-series transcriptomic responses to photoperiod and nutrient regimes that induced growth cessation and then growth resumption. To validate the utility of this approach for identifying key regulators of growth, wood chemistry, and environmental responses, we altered gene expression or activity in transgenic *Populus*. Moreover, results from a three-year field study support that manipulation of regulatory control points can advance genetic improvement goals.

P-11

Challenges in Micropropagation of *Musa* Tissue Culture. MARIA M. JENDEREK and Dianne M. Skogerboe. USDA-ARS, National Laboratory for Genetic Resources Preservation, Fort Collins, CO 80521. Email: maria.jenderek@usda.gov

Musa (banana and plantain) fruits are a highly valued staple food and important in economies in many tropical countries. Shoot culture of the genus are easily established in vitro. However, in comparison to several other species, *Musa* culture require additional steps to ensure success. Almost all *Musa* cultures contain endophytes, often not visible until exposed to an abiotic stressor. Hence, before extended micropropagation, the shoots have to be screening for endophytes. Only endophyte free cultures are used for shoots intended for cryopreservation. Phenolic compounds are secreted by most *Musa* plants. The resultant blackening has deleterious effects, including death of the cultured plants. To some extent activated charcoal, when added to tissue culture medium absorbs the phenolic but charcoal also conceals low levels of endophytes when present. The shoot multiplication progress depends on the level of benzyl adenine (BA) in the medium; the higher the content ($\geq$6 mg/L) the more rapid and a larger number of shoots are developed but high BA concentration supports somaclonal variation. To conserve the genetic fidelity of cryopreserved *Musa* accessions, in vitro culture are micropropagated on 2.25 mg/L of BA. This low BA level lengthens, by several months, the time needed to produce the quality and quantity of *Musa* plantlets needed for cryogenic processing and storage. Current research on *Musa* culture focuses on replacing charcoal with other antioxidants and optimizing BA content in the culture medium.

P-13

Genetic Improvement of Potato Using Innate® and GE Technology in a Challenging Landscape. JEFF HABIG. Simplot Plant Sciences, 5369 W. Irving Street, Boise, ID 83706. Email: Jeffrey.habig@simplot.com

Genetic improvement of the potato using conventional breeding is a time-consuming and difficult endeavor because it is a highly heterozygous crop. Making genetic gains is complicated by tetrasomic inheritance and the fact that it is a vegetatively propagated crop. Additionally, market forces have kept very old varieties prominent despite the best efforts of breeders. Genetic modification provides an opportunity to improve a few genetic deficits while retaining the familiar benefits of traditional varieties. The Simplot Plant Sciences division of the J. R. Simplot Company has developed Innate®-branded, genetically modified potatoes using marker-free transformation methods and gene edited potatoes using CRISPR/Cas9. The genetically modified potatoes display improvements in quality traits that benefit growers, processors, retailers, and consumers as well as providing input traits that lead ultimately to sustainable benefits for the land. The strategy used to develop Innate® and GE potatoes, traits engineered, and the successes and challenges to commercialization will be discussed.

P-14

The Effect of Scientific Information and Narrative on Preferences for Possible Gene-edited Solutions for Citrus Greening. B. R. MCFADDEN, B. N. Anderton, K. A. Davidson, and J. C. Bernard. University of Nevada – Reno, Reno, NV. Email: bferguson@unr.edu

This study used a national survey to examine how information that compared and contrasted gene editing with other breeding techniques, as well as a narrative, influenced both attitudes towards gene editing generally and preferences between a gene-edited insect and gene-edited tree to combat citrus greening. Consumers had low familiarity with gene editing but linked it to genetic modification. For citrus greening, respondents equally supported a gene-edited insect or tree, but the narrative decreased the perceived safety of both. These findings suggest that in general, consumers may support gene editing approaches to combat citrus greening.

P-15

A Review of Factors Affecting Successful Hardening of Ttissue Cultured Plantlets, Including Experiences with Tropical Plants, So as to Acclimatize Them to Ex Vitro Conditions. SYLVIA MITCHELL. Medicinal Plant Research Group, The Biotechnology Centre, University of the West Indies, Mona Campus, Kingston 7, JAMAICA. Email: sylvia.mitchell@uwimona.edu.jm

In vitro plantlets grown in glass vessels at relatively low light intensity and high humidity) transferred to ex vitro conditions (having a higher light intensity but lower humidity) need to adapt to the new environment in order to survive. These adaptations include acquiring roots, functioning stomata, resumption of full photosynthetic ability, and establishing flow of water through roots into the stem. The plantlet removed from in vitro vessels is tender and needs handling with care and the adaptation process 'hardens' the plant as the wax layer on the leaves increases thus reducing loss of water via evapotranspiration. Some of the factors found to affect the level of success of hardening include: plant species, plant growth hormones of previous media, other previous media factors, size of plantlets, whether rooting is done in vitro or ex vitro, speed at which humidity is reduced and light intensity increased, type of potting mix, method of planting, length of the juvenile period of the plant, and number of plantlets being hardened. These factors will be reviewed and areas needing further research to be pursued suggested in order to further increase the efficiency of the hardening/acclimitization process for in vitro plantlets.

