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Plant Symposia

P-1

The Role of Secondary Metabolites and COR Proteins in Plant Freezing Tolerance. DIRK K. HINCHA, Elisa Schulz, Anja Thalhammer, Takayuki Tohge, Ronan Sulpice, Alisdair R. Fernie, and Ellen Zuther. Max-Planck-Institute of Molecular Plant Physiology, Am Mühlenberg 1, D-14476 Potsdam, GERMANY. Email: hincha@mpimp-golm.mpg.de

Freezing tolerance is a key factor limiting the geographical distribution of plant species and agricultural yield. Plants from temperate regions increase their freezing tolerance in response to low, non-freezing temperatures in a process termed cold acclimation. Natural variability in the freezing tolerance of *Arabidopsis thaliana* was phenotyped by electrolyte leakage analysis in 54 accessions from throughout the Northern hemisphere under non-acclimated and acclimated conditions. Additionally, analysis of secondary metabolites was performed using liquid chromatography- mass spectrometry (LC-MS). This revealed a massive accumulation of flavonols and anthocyanins during cold acclimation. The patterns of these compounds differed significantly between accessions. Further, the expression of genes encoding transcription factors and enzymes involved in flavonoid biosynthesis was investigated by qRT-PCR. Comprehensive correlation analysis allowed the identification of genes and metabolites that are closely connected in their respective pathways. Comprehensive studies of k.o. mutants and over-expressors of different steps in the flavonol and anthocyanin biosynthesis pathway indicated a functional role of specific groups of these molecules in plant freezing tolerance. In addition to many metabolites, COR proteins are massively induced during cold acclimation. We investigated the role of chloroplast-localized COR15A and COR15B in RNA interference knock-down and in over-expression lines, which showed that COR15 proteins are necessary for *Arabidopsis* to achieve full cold acclimation. Enzyme activity measurements indicated that different chloroplast enzymes differ in their in-vivo freeze-thaw stability. However, while cold acclimation increased the freezing stability of some enzymes, the absence or presence of COR15 proteins had no influence on enzyme activity after freezing, ruling out a role of COR15 proteins in enzyme stabilization in vivo. Rather, our data support a role of COR15 proteins in stabilizing cellular membranes.

P-2

Regulatory Pathways that Control Plant Freezing Tolerance. MICHAEL F. THOMASHOW. MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824. Email: thomash6@msu.edu

Plants from temperate regions increase in freezing tolerance in response to low non-freezing temperatures, a phenomenon known as cold acclimation. In Arabidopsis, cold acclimation is associated with the induction and repression of hundreds of genes. Our goal is to identify the regulatory pathways that bring about these changes in gene expression and determine which pathways have roles in freezing tolerance. One such pathway, the CBF pathway, involves action of three regulatory genes-*CBF1*, *CBF2* and *CBF3*-that are rapidly induced in response to low temperature. The *CBF* genes encode closely related transcription factors that control the expression of more than 100 cold-regulated genes designated the CBF regulon. Expression of the CBF regulon increases freezing tolerance. Regulation of the CBF pathway is complex. We established a role for CAMTA family transcription factors in cold induction of the *CBF* genes and have proposed a possible molecular mechanism for the long sought link between low-temperature calcium signaling and cold-regulated gene expression. In addition, we identified components of the circadian clock that have roles in both circadian regulation and cold induction of the CBF pathway and have found that these clock components are required for plants to attain maximum freezing tolerance at both non-acclimating and cold acclimating temperatures. Finally, we have found that the pathway is regulated by photoperiod. During the warm growing season when day-length in long, the pathway is repressed by phytochrome B and two phytochrome-interacting factors-PIF4 and PIF7-thus mitigating allocation of energy and nutrient resources toward unneeded frost protection. This repression is then relieved by shortening day-length resulting in up-regulation of the CBF pathway and an increase in freezing tolerance in preparation for coming cold temperatures. Taken together, these results indicate that Arabidopsis freezing tolerance is conditioned by multiple environmental factors that mediate their effects through regulating expression of the CBF pathway.

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P-3

Live *In Planta* Monitoring of Cold-responsive Gene Expressions at Low Temperature. Yoko Tominaga, Yukio Kawamura, and MATSUO UEMURA. Cryobiofrontier Research Center, Iwate University, Morioka 020-8550, Japan. Email: uemura@iwate-u.ac.jp

Large-scale omics studies such as transcriptomics, proteomics and metabolomics have provided comprehensive information on the quantitative changes of gene products in response to environmental stresses. However, the outcome of these studies was limited to the particular time point of the treatment since the isolation of RNAs, proteins or metabolites should disrupt plant tissues and cells. To understand responses to environmental signals, it is important to investigate continuously how the gene expression is regulated spatially and temporarily in the plant. Here, we have developed *in planta* monitoring system of cold-responsive gene expressions under controlled temperature and photoperiod conditions using *luciferase* (*LUC*) connected to the promoter of the target gene. The promoter of *COR15a*, a well-investigated cold-inducible gene in *Arabidopsis*, was selected as the indicator of the responsiveness to low temperature in order to observe spatial distribution and temporal regulation of the gene expression during cold acclimation. Monitoring the changes of the luminescence intensity from the individual plants revealed that the cold-inducible gene have characteristic temporal expression patterns in response to various temperatures, which are closely associated with the light status and photosynthesis. These results demonstrated the molecular events of how plant responses to temperature and light signals at the whole-plant level in non-destructive way. This research was supported by Grants-in-Aid for Scientific Research (#22120003 and #24370018 to MU, and #25292205 to YK).