P-16

Transplanting Tissue-cultured Banana Plants to Field. MARIA M. JENDEREK[1], Ricardo Goenaga[2,3], and Yaleidis Mendez[2]. [1]USDA-ARS, National Laboratory for Genetic Resources Preservation, Fort Collins, CO 80521; [2]USDA-ARS, Tropical Agriculture Research Station, Mayaguez, PR 00680; and [3]USDA-ARS, Subtropical Horticulture Research Station, Miami, FL 33158. Email: maria.jenderek@usda.gov

Tissue culture derived plantlets have to be adapted to outside environmental conditions before transplanting to field. They need to be adjusted to growing in a lower RH and higher light intensity than in vitro. Before planting into a soil mixture, the culture medium should be washed off from the plantlet roots and the shoot base (rhizome) under running water until all agar is removed. A successful acclimation procedure for *Musa* (banana) plantlets involves dipping the roots in a fungicide [e.g., Methyl 1-(butylcarbamoyl)-2-benzimidazo lecarbamate] and in a root-inducing auxin (IBA and NAA) product. The medium-free and treated plantlets are transferred to small pots with a sterilized substrate and covered with a clear plastic cup or misted intermittently; the misting time is gradually reduced as the plants grow. After ca. 2 weeks, the plants are fertilized with a 1/3 strength of a 20–20-20 soluble fertilizer and the plastic cup is removed. Following a 3–4 months growing in a greenhouse, 1 to 2 ft. tall plants with

3–4 leaves are sprayed with an anti-transpirant and planted in the field where normal agronomic practices for banana cultivation are applied.

P-17

Laboratory Changes for Greenhouse Success. A. LABRUM. Bailey Nursery, 1325 Bailey Rd., St. Paul, MN 55119. Email: Angela.labrum@Baileynursery.com

Being able to successfully acclimatize planlets is the final and arguably most important step. It doesn't matter if you can produce a million plants in your lab if you are not able to acclimatize them and get them out to the field at a high rate of success. In the laboratory we can effect root quality, stem caliper, leaf color, overall vigor and more. An Acer sent to a greenhouse with a bent tip, a Pyrus with one sided roots, a Prunus with small caliper that melts down are all examples of poor plantlet quality. We have had acclimation rates as low as 30% jump to 95% with changes we made in the laboratory. A weak plant in the lab will be a weak plant in the greenhouse and will probably never make it to the field. Effective evaluation of plantlet quality in the lab is critical.

P-18

Plant Cis-regulatory Elements at Single Cell Resolution. BOB SCHMITZ[1], Alex Marand[1], Xuan Zhang[1], Haidong Yan[1], Pablo Mendieta[1], Sohyun Bang[2], Andrea Gallavotti[3], and Zongliang Chen[3]. [1]University of Georgia, Department of Genetics; Athens, GA, 30602; [2]University of Georgia, Institute of Bioinformatics, Athens, GA, 30602; and [3]Waksman Institute, Rutgers University, Piscataway, NJ, 08554. Email: schmitz@uga.edu

Cis-regulatorya elements (CREs) encode the genomic blueprints for coordinating spatiotemporal gene expression programs underlying highly specialized cell functions. To identify CREs underlying cell-type specification and developmental transitions, we implemented single-cell sequencing of Assay for Transposase Accessible Chromatin (scATAC-seq) in *Zea mays, Oryza sativa, Glycine max* and *Arabidopsis thaliana.* The single-cell type nature of scATAC-seq provided an unprecedent view into cell type-specific CREs in these plants. These data have been used to construct cell type-specific atlases of CREs across a range of tissues enabling the reconstruction of 3-dimensional chromatin interaction maps, pseudotime developmental trajectories and identification of key transcription factors that define these transitions. Cell-type specific accessible chromatin regions in maize were enriched with phenotype-associated genetic variants and signatures of selection, revealing the major cell-types and putative CREs targeted by modern maize breeding. Implementation of a genotype-dependent pooling method enabled the detection of accessible chromatin regions from over 250 unique maize genotypes that were used for detection of CREs underlying phenotypic variation and chromatin accessible QTL at single cell resolution. Collectively, our analysis affords a comprehensive framework for understanding cellular heterogeneity, evolution, and cis-regulatory grammar of cell-type specification in plant genomes.

P-19

Transcriptional and Imprinting Complexity in Arabidopsis Endosperm at Single Nucleus Resolution. MARY GEHRING, Colette L. Picard, Rebecca Povilus, and Ben P. Williams. Whitehead Institute for Biomedical Research, 455 Main St., Cambridge MA 02142. Email: mgehring@wi.mit.edu

Seeds are a key lifecycle stage for many plants. They are also the basis of agriculture and the primary source of calories consumed by humans. Here, I will describe our efforts to generate a transcriptional atlas of developing *Arabidopsis thaliana* endosperm, a seed tissue, using single-nucleus RNA-seq (snRNA-seq). Endosperm is a site of gene imprinting in flowering plants and mediates the relationship between the maternal parent and embryo. Gene imprinting is an epigenetic phenomenon where alleles of genes are expressed at different levels depending on whether they are inherited from the male or female parent. Using snRNA-seq we identified transcriptionally-uncharacterized nuclei types in the chalazal endosperm region, which interfaces with maternal tissue for nutrient unloading. We demonstrated that the extent of parental bias of maternally expressed imprinted genes varied with cell cycle phase, and that imprinting of paternally expressed imprinted genes was strongest in chalazal endosperm. Thus, imprinting is spatially and temporally heterogenous. Increased paternal expression in the chalazal region suggests that parental conflict, which is proposed to drive imprinting evolution, is fiercest at the boundary between filial and maternal tissues.

P-20

Single Cell Processing and Analysis: Sample Preparation, Enrichment, and Engineering of the Minimal Representative Unit of Life. ABRAHAM LEE. University of California, Irvine, NSF I/UCRC CADMIM, The Henry Samueli School of Engineering, Irvine, CA 92697–2715. Email: aplee@uci.com

Single-cell analysis has become a powerful paradigm in revealing population heterogeneity, identifying minority subpopulations of interest, as well as discovering unique characteristics of individual cells. That is, one must not only be able

to identify molecular and cellular targets that are the source of disease but also understand how these targets behave at the organ and physiological systems level. Recent developments in microfluidics have contributed to burgeoning precision medicine fields such as liquid biopsy, immunotherapy, single-cell analysis, genotyping and gene sequencing, and micro-physiological systems. This is due to the fact that microfluidics bridges the scales of molecular, cellular, tissue, and can even recapitulate organ and circulatory functions of the living system. A key bottleneck is to identify the critical subpopulation of cells, often at single-cell resolution among billions of cells. Here we present a microfluidic trapping array that can rapidly and deterministically trap single-cells in highly-packed microwells. We first describe the design and fabrication protocols of the trapping array, and then presents its two representative applications: single-cell mRNA probing when integrated with a dielectrophoretic Nano tweezers (DENT), and live-cell real-time imaging when combined with fluorescence lifetime imaging microscopy (FLIM). As the single-cell trapping efficiency is determined by the channel design instead of the flow rate, this trapping array can be coupled with different microfluidic sample processing units with varying rates of flow for various single-cell analyses. We will show results obtained by the microfluidic devices with plant cells as well as mammalian cells.

P-21

Immature Embryo Transformation of Recalcitrant Maize Inbreds Using WUS/BBM. Minjeong Kang[1], Alicia Masters[2], Morgan McCaw[1], Jacob Zobrist[1], William Gordon-Kamm[2], Todd Jones[2], and KAN WANG[1]. [1]Iowa State University, Ames, IA and [2]Corteva AgriScience, Johnston, IA. Email: kanwang@iastate.edu

Morphogenic genes such as *Baby boom* (*Bbm*) and *Wuschel2* (*Wus2*) haves been used to produce transgenic plants from recalcitrant maize inbred lines (Lowe et al., 2016, 2018). This method (named QuickCorn), developed by Corteva AgriScience, is fast and less genotype-dependent than traditional maize transformation. Here we report an effort in evaluating the robustness of the protocol by comparing transformation frequencies of three public maize inbred lines obtained by multiple researchers in two different laboratory settings. The goal is to identify factors contributing to protocol reproducibility for newly trained researchers when transforming public maize genotypes. *Agrobacterium*-mediated transformation method was used to infect immature zygotic embryos (IZEs) of B73, Mo17 and W22, three important inbred lines of great transformation recalcitrance. Total frequencies of regenerated events (# of rooted plantlets per 100 infected IZEs), transgenic events (# of transgenic events per 100 infected IZEs) and quality events (# of events with single copy integration and morphogenic gene excised per 100 infected IZEs) were calculated. Our comparison show that all three recalcitrant public inbred lines can be transformed, but with varied frequencies. Average transformation frequencies were ~ 14% for W22, ~4% for both B73 and Mo17. Compared to traditional transformation methods, the QuickCorn method does not demand highly trained researchers, making it more amenable for academic laboratories. Quality of the starting material is one of the major factors for the success of transformation. Healthy ears with the correct developmental stage, media that are correctly prepared and explant materials that are properly handled in the process are fundamentals to the protocol reproducibility (Masters et al., 2020).