P-4

Enhanced Methods for Doubled Haploid Production in *Triticum aestivum*. T. SUELTER[1], F. Chumley[1,2], N. Rothe[1], R. Berard[1], and H. N. Trick[2]. [1]Heartland Plant Innovations, Inc., Manhattan KS 66502 [2]Department of Plant Pathology, Kansas State University, Manhattan KS 66506. Email: tsuelter@heartlandinnovations.com

Doubled haploid (DH) technologies have become an integral part of crop improvement systems for many diverse species, including cereals. The ability to produce 100% homozygous DH lines quickly and efficiently has greatly accelerated plant breeding, trait discovery, and marker development. In maize, DH production is easy and inexpensive, owing to the unique reproductive biology of maize flowers and the wide availability of maize lines that efficiently induce formation of haploid seeds. Major corn breeding companies routinely produce and use more than 1 million DH lines per year. In contrast, the world's biggest wheat breeding operations produce 10- to 20-fold fewer DH lines, and many wheat breeders are not able to access the technology at all. Many unique challenges complicate and reduce the efficiency of wheat DH production. Standard wheat DH methods utilize maternally-derived haploids generated via maize pollination and haploid embryo culture, followed by colchicine-induced chromosome doubling. Opportunities will be discussed for innovation using *in vitro* biology to improve efficiency, reduce labor, shorten timelines, and reduce costs for each step of wheat DH production. Achieving these goals are essential to stabilizing and increasing the yield of wheat, which is the world's most-widely grown crop.

P-5

Microspore Embryogenesis in Maize. JOSEPH F. PETOLINO. Dow AgroSciences, 9330 Zionsville Rd., Indianapolis, IN. Email: jfpetolino@dow.com

Maize micrspores are competent to shift their development from their normal gametophytic pathway of pollen formation, germination and fertilization to undergo direct embryogenesis resulting in haploid embryo-like structures. As with zygotic maize embryos, these microspore-derived embryo-like structures can be used to generate haploid tissue cultures capable of *in vitro* proliferation and subsequent plant regeneration. The ability to undergo microspore embryogenesis is genotype-specific and heritable. Highly androgenic germplasm has been developed for use in both basic and applied aspects of genetic modification.

P-6

Towards a Non-transgenic CENH3-mediated Genome Elimination System. SHAMONI MAHESHWARI, Simon Chan & Luca Comai. University of California, Davis, Davis, CA. Email: smaheshwari@ucdavis.edu

In the model plant *Arabidopsis thaliana*, a cross between a wild-type parent and a plant with engineered modifications in the centromere-specific histone H3 (CENH3) leads to extensive mis-segregation. The most dramatic outcome of which is the production of haploids through the elimination of the parental genome marked with the modified CENH3. Unlike canonical histone proteins that are highly conserved across the plant and animal kingdoms, CENH3 is evolving at an accelerated rate. This raises the intriguing possibility that naturally

derived variation in CENH3 could also cause segregation errors potentially leading to genome elimination. I will be presenting data that demonstrates that a wild-type CENH3 gene when introduced into a different but related species can complement the loss of the local CENH3 gene, however when the complemented plant is crossed to an individual expressing the native CENH3, the one with the foreign CENH3 acts as a haploid inducer. The implication of this result is that natural alleles of CENH3, when introduced into a non-native genetic background, can act as haploid inducers because they result in "weaker" centromeres. We are currently surveying CENH3 alleles from a broad range of taxa to better understand what features of CENH3 are responsible for genome elimination with a view to generating a non-transgenic haploid induction system.

P-7

Precision Breeding of Grapevine: A Biologically-sound Method for Genetic Improvement. D. GRAY[1], Z. Li[1], J. R. Jasinski[1], M. Dutt[1], and S. Dhekney[2]. [1]Mid-Florida Research & Education Center, University of Florida/IFAS, University of Florida, 2725 S. Binion Rd., Apopka, FL 32703 and [2]University Wyoming, Department of Plant Sciences, Univ. Wyo., Sheridan REC, 663 Wyarno Road, Sheridan, WY 82801. Email: djg@ufl.edu

Precision breeding, also termed cisgenic or intragenic cultivar development, utilizes only defined genetic fragments from sexually-compatible parents. Precision breeding is the logical extension of conventional breeding inasmuch as it is fully consistent with the grapevine lifecycle. Grapevine is unique among all crops because of its special sensory attributes. A relatively small number of well-known elite cultivars and their landraces account for the majority of world wine production. They are subject to significant disease pressures, making substantial chemical control and sanitation necessary in many regions. Heightened genetic resistance is urgently required to ease production. However, it is difficult-to-impossible to add only resistance traits to elite cultivars using conventional breeding techniques due to the obstacles of inbreeding depression, self-incompatibility and the long grapevine lifecycle. However, technology to bypass these obstacles is finally available. Over the years we have developed crucial cell culture and gene insertion systems for a wide range of grapevine cultivars. The genomic sequence of 'Pinot Noir' and advanced computational tools only recently provided the final piece needed to enable precision breeding of grapevine. A concern expressed against using precision breeding has centered on the fact that insertion sites are random and may cause "unintended consequences". However such concerns overlook the fact that plant sexual reproduction randomly induces far greater variability through the many mechanisms of meiotic crossing over and transposition. Such well-known and expected variability is one reason that plant breeders must make so many controlled pollinations and evaluate often thousands of progeny in order to find one desirable individual. Precision breeding, which instead utilizes the comparatively stable mitotic pathway, is more predictable, efficient and less disruptive than conventional breeding because only specific traits are transferred and key obstacles are avoided. However, with new varieties under development, substantial field evaluation, as is the norm for conventionally-bred crops, will be required to determine whether precision bred versions of elite cultivars will possess desirable attributes and/or otherwise be useful.