P-22

Leaf Transformation in Maize and Sorghum. BILL GORDON-KAMM, Ning Wang, Emily Wu, Ping Che, Nagesh Sardesai, Lrisa Ryan, Ajith Anand, and Todd Jones. Corteva Agriscience, 8305 NW 62nd Ave, Johnston, IA 50131. Email: William.gordon-kamm@corteva.com

Researchers working on crops within the *Poaceae* have lacked a routine, consistent (and most importantly – accessible) method for transformation and genome editing. For large plants such as maize and sorghum, using immature embryos as the starting explant places an extra burden on research labs due to the costly and capricious nature of maintaining consistent embryo-donor plants year-round. Using maize and sorghum as our models for improvement and proof-of-concept, we have developed a method that relies on 2-week old growth chamber-grown seedlings to provide the target explant for *Agrobacterium*-mediated transformation. Currently, our leaf transformation frequency for maize (T0s plants/researcher-hour) is approaching parity when using either immature embryos or seedling-derived leaf segments as the transformation targets (only 2–3-fold lower). In this presentation, I will discuss what makes this work in seedlings, similarities (and differences) between the two explants, and which aspects are critical if you want to try this in your lab. Additionally, we have demonstrated leaf transformation across many species within four grass sub-families (with no protocol modifications beyond that developed for maize), which bodes well for extending this across many cereal crops.

P-23

GRF-GIF: A New Transformation Technology That Improves Regeneration Efficiency of Genome Edited Plants. JUAN MANUEL DEBERNARDI[1,2], David Tricoli[1], Maria Florencia Ercoli[3], Pamela Ronald[3], Javier F. Palatnik[4], and Jorge Dubcovsky[1,2]. [1]Dept. Plant

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Genome editing allows precise DNA manipulation, but its potential for innovation is limited in many crops by low regeneration efficiencies and few transformable genotypes. In this work, we show that expression of a chimeric protein including a wheat Growth-Regulating Factor (GRF) transcription factor and one of its cofactors, GRF-Interacting Factor (GIF) *(GRF-GIF* henceforth) dramatically increases regeneration frequencies in wheat, triticale and rice. Moreover, by combining *GRF-GIF* and CRISPR-Cas9 technologies, we were able to generate large numbers of edited wheat plants. The *GRF-GIF* transgenic plants were fertile and without obvious developmental defects, likely due to the multiple post-transcriptional regulatory mechanisms operating on *GRF-GIF* in adult plant tissues. Finally, we show that dicot versions of *GRF-GIF* chimera improve regeneration efficiency in citrus, grape and pepper suggesting that this strategy can be expanded to dicot crops.

P-24

Supervised Machine Learning Techniques Utilized in Plant Tissue Culture Research. MELEKŞEN AKIN[1], Sadiye Peral Eyduran[1], Ecevit Eyduran[2], and Barbara M. Reed[3]. [1]Department of Horticulture, Iğdır University, Iğdır, TURKEY; [2]Department of Business Administration, Quantitative Methods, Iğdır University, Iğdır, TURKEY; and [3]Department of Horticulture, Oregon State University, Corvallis, OR 97370. Email: akinmeleksen@gmail.com

Generally, tissue culture data are complex and inherently non-linear. Therefore, the classical linear models can be subject to severe limitations. Tree-based models and Multivariate Adaptive Regression Splines (MARS) are non-parametric algorithms that can handle multidimensional linear and non-linear relationships between input and output variables without requiring the restrictive distributional assumptions of the traditional linear models. These machine learning approaches are appropriate to analyze both numerical and categorical multivariate data sets. Decision trees generate easy to interpret elegant trees reflecting both main and interaction effects of each explanatory variable on the output. The trees are built according to the principle of recursive partitioning, where the heterogeneous data are recursively split into subsets of homogeneous groups (observations with similar response values). The MARS algorithm displays the relative contribution of each explanatory variable on the response by generating a transparent mathematical equation. The region of association change between the input and output variables are defined by a series of piecewise linear splines providing flexibility to the model and allowing to map non-linear relationships. Besides showing the effect size of the variables, both of these machine learning algorithms are categorized as supervised learning and can be employed to predict and optimize the tested factors on a tissue culture experiment for a better plant growth. This study aims to provide a soft introduction to the principles of decision trees and MARS algorithm and show how to implement them utilizing various packages in R software, thus inspiring future application of these promising supervised machine learning approaches on multi-dimensional *in vitro* data.

P-25

Process Optimization: Moving from Process Control to DoE. SCOTT BURGMEYER. Creative Solutions Group, Norwalk, IA 50211. Email: scott@creativesolutionsgp.com

Organizations strive to build more value, revenue and throughput in their key and support operations. This is where Process Optimization enters. Process Optimization focuses on ensuring information and product flows smoothly within your organization. Operationally we focus on process control and through use of Lean Six Sigma via The DMAIC Way® we ultimately apply Design of Experiments (DoE) to yield the best outcomes of the process possible. During this session learn how these tools can optimize your organization by exploring: The DMAIC Way®, Process Control, and Design of Experiments.

P-26

The DOE Approach to Experimentation and Quality Control. R. P. NIEDZ. U.S. Department of Agriculture, Agricultural Research Service, U.S. Horticultural Research Laboratory, Ft. Pierce, FL. Email: randall.niedz@usda.gov

Design of Experiments (DOE) is a framework for conducting experimentation. The basic framework is not a single "DOE experiment" but a sequence of efficient iterative experiments. At each stage, an experiment is designed using the information learned from the previous experiment. There are 4 stages, sometimes called SCOR – Screening, Characterization, Optimization, and Ruggedness testing (Mark J. Anderson, The ITEA Journal of Test and Evaluation 2019; 40: 56–61). Each stage has its own set of experimental design structures that are deployed sequentially. The result is an optimized and repeatable process. A micropropagation system will be used to illustrate each of the SCOR steps in the DOE framework. The micropropagation example will include the logic of each of

the SCOR steps, explanations of the various designs, where they fit into SCOR, how they are used, and the various considerations at each stage. Though rarely seen in the literature, ruggedness testing is included as the final SCOR step as it will ensure that the resulting process is robust and will hold up when used in the field.

P-27

Impacts of the Regulatory Environment for Gene Editing on Delivering Beneficial Products. D. JENKINS[1], CHLOE PAVELY[2], A. Atanassova[3], and R. Dobert[4]. [1]Pairwise Plant Services, Inc., 807 East Main Street, Suite 4-100, Durham, NC 27701; [2]Calyxt, Inc., 2800 Mount Ridge Road, Roseville, MN 55113; [3]BASF Business Coordination Centre – Innovation Center Gent, Technologiepark 101, 9052 Gent, BELGIUM; and [4]Bayer Crop Science. 700 Chesterfield Parkway West, St. Louis, MO 63017. Email: djenkins@pairwise.com, chloe.pavely@calyxt.com

Plant breeding innovations, such as various forms of genome editing, offer tremendous potential to deliver crops that provide consumer benefits, improve sustainability and democratize access to this Nobel Prize winning technology. However, while many governments are in the early stages of formulating policy with respect to genome edited products, some have put forth regulatory frameworks that take different approaches. Central to the discussion is the principal of "like products should be treated in like ways" and the subsequent employment of exemptions for products that could have been produced through conventional breeding. There are misconceptions about the justifications for exemptions and what they mean from both a food safety and overall food system regulation standpoint. In this paper we provide a short overview of the different classes of global regulatory systems, a discussion on the use of exemptions, insight into post-market regulatory systems and their applications to these products, and the benefits of such approaches.

P-28

Is it CRISPR? – Detection of Genome Edits. R. SHILLITO[1], F. Meulewaeter[2], A. Van Hoecke[2], Katelijn Dhalluin[2], and S. Whitt[1]. [1]BASF Corporation, 407 Davis Drive, Morrisville, NC 27560 and [2]BASF Innovation Center Gent, Technologiepark 101, 9052 Gent Zwijnaarde, BELGIUM. Email: raymond.shillito@basf.com

Genome editing tools such as TALENs, and CRISPR/Cas are increasingly being applied to research in plant sciences and to breeding improved plants. The advent of this technology has led to the need to develop detection and diagnostic methods for use during research, at the cell and plant level, and during subsequent breeding and possible development of a commercial product.Detection of genome edits in a research context employ a wide range of methods, including PCR, digital PCR and sequencing approaches.

P-29

Contributions of Transformation and Genome Editing in Maize to Global Agriculture. ALBERT P. KAUSCH. The Plant Biotechnology Laboratory, Department of Cell and Molecular Biology, University of Rhode Island, West Kingston, RI 02892. Email: apkausch@uri.edu

The importance of maize (*Zea mays* L.) to global agriculture, world economy, and food security is widely known. All current maize breeding programs are now deeply integrated with the recent and rapid technological advances in genome sequencing, computational biology, new genotyping and phenotyping technologies, transformation technologies and advanced molecular and breeding approaches including genome editing, and doubled haploid technology. The synergistic applications of maize transformation, advanced genomics and genome editing provide a potent interdependent triad for functional genomics research and advanced molecular breeding. Implementation of the capabilities to transform maize and conduct genome editing will profoundly influence the dynamics of global agriculture ushering in a new era of varietal development and molecular breeding. Since the introduction of transgenic maize into advanced breeding programs over 53.6 Mha accounting for 29% of the world's maize production have been planted globally. Up to 10% higher yields are achieved using new varieties generated using genetic modification technologies compared to similar conventional varieties By extension, the impact new varietal releases developed through genome editing will likely be more significant. Recent advances in transformation and genome editing technologies facilitate an even wider applicability the development of new varieties with increasing complex traits, the introduction of biochemical pathways and the use of synthetic biology. The future is of genotype independent maize transformation and genome editing, as the working platform will impact world agriculture, global food security and plant science forever.

P-30

Development of a Tissue Culture Based *Agrobacterium*-mediated Transformation System for Cannabis sativa L.