Disclosure: Author received research grant from FDACS VTF.

P-8

'FasTrack' – A Revolutionary Approach to Long-generation Cycle Tree Fruit Breeding. Ralph Scorza[1], DOUG RAINES[1], Chris Dardick[1], Ann M. Callahan[1], Chinnathambi Srinivasan[1], Ted M. DeJong[2], Jay Harper[3], Sarah Castro[2], and Mark Demuth[1]. [1]USDA Appalachian Fruit Research Station, Kearneysville, WV; [2]University of California, Davis, CA; and [3]Penn State University, University Park, PA. Email: rscorza@afrs.ars.usda.gov, doug.raines@ars.usda.gov

The American tree fruit industry is facing challenges of climate change, reductions in available labor, the need for reduced chemical inputs, the spread of exotic pests and pathogens, and consumer demands for improved fruit quality. To meet these challenges breeding new adapted fruit cultivars is critical. Current limitations of fruit breeding include long juvenility periods, significant field costs, and yearly limitations on flowering and fruiting related to dormancy. Much research has focused on marker assisted selection (MAS), germplasm characterization, and genetic engineering (GE) as means to advance tree fruit breeding. However, these strategies are all still limited by long generation cycles. We have developed a system to shorten the breeding cycle of fruit trees and other long-breeding-cycle crops. We have overcome the juvenility and environmental limitations of flowering and fruiting by incorporating a gene that induces trees to flower early and continually. This "FasTrack" breeding system has reduced the generation cycle of plum from 3-7 years to less than one year. The system allows for the rapid incorporation of important traits into plums and other long-generation-cycle crops and then in the final generation, when substantial improvements are clearly evident, only seedlings that do not contain the early flowering gene are selected, and these are not considered to be genetically engineered. The selected trees may then be used directly as new varieties, or improved lines for further breeding. Such an approach provides tree fruit and

other long-cycle crop breeders with the ability to respond to new market demands, climate changes, and invasions of new diseases and pests in a way never before possible.

P-9

Recombinase-directed Plant Gene Transfer. DAVID W. OW. South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, CHINA. Email: dow@scbg.ac.cn

For many important crops, classical breeding is used to move the transgene from a transformable laboratory variety to diverse field cultivars. Though fruit crops are generally propagated by clonal cuttings, the development of new varieties often takes it through the genetic breeding process. The probability to assort the 'n' number of transgenic loci into a single genome, $(¼)^n$ for a diploid species, may not seem onerous. However, given the 'x' number of other nontransgenic traits that a breeder needs to assemble into the same genome, the $(¼)^{n+x}$ number could reach infinitesimally small. We favor a strategy where the number of segregating transgenic locus is kept to a minimal through the site-specific appending of new DNA next to previously placed transgenes, followed by subsequent breeding of the transgenic locus from the transformable variety to the numerous field cultivars. In tobacco, we had completed 2 rounds of integrase-mediated DNA stacking, and lines with faithful structure and function were obtained at a workable frequency, even though gene silencing was observed in other lines. To translate this research into crop plants, we have begun creating target lines in rice. Progress to date will be presented.

P-10

Genetic Improvement of Stone fruits Using SNPs. S. JAYASANKAR[1,2,] H. El Sadr[1,2], I. El-Sharkawy[1,2], S. Sherif[l], and T. Banks[2] · [1]Department of Plant Agriculture, University of Guelph, Guelph ON, [2]Vineland Research Station, Department of Plant Agriculture, University of Guelph, Guelph ON. Email: jsubrama@uoguelph.ca

Stone fruits such as peach [*Prunus persica* (L.) Batsch] and plum [*P. salicina* L.] have a long breeding cycle. Further factors such as heterozygosity of the existing cultivars, incompatibility issues and an abstract flowering period makes conventional breeding very cumbersome and often with little, predictable results. Molecular markers (MM), offer promise to increase the predictability of the hybrids in the seedling stages itself. This can save a lot of time, space and money for stone fruit breeding programs. In addition such MM approaches also help to concentrate the population towards a particular trait of interest such as disease resistance etc. In this study we used a peach population consisting of 133 peach accessions to conduct an association mapping study. Each accession was phenotypically evaluated for (i) flowering date (FD) (ii) Maturity date (MD). DNA was collected from each accession and the accessions were sequenced using the genotyping by sequencing (GBS) protocol. The phenotypic and genotypic data collected were used to conduct association analyses using the GBS bioinformatics pipeline. The objective of the analyses was to identify the genomic region(s) that hold major loci for MD. Similarly, we compared two plum cultivars with varying dates of fruits maturity, where we hypothesized the change in fruit maturity to be a function of growth hormones, especially auxin. Our results revealed that in both peach and plum the difference between early and late accessions could be due to single nucleotide polymorphism (SNP) or an insertion/deletion (INDEL). The results reported here will aid stone fruit breeders in accelerating breeding for MD.