(marijuana). JANESSE HOLMES and Zamir K. Punja. Department of Biological Sciences, Simon Fraser University, Burnaby, CANADA. Email: janesseh@sfu.ca

Cannabis sativa L., which has risen in prominence in recent years, is valued globally for its seed protein, fiber, and cannabinoid properties. THC-containing cannabis (marijuana) has been continually crossbred to develop strains with increasingly higher THC content and for an overall higher quality product. Tissue culture methods are one way in which these desirable strains can be propagated, stored, and maintained using relatively little space and resources. Previous research has shown that C. sativa L. can be recalcitrant to tissue culture methods, depending on the strain used. In addition, genotype differences and endophyte contamination can present challenges to reliable tissue culture protocols. We evaluated leaf and petiole explants for callus and root induction response in five cannabis strains and observed that strains White Rhino and Pennywise were more responsive than Death Bubba, Island Honey, and Afghan Kush. We also evaluated nodal explant growth on tissue culture media supplemented with MS basal salts compared to DKW basal salts and found little difference. Our research suggests that mother plants treated with fluopyram, a systemic fungicide, show decreased endophytic contamination in nodal explant tissue culture compared to an untreated control. Cannabis is susceptible to phytopathogens and due to strict pesticide and microbe threshold levels, producers are limited in options for pathogen control. We provide an update on transforming cannabis with Arabidopsis thalianaNPR1, a gene encoding a transcription factor involved in turning on various defense genes in the Salicylic acid (SA) dependent pathway of Systemic Acquired Resistance (SAR).

P-33

Building Partnerships and Approaches for Overcoming Bottlenecks that Prevent Efficient Genetic Engineering and Gene Editing. M. ANNIE SALTARIKOS. Bayer U.S. - Crop Science, 700 Chesterfield Pkwy West, Chesterfield, MO 63017. Email: annie.saltarikos@bayer.com

As the world's population continues to rise and the amount of available farmland becomes increasingly limited, there is a continued need to develop high yielding crops in sustainable manner. This need has driven the rapid adoption and deployment of using plant biotechnology practices across the major crop species to deliver genes or favorable traits (both plant-derived from other organisms) to increase yield and provide resistance to both biotic and abiotic stresses. However, the technical approaches to generating transgenic and gene editing events and launching these breakthroughs is still quite challenging and can limit the pace with which society can benefit from these solutions. In this session, both the bottlenecks as well as potential solutions for addressing these challenges to high-throughput transgenic and gene edited plants in the major crop species will be presented. These two presentations will serve as a basis for a panel discussion immediately following with representatives from both the public and private sector to explore both technical solutions and how partnerships between sectors can help drive and enable new solutions.

P-34

Challenges that Hinder Progress of Genetic Engineering and Gene Editing. JOYCE VAN ECK. The Boyce Thompson Institute, Ithaca, NY and Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, NY. Email: jv27@cornell.edu

With the growing population, increases in biotic and abiotic stresses from climate change on plants, and factors causing the loss of plant biodiversity, implementation of all approaches for rapid crop improvement has become even more critical. Genetic engineering and gene editing play vital roles in this improvement, however, efficient approaches are still difficult to achieve in many plant species, especially in some important food crops. Bottlenecks can arise at various stages of the process including plant regeneration, gene delivery, and recovery of high-quality modified lines. The premise of the session in which this presentation will take place is to facilitate discussion on strategies to best address these bottlenecks and to identify ways to build partnerships within and between public and private research organizations to break the bottlenecks. Therefore, this presentation will set the stage for discussion by outlining the various difficulties encountered that prevent achieving routine, highly-efficient methods. Issues presented will start with those related to response in tissue culture followed by transformation and recovery of transgenic and edited lines. In addition, difficulties encountered beyond the methodologies such as finding personnel with desirable skill sets and funding availability will also be presented.

P-35

Somatic Transgene Excision Strategies for Gene Editing in Clonally Propagated Plants: Inducible Excision Performance in Transgenic Poplar. STEVEN H. STRAUSS, Greg S. Goralogia, Anna Brousseau, Daniel Casey-Hain, Cathleen Ma, and Michael Nagle. Department of Forest Ecosystems and Society, Oregon State University, Richardson Hall, Corvallis, OR 97331. Email: Steve.Strauss@oregonstate.edu

CRISPR/Cas9-based approaches for gene editing in plants most commonly involve the insertion of editing components stably in the genome, followed by sexual segregation to remove the transgenic components. In clonally propagated or sterile crops, options for removal of editing transgenes are limited, and transient approaches inefficient. To expand options for editing in such crops, we developed a vector system based on developmental and chemical control of Cre recombinase expression that would excise the majority of transgenic components.To find promoters suitable for expressing Cre recombinase during shoot organogenesis, we cloned several Arabidopsis and *P. trichocarpa* promoter regions including *WUSCHEL (WUS)*, *SHOOT MERISTEMLESS (STM)*, and *COLD SHOCK PROTEIN (CSP3)*, placed them upstream of a GFP coding sequence and transformed them into *Populus tremula x alba* 717-1B4. Analysis of these promoter:GFP constructs found the *CSP3* promoter to be best suited to driving Cre expression, however expression in later stages of callus development prompted us to also include chemical-inducible Cre activity control where the *CSP3* promoter drove a Cre protein fusion to the rat glucocorticoid receptor. As a proof-of-concept we sought to produce a gene edited and "clean" semi-dwarf and sterile horticultural variety. We generated dwarf and sterile 717 *P. tremula x alba* hybrid poplars by introducing in-frame deletions of the DELLA domain in a homolog of the Arabidopsis *REPRESSOR OF GA 1 (RGA1)* gene, and loss of function mutations in the *LEAFY* gene. We used fluorescent marker genes to monitor the rate and spatial extent of Cre excision. Using a LoxP flanked fluorescent switch system with a 35S or *CSP3* promoter-driven CRE-GR gene, we found less than 1% premature excision events during transformation up to the late callus stage. This suggests adequate control, thus allowing Cas9-mediated editing prior to excision and regeneration. We further discuss the rate of editing and transgene excision, and our ongoing efforts to improve the system using different marker systems and methods of chemical induction.

P-36

DNA-free Genome Editing with Preassembled CRISPR/Cpf1 Ribonucleoproteins in Corn, Soybean and Canola. ASHOK SHRAWAT, Dafu Wang, Michelle Valentine, Vladimir Sidorov, and Peizhen Yang. Bayer Crop Science, 700 Parkway West, Chesterfield, MO 63017. Email: ashok.shrawat@bayer.com

Genome Editing for targeted gene improvement is widely used in the field of plant science for basic research as well as for trait improvements in commercial crops. In this study, we present the recent developments and techniques in the DNA-free genome editing of row crops. The preassembled Cpf1–gRNA ribonucleoproteins without selection markers were introduced into maize and soybean embryos via biolistic delivery, and into canola protoplast by transfection. Using these methods of delivery, we have successfully demonstrated the recovery of edited plants in maize, soybean, and canola.

P-37

Generation of Agrobacterium Strains that Introduce T-DNA into the Nucleus But Do Not Efficiently Integrate It into the Plant Genome. L.-Y. LEE and S. B. Gelvin. Department of Biological Sciences, Purdue University, West Lafayette, IN 47907–1392. Email: lee34@purdue.edu

Agrobacterium-mediated transformation is the most commonly used method to introduce genome editing reagents into plants. For both regulatory and technical reasons, plants lacking integrated T-DNA that encodes these reagents are highly desirable. Removal of integrated T-DNA is routinely accomplished by segregating out T-DNA insertions in subsequent generations. However, genetic segregation of chromosomes containing T-DNA can be cumbersome, time-consuming, and expensive, especially for those species with long generation times (e.g., many tree species) and those crops routinely propagated vegetatively. It would therefore be useful to employ an *Agrobacterium* strain that efficiently delivers T-DNA and transiently expresses genome engineering reagents, but does not integrate the genes encoding these reagents. We focused on mutagenizing VirD2, the protein linked to T-strands which leads them from *Agrobacterium* into plant cells, then targets T-strands to the plant nucleus. We generated more than 180 VirD2 mutants with either single or double amino acid substitutions throughout the protein, and tested them on Kalanchoe and tobacco plants for their abilities to facilitate stable and transient transformation, respectively. VirD2 mutants that maintained transient transformation activity but lost efficient stable transformation activity were further tested using quantitative *Arabidopsis* root transformation assays. We are currently in the process of testing several mutants for their ability to enable genome editing without concomitant T-DNA integration.

P-38

Developing Ensifer-mediated Plant Transformation as an Alternative to Agrobacterium-mediated Transformation. S. B. GELVIN[1], L.-Y. Lee[1], R. A. Lapham[1], C. Roleck[1], A. Govindan[2], and E. Mullins[2]. [1]Department of Biological Sciences, Purdue University, West Lafayette, IN 47907–1392 and [2]Crop Sciences Department, Teagasc, Oak Park, Carlow, R93 XE12, IRELAND. Email: gelvin@purdue.edu