P-11

Plant Adaptation to Mineral Nutrient Stresses in the Soil. LEON KOCHIAN[1], Jiping Liu[1], Miguel Piñeros[1], and Jurandir Magalhaes[2]. [1]Robert W. Holley Center for Agriculture and Health, USDA-ARS, Cornell University, Ithaca NY 14853 and [2]Embrapa maize and Sorghum, Sete Lagoas, BRAZIL. Email: LVK1@Cornell.edu

Because terrestrial plants are sessile organisms, they must directly deal with a wide range of environmental stresses, including abiotic stresses associated with insufficient essential mineral nutrients and excessive levels of toxic metals in the soil. Hence it is not surprising that plants have evolved quite sophisticated and elegant strategies to deal with an often harsh soil environment. Understanding the molecular and genetic control of these strategies is essential for the development of stress tolerant, high yielding, and locally adapted crop varieties. Some of the best understood plant adaptations to mineral stresses involve the evolution of mechanisms for dealing with toxic metals in the environment. These can involve either avoidance (exclusion of the metal from a plant tissue/organ) or true tolerance, which appears to involve chelation and/or sequestration of the metal in an internal compartment. One of the best characterized mechanisms of plant metal tolerance is associated with crop aluminum (Al) tolerance. Al toxicity is a worldwide problem that arises when soil pH values drop to 5 or below; in these acidic soils the rhizotoxic Al^{3+} is solubilized from clay minerals into the soil solution, damaging roots and resulting in reduced water and nutrient uptake. This talk will focus on a major Al tolerance mechanism which involves Al exclusion from the root tip

mediated by Al activation of specialized transporters that release organic acids into the rhizosphere, where they chelate and prevent Al^{3+} from entering the root. Several Al tolerance genes have been cloned recently and these belong to two different families of novel membrane transporters. The function and regulation of these tolerance genes will be discussed, as well as the implications of these findings in facilitating plant improvements for growth on degraded or marginal soils.

P-12

A Systematic Approach for Optimizing *in Vitro* mineral Nutrition in Diverse Red Raspberry Germplasm. S. POOTHONG[1] and B. Reed[2]. [1]Oregon State University, Department of Horticulture, 4017 ALS, Corvallis, OR 97331 and [2]USDA-NCGR, USDA-ARS National Clonal Germplasm Repository, 33447 Peoria Road, Corvallis, OR 97333-2521. Email: sukalya_p@hotmail.com

Growth medium improvement is critical for more efficient micropropagation of a wide range of nursery crops. Genetic variation in species or cultivars makes it difficult to successfully apply a standard *in vitro* growth medium to all plant types. Red raspberry cultivars show a broad response to Murashige and Skoog medium (MS). Some cultivars display stunted growth, hyperhydricity, leaf discoloration, callus, leaf spots or necrosis; all are likely caused by non-optimum concentrations of essential nutrients. Optimization of growth media based on mineral nutrition is very challenging due to the diverse requirements of various plant species and the properties of the chemical nutrients. Response surface methodology (RSM), computer aided experimental design and modeling, provides a new tool for optimizing nutrients. An initial study modeling five groups of MS minerals was conducted using RSM to determine which ones produced the most significant plant responses. Ammonium nitrate, potassium nitrate, mesos ($CaCl_2$, $MgSO_4$ and KH_2PO_4) iron and minor nutrients were tested over a range of concentrations and modeled to represent all possible combinations. The mesos component had the greatest effect on quality and growth, but there were also significant effects from the nitrogen components. Optimization confirmed that all cultivars require significantly higher concentrations of all three mesos components (2.5× to 3×) than those available in MS. Increased mesos altered mineral uptake and a metabolic analysis indicated an increase in three antioxidant compounds. Increased concentrations of nitrate also resulted in significant improvements in plant growth and quality for most genotypes. This systematic approach illustrated that the optimization of growth media is highly effective and can also be applied to other plant species or crops.

P-13

Non-Nutrient Factors That Affect Optimal Nutrient Concentrations. JEFFREY ADELBERG. School of Agriculture Forest and Environmental Sciences, Clemson University, Clemson SC. Email: jadlbrg@clemson.edu

Maximizing growth responses by altering mineral elements in basal tissue culture media is a goal that is seldom experimentally approached. Interactions among nutrient elements suggest multifactor experimental designs to solve for the many independent variables, simultaneously. There are non-nutrient factors that should also be considered as affecting optimal nutrient concentrations. In our model system, micropropagation of turmeric in liquid media, an experiment was conducted to maximize growth by altering macronutrients, while maintaining MS concentrations of meso- and micro-elements. The use of NH_4^+ and NO_3^-, but not K^+, was affected by media volume in the vessel. Nutrient use would only partially be explained by the linear product of the amount of nutrient in a vessel (concentration x volume) as other limitations were imposed by high media volume in the system. Optimal growth was also likely limited by an inadequate supply of P and Mg. A second was experiment was conducted, where P, Mg, Ca and KNO3 were factors (at optimal media volume and NH_4^+ concentrations). P content of plant tissue was raised to accepted agronomic standards (3750 ppm) by increasing P concentration in media (to 2-5x MS levels) or decreasing the amount of plants in a vessel by a response surface that indicated other limitations existed to P accumulation at high plant densities. Similarly, Mg content of plant tissue was raised to accepted agronomic standards (5000 ppm), only when Mg in medium was at highest levels tested (3x MS) concentrations, with reduced plant density, high concentrations of Ca, and moderate levels of KNO3. Growth responses including multiplication rate, the size of plants, and growth in the greenhouse are affected by the internal balance of nutrients in plant tissue. The efficiency of a system, in this case a smallvolume, batch culture system may be optimized by looking at factors such as plant density and media volume, along with the synergistic and antagonistic responses to pairs of other nutrient elements. Systematic multivariate approaches are feasible in small, well-designed experiments.

P-14

Evolution of Molecular Breeding at Monsanto. JEFFREY PAUL WOESSNER. Monsanto Company, Applied Molecular Breeding Center, 3302 SE Convenience Blvd, Ankeny, IA, 50021. Email: jeff.woessner@monsanto.com

Advances in breeding technology have contributed to significant crop improvement over the last 60 years, but with the

world's population projected to grow another 2 billion by 2050, farmers will need to produce more food in the next 50 years than they have in the last 10,000 years combined. With limited arable land, variable climate and changing world diets, it is imperative to enhance crop productivity. Monsanto is working to help farmers achieve this objective through a combination of breeding, biotechnology and improved farm management practices. In this talk, I will focus on the advances in molecular breeding as deployed at Monsanto over the last decade and highlight some of the ways that these tools have impacted the breeding and biotechnology pipelines. Molecular markers are allowing us to mine extensive collections of germplasm quickly and efficiently to increase the rate of genetic gain over conventional breeding, screen for challenging phenotypes like disease tolerance, and combine desirable traits to generate elite seeds more rapidly.

Disclosure: Author was or is employed by Monsanto Company.

P-15

Science of Seed Production Technology: A Transgene Process for Hybrid Seed Production. A. MARK CIGAN. Trait Technologies, Dupont Pioneer 7300 NW 62nd Ave. Johnston, IA. Email: Mark.Cigan@pioneer.com

Development of hybrids has not been fully enabled in crops where current methods of hybrid production are either inefficient or limit the full range of germplasm utilization. At Pioneer a directed molecular genetic approach has been taken, using maize as a model system, to develop several genetic hybridization platforms that not only produce male-sterile plants, but that enable these male-sterile plants to produce nearly 100% male-sterile progeny upon increase. This includes both recessive and dominant-acting systems that can be applied not only to maize but to other crops where the reproductive biology of that crop has been an impediment to wider utilization of hybrids in those crops. One of these is a process designated as SPT (Seed Production Technology). The SPT process utilizes a recessive mutation in a sporophytic gene required for male fertility, creating female parent lines that are male sterile when the mutant allele is homozygous, yet enabling the production of nearly 100% male sterile, non-transgenic progeny. Full male fertility is restored in hybrid progeny upon pollination of the male-sterile female parent plants with pollen from any male parent carrying a wild-type allele of the mutant male-sterility gene. The SPT process offers a reliable, cost-effective method to propagate pure populations of homozygous recessive male-steriles that are non-transgenic for the SPT process. This process is well-suited crops with sequenced genomes and can be a first step in developing or expanding the development of hybrids in those crops. Advances in hybridization systems and progress for producing new crop hybrids will be discussed.

Disclosure: Author was or is employed by DuPont Pioneer.

P-18

Micropropagation Production at Oglesby Plants International, Inc. MURDOCK "RAY" GILLIS. Lab Director, Oglesby Plants International, Inc., 26664 SR 71N, Altha, FL 32421. Email: rayg@oglesbytc.com

Recognizing that there is often a disconnect between institutional research and the frequent end consumer, commercial micropropagation labs, this presentation will seek to highlight the aspects involved in large-scale commercial production of ornamental plants at Oglesby Plants International, Inc. The presentation will feature key areas related to the planning, preparation, and implementation of micropropagation procedures in order to meet large-scale production goals. Special focus will be given to how obstacles specific to commercial plant production are overcome within a micropropagation company.

P-19

Micropropagation Production at Microplant Nurseries, Inc. GAYLE SUTTLE. Microplant Nurseries, Inc., PO Box 237, Gervais, OR 97026. Email: gayle@microplantnurseries.com

Author will take you through a virtual tour of the commercial micropropagation facilities at Microplant Nurseries, Inc. The company has been producing millions of trees, shrubs and perennials over the past 34 years for both the horticultural and agricultural trade in the United States. There will be a discussion of what, how and why the company has grown various crops, lessons learned, as well as the market impacts that have occurred. The development of plant management software and the use of cold storage will also be discussed.

P-20

A Practical Approach of Tissue Culture Automation. JOHN BIJL. Vitro Plus, NETHERLANDS. Email: john@vitroplus.nl

Plant Tissue Culture never must be the goal, see it as just a simple method to reach a much higher goal namely; make the most happy customer ever. If Tissue Culture is too difficult it has to be simplified or otherwise forget automation. How? if sugar causes contamination: just omit the sugar or replace it for something else. If you have 100 crops to focus on: become a specialist, and try to become the best in the World in the product you love. If you ever want to come to the level of real automation of Tissue Culture you have to simplify and

industrialize your processes. Look around you, everything has been invented already by others only not by Tissue Culture people. The only thing: it was not mentioned for Tissue Culture so you have to adapt it. This also means you have to leave your island and start to co-operate with others. My story is a good example about how automation of Tissue Culture can induce market leadership, but it would never have happened if our customers would not be happy too.