Agrobacterium-mediated plant transformation (AMT) has been the major technology for producing transgenic plants for the past 35 years, and more recently has been used for introducing genome engineering reagents into plants. However, AMT has limitations, including the host range of susceptible plants and the propensity for particular plant species/cultivars to react against Agrobacterium with a necrotic response. Other bacteria, when properly engineered, can also transfer DNA to plants, although these bacteria are less efficient than is Agrobacterium. Recently, Ensifer adhaerens has been shown to transform plants (Ensifer-mediated transformation, EMT) when supplied with plasmids containing Agrobacterium T-DNA and vir genes. Because Ensifer is not a pathogen, plants may not recognize it and mount a defense response. EMT may therefore be useful to extend the host range of AMT. We conducted RNAseq analysis of Arabidopsis roots incubated with Agrobacterium or Ensifer. Agrobacterium infection resulted in >1500 differentially expressed plant genes (DEG), including many involved in defense and signaling. Ensifer infection resulted in ~90% fewer DEG, and almost none of these is involved in defense and signaling. Most Arabidopsis mutants transform similarly to wild-type using AMT or EMT, although the efr mutant, which transforms better than wild-type using AMT, transforms similarly to wild-type using EMT. Arabidopsis therefore may not recognize the Ensifer Ef-Tu protein as a PAMP. Because of this differential defense response of Arabidopsis to Agrobacterium and Ensifer, we wish to develop EMT as an alternative to AMT. However, EMT is less efficient than is AMT, and Ensifer is difficult to manipulate in the laboratory because it naturally harbors numerous antibiotic resistances. We are currently engineering removal of kanamycin resistance from Ensifer to permit the introduction of plasmids harboring various vir genes important for strong Agrobacterium virulence. We shall test various vir gene combinations in Ensifer for their effect on virulence of model plants and crop species.

P-39

Launching Cannabis Research in a Cell- and Molecular-Biology Lab. BASTIAAN BARGMANN, Keely Beard, and Audrey Boling. Virginia Tech School of Plant and Environmetal Sciences, 220 Ag Quad Lane, Blacksburg, VA 24061. Email: bastiaan@vt.edu

Cannabis sativa L. is a valuable, up-and-coming industrial crop with a substantially growing market. However, due to an extended period of legal restriction, research with cannabis has been limited, particularly in laboratory settings. Expanding the application of biotechnological techniques to cannabis can facilitate addressing species-specific impediments to improving crop traits and further fundamental understanding of its intricacies. Here, we describe application of protoplast transformation for the study of transient gene expression in a low-THC cannabis cultivar. To produce explant tissue as a source of protoplasts, a method for hormone-free *in vitro* micropropagation is established. Protoplasts are isolated from young leaves of the micropropagated stocks and transiently transformed with plasmid DNA carrying a fluorescent marker gene. This is the first report of protoplast transformation in this species. A protoplast isolation yield is achieved of up to 2×10^6 cells per gram of leaf material, vitality staining shows that up to 82% of isolated protoplasts are viable, and quantification of the cells expressing a fluorescent protein indicates that up to 31% of the cells can be successfully transformed. Additionally, protoplasts are transformed with an auxin-responsive reporter gene and the reaction to treatment with indole-3-acetic acid is quantified using flow cytometry. This work demonstrates that relatively minor modification of standard techniques can be used to study this important emerging crop.

P-40

Coping with Hyperhydricity, Culture Decline, and Challenging Rooting in Hemp Micropropagation. J. LUBELL-BRAND. University of Connecticut, 1376 Storrs Road, Unit 4067, Storrs, CT 06269. Email: jessica.lubell@uconn.edu

We developed an efficient method for generating clonal liner plants for commercial scale hemp (*Cananbis sativa*) production that uses micropropagation and retipping (taking cuttings using new shoots from recently micropropagated plants). Our micropropagation protocol consists of four stages: culture initiation, shoot multiplication, in vitro pre-rooting, and ex vitro rooting in rockwool. During culture initiation we use vented lid vessels and medium containing agar at 1% (*w/v*) to prevent hyperhydricity of shoots. For shoot multiplication, good quality cultures may be maintained for four months or more using MS plus 2.5′ MS mesos, 500 $mg{\cdot}L^{-1}$ ammonium nitrate, 0.5 $mg{\cdot}L^{-1}$ metatopolin, 0.1 $mg{\cdot}L^{-1}$ gibberellic acid, 3% (w/v) sucrose, and 0.8% (w/v) agar. Cultures are subcultured every 21 d. Microshoots are pre-rooted in vitro using MS with 1 $mg{\cdot}L^{-1}$ indole-3-butyric acid (IBA) for 14 d. Next, they are transferred ex vitro to rockwool and 75 to 100% rooting may be achieved. All in vitro micropropagation stages utilize vented lid vessels. Retip cuttings root over 90% in rockwool and the retipping process can produce 9 times as many liner plants as stem cuttings derived from traditional stock mother plants.

P-41

Optimizing Tissue Culture for Commercial Production. I. B. COLE. Zenlabs, 7745 Arjons Dr., San Diego, CA 92126. Email: ian@zenleafca.com

The commercial cannabis market in California has been established for many years, and has exploded following statewide legalization. Several companies have turned to tissue culture to provide plant material to growers and have quickly realized that developing protocols for cannabis TC requires substantial optimization. We have a genetic bank of over 180 varieties and have encountered many of these problems. Additionally, our lab focuses on pathogen elimination and development of clean mother stock and working through these protocols has been challenging as well. In this presentation we will share some of our challenges, successes and strategies for producing clean, healthy plants from the lab.