P-21

Considerations for Contamination Cleanup in Plant Tissue Culture. DAVID S HART, Gary R Seckinger, and Kenneth C Torres. *Phyto*Technology Laboratories, 9245 Flint Street, Overland Park, KS 66214. Email: david@phytotechlab.com

There are a number of antimicrobials, antifungals, antivirals, as well as products to characterize these pathogens that are commercially available. It is both academically and commercially important to understand how to use these products to their full efficacy and the potential side effects. The molecular mechanisms of action behind these products as well as some of the stability issues that can arise will be presented from a plant tissue culture perspective.

P-22

Best Practices in Tissue Culture for the Control of Biological Contamination. YVONNE A. REID. ATCC, Manassas, VA 20110. Email: yreid@atcc.org

Culturing of plant cells is widely used by scientist in the life sciences. However, one of the most common problems of cultured cells is biological contamination. Regular testing of cells for contamination is crucial, yet this activity is often underappreciated by most research scientists. The adverse effects of cell cultures exposed to biological contaminations (bacteria, fungi, yeasts, viruses and cellular) can be devastating. However, best practices in tissue culture can overcome these contaminations. The obvious consequences of cell culture contaminations are not only the loss of time and money, but obtaining inaccurate and irreproducible results. Different types of biological contaminations and how to prevent them will be discussed.

P-23

Genetic Improvement of the Biofuel Feedstock Sugarcane with Intragenic, Targeted Mutagenesis and Transgenic Biotechnologies. FREDY ALTPETER[1], Je Hyeong Jung[1], Ratna Karan[1], Janice Zale[1], Jae Yoon Kim[1], Hao Wu[1], Hugo Dermawan[1], Bhuvan Pathak[1], Raechelle Gretencord[1], Hui Lui[2], Jason Candreva[2], and John Shanklin[2]. [1]University of Florida, 3062 McCarty Hall, Gainesville, FL and [2]Brookhaven National Lab, Upton, NY. Email: faltpeter@ufl.edu

Sugarcane is one of the most productive biofuel crops due to its superior photosynthetic efficiency and accumulation of large amounts of sucrose in the stem internodes. Sugarcane has a C4 type metabolism for fixation of carbon, allowing it to be very well adapted to biomass production in tropical and subtropical regions. We are exploring the prospects of diverting the carbon storage in vegetative tissues from sucrose to triacylglycerol (TAG) for increased energy density and development of an advanced biofuel. This strategy involves the analysis of combinations of gene expression cassettes for metabolic engineering, supporting biosynthesis and storage of TAG. Correlations between TAG accumulation and gene combinations and their expression levels will be presented. These data indicate the feasibility of genetically engineering the high biomass crop sugarcane to produce TAG, which can be readily converted to biodiesel transportation fuel. In addition to the above transgenic approaches both an intragenic approach and a targeted mutagenesis approach for altered cell wall composition to enhance conversion of lignocellulosic sugarcane residues to biofuel will also be discussed.

P-24

Engineering Sesquiterpene-based Biofuels in Sorghum. I. S. CURTIS. Chromatin Inc., 2109 S. Oak St., Suite 101, Champaign, IL 61820. E-mail: icurtis@chromatininc.com

Sorghum is a highly productive, drought-tolerant, C4 photosynthesis cereal that has an unmatched versatility by providing high yields of starch, sugar and lignocelluloses for biofuel production as compared to other bioenergy crops. Production of β-farnesene in plants, a precursor for biodiesel has distinct advantages such as eliminating the need for expensive downstream processing as well as reduction in production costs. The focus of this project is on generating β-farnesene enriched sesquiterpenes in sorghum by expressing genes involved in the mevolanate (MVA) and methylerylerythritol 4-phosphate (MEP) pathways. Transgenic sorghum plants with multiple transgenes were produced using embryogenic calli as explants. Transformed events carrying key mevalonate and non-mevalonate pathway genes or the entire MVA pathway of nine genes were subjected to chemical induction to elevate farnesene production. An overview on the progress for engineering sesquiterpene based biofuels through transgenic plant technology in sorghum will be presented.

P-25

Genetic Modification of Switchgrass for Improved Biofuel Production. Z. WANG[1], C. Fu[2], F. Hardin[2], J. Gou[2], A. Mason[2], and X. Xiao[2]. [1]The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401 and [2]The Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401. Email: zywang@noble.org

Switchgrass has been developed into a dedicated herbaceous bioenergy crop. We have developed a highly efficient transformation system for the widely used switchgrass cultivar Alamo by identifying tissue culture responsive genotypes and optimizing transformation parameters. The transformation efficiency of this system exceeds 90%. We take transgenic approaches to genetically modify the quality or quantity of switchgrass biomass suitable for biofuel production. Major lignin biosynthetic genes were isolated from switchgrass and transgenic plants carrying RNAi constructs were produced. The down-regulation of lignin biosynthesis with certain transgenes resulted in improved sugar recovery and ethanol yield. The miR156b precursor and some *SPL*genes were misexpressed in switchgrass. Non-flowering plants with improved biomass yield were obtained. A standardized protocol for sample collection for cell wall and biomass analyses was developed to maintain consistency in switchgrass evaluation methods and to enable comparisons of data obtained from different studies.

P-26

Planted Hardwood Bio-energy Forests for the Southeastern United States: Deployment Strategies in Hybrid Poplar and *Eucalyptus* sp. JEFF WRIGHT. ArborGen Inc, 2173 Salt Wind Way, Mount Pleasant, SC 29466. Email: patula.wright@gmail.com

Bio-energy forest plantations will supplement woody biomass from other sources such as logging residues. In the southern US, projections are for an increase of up to 25 million "new" tons of woody biomass demand for bio-energy. To supply this woody biomass demand will require purpose grown plantations of various species including pine, eucalypts, sweetgum, hybrid poplar and cottonwood, amongst others. Forest plantation yields can be greater than 30 green tonnes/ha/year (MAI 30) on rotations of 5-12 years. Utilization of this renewable and sustainable biomass resource will be as feedstock "designed" for a large number of bio-energy applications. Much of the emphasis has been on hardwood plantations due to their ability to coppice, continued genetic improvement programs as well as the opportunity to combine fast growth and wood properties in selected clones. In the specific case of *Eucalyptus* and *Populus*, there are a large number of commercial planting programs in countries outside the US. Feedstock characteristics are important in bio-energy hardwood plantations. Firstly, the plantation hardwood species has to be adapted to the soil and climate conditions. The hardwood feedstock has to be acceptable in harvesting, field processing and ultimately for conversion to bio-energy. Lastly, the growing (stumpage), harvest, haul and preparation costs have to be favorable compared to other biomass options.

P-27

Genome Editing in Plants via Designed Zinc Finger Nucleases. JOSEPH F. PETOLINO. Dow AgroSciences, 9330 Zionsville Rd., Indianapolis, IN. Email: jfpetolino@dow.com

Creating DNA double-strand breaks (DSBs) at investigator-specified genomic locations, and thereby stimulating the cell's naturally-occurring DNA repair processes, has opened up some intriguing possibilities for genetic modification. Zinc finger nucleases (ZFNs) are designed restriction enzymes consisting of a non-specific cleavage domain fused to sequence-specific DNA binding domains. ZFN-mediated DSB formation at endogenous genomic loci followed by error-prone non-homologous end joining (NHEJ) repair can result in nucleotide base pair insertions or deletions at a specified locus. Similarly, specific DNA sequence modifications can be made by providing donor DNA templates homologous to sequences flanking the cleavage site via homology-directed repair (HDR). ZFNs can also be used to create concurrent DSBs leading to targeted deletions of intervening DNA sequence. In addition to these targeted mutations, site-specific transgene integration into ZFN-induced DSBs is possible via either NHEJ or HDR. Such advances in genome editing can enhance basic understanding of plant gene function as well as augment applied crop improvement.

P-28

Targeted Trait Introduction and Mutagenesis in Crop Plants by Double-strand-break Technologies. A. MARK CIGAN. Trait Technologies, Dupont Pioneer 7300 NW 62nd Ave Johnston, IA. Email: Mark.Cigan@pioneer.com

Analogous to the introduction of dwarfing genes during the Green Revolution in wheat, a simple method to precisely modify plant genes and insert traits in crop plants would radically change agriculture. The development and implementation of methods to increase seed yield are important as studies suggest that by 2050, global agricultural production may need to be doubled due to world population growth. To meet this goal, agronomically important traits have been classically

identified by screening large populations of naturally occurring mutations or more recently by introducing transgenes that confer insect resistance or herbicide resistance into plant genomes at random sites along chromosomes. Both processes require extensive screening and introgression to identify and deliver commercially important traits in elite germplasm. This process is slow, expensive and inefficient, ultimately increasing time to the farmer. Furthermore, the trend in plant biotechnology has been to increase the number of transgenic traits which are typically located on multiple unlinked chromosomes. Combining multiple unlinked traits is costly to plant breeding both from the standpoint of intensive backcrossing and the potential of reducing seed yield due to co-segregation of sub-optimal genetics (linkage drag). Methods which would allow specific changes in a plant's genome would overcome many of these challenges. With the recent introduction of highly-specific double-strand-break reagents that are relatively affordable and easily programmable, plant scientists now have the means to rapidly direct gene inactivation, editing and integration in crop plants with elite genetics. In addition to creating loci where multiple transgene products may be positioned and linked, improved agronomic performance and novel product concepts are conceivable through precise alteration of native genes. Double-strand-break and gene integration technologies enabling editing of genes and building of multi-trait loci at DuPont Pioneer will be presented as a means to meet future global food security needs.

Disclosure: Author was or is employed by DuPont Pioneer.

P-29

Genome Editing in Plants Using CRISPR/Cas9/sgRNA Technologies. DONALD P. WEEKS and Wenzhi Jiang. Department of Biochemistry, University of Nebraska, Lincoln, NE 68588-0664. Email: dweeks1@unl.edu

The CRISPR/Cas9/single guide RNA (sgRNA) system has emerged as a potent new tool for targeted gene editing in numerous eukaryotic organisms, including plants. In our initial studies of the Cas9/sgRNA system with two dicot plant species, Arabidopsis and tobacco, we used *Agrobacterium tumefaciens* to deliver genes encoding Cas9, sgRNA and a nonfuctional, mutant green fluorescence protein to Arabidopsis and tobacco leaves to determine if transient expression of the Cas9 and sgRNA genes could be detected. The mutant GFP gene contained a target site in its 5' coding region that was successfully cleaved by a CAS9/sgRNA complex. Error-prone DNA repair at the cleavage site resulted in creation of functional GFP genes that could be readily detected by fluorescence microscopy. DNA sequencing confirmed Cas9/sgRNA-mediated mutagenesis at the target site. Follow-up experiments with similar constructs delivered to Arabidopsis using the floral dip method of transformation resulted in several T1 plants displaying GFP fluorescence in a mosaic pattern over the surface of their leaves. Examination of the T2 and T3 generation progeny of multiple T1 plants confirmed stable inheritance of mutagenized GFP genes that had been caused by the action of Cas9/sgRNA soon after the initial transformation event. Successful demonstration of the Cas9/sgRNA system in model plant and crop species bodes well for its near-term use as a facile and powerful means of plant genetic engineering for scientific and agricultural applications.

P-30

Nanoparticle-mediated Recombinase Delivery in Maize. KAN WANG[1], Susana Martin-Ortigosa[1], David J. Peterson[2], Justin S. Valenstein[3], Brian G. Trewyn[3], and L. Alexander Lyznik[2]. [1]Center for Plant Transformation, Plant Sciences Institute, and Department of Agronomy, Iowa State University, Ames, IA 50011-1010; [2]DuPont Pioneer, 7000 NW 62nd Avenue, Johnston, IA 50131-1000; and [3]Department of Chemistry, Iowa State University, and Ames Laboratory, US Department of Energy, Ames, IA 50011-3111. Email: kanwang@iastate.edu

Delivery of proteins instead of protein-encoding DNA fragments into plant cells is of particular interest for genome editing because it can avoid DNA (transgene) integration into the genome and generate precisely modified "non-transgenic" plants. The transient presence of the enzymes or biomolecules in the cells can be advantageous for applications in which long term expression of transgenes are not desired. We have been explored the utilization of surface-functionalized nanoparticles for intracellular delivery of biomolecules in plant tissues. We report here a gold-plated mesoporous silica nanoparticle (Au-MSN) platform for co-delivery of proteins and DNA to plant tissues using a biolistic particle bombardment method. Au-MSN with large average pore diameters (10-nm) are shown to deliver and subsequently release marker proteins and plasmid DNA to the same cell after passing through the plant cell wall upon bombardment. Release of fluorescent eGFP indicates the delivery of active, non-denatured proteins to plant cells. We also demonstrate that the Au-MSN can be used to deliver recombinase to plant genome, leading to site-specific recombination. Purified Cre recombinase was loaded into the pores of Au-MSN and biolistically delivered into maize embryos containing *loxP* sites integrated into chromosomal DNA (Lox-corn). Lox-corn expressed the glyphosate-resistance *gat* and the blue fluorescent protein *AmCyan1* genes flanked by *loxP* sites. The MSNs-released Cre enzyme recombined the *loxP* sites thus removing the DNA fragment flanked by these sequences. Such excisions led to the expression of the red fluorescent protein

DsRed2 gene and the loss of the selectable marker gene. Visual selection was used to recover the recombination events. Subsequently, fertile maize plants were regenerated from the recombined events and DNA analyses confirmed the recombination events. To our knowledge, this is the first time that MSNs have been used for the delivery of a functional recombinase into plant tissues leading to successful genome editing.

P-31

Innovative Ways to Successfully Initiate Tropical Plant Species Including Ginger (*Zingiber Officinale*), Turmeric (*Curcuma Longa*), Sarsaparilla and Chainy Root (*Smilax Spp*), and Yam (*Dioscorea* Spp). SYLVIA ADJOA MITCHELL. Medicinal Plant Research Group, The Biotechnology Centre, University of the West Indies, Mona Campus, JAMAICA. Email: sylvia.mitchell@uwimona.edu.jm

The greatest challenge to in vitro culture of tropical plants is the initiation process with the growth of fungi and bacteria usually winning. High levels of contamination, especially after rainy, humid episodes, have prevented the use of tissue culture for many tropical plants. Most of this contamination is obvious within two weeks of culture and can be confirmed by use of enriched media. Some techniques used to overcome contamination during initiation include: use of fast growing shoots; avoidance of rainy periods by ensuring at least four days of sunshine; potting the plants and initiating fresh growth; a double sterilization regime; and appropriate levels of sterilizing agents. While these techniques have worked for ginger, turmeric, sarsaparilla, chainy root and other medicinal plants, others such as *Dioscorea* have proven more difficult and have required even more innovative methods. A method has been developed (water culture) that has resulted in the initiation of 100% clean cultures of *Dioscorea* spp. nodal explants and has produced more than 20 clean cultures from one tuber of Negro Yam (*Dioscorea rotundata*). These and other innovations will be explained in detail.

P-32

Micropropagation of Ornamental Tropical Foliage Plants: Not Just Multiplication. JIANJUN CHEN and Richard J. Henny. University of Florida, IFAS, Department of Environmental Horticulture and Mid-Florida Research and Education Center, 2725 S. Binion Road, Apopka, FL 32703. Email: jjchen@ufl.edu

Ornamental tropical foliage plants are those with attractive foliage and/or flowers that are able to survive and grow indoors. The ornamental tropical foliage plant industry is a viable sector in U.S. agriculture. Micropropagation has played an important role in the expansion of the foliage plant industry. The industry was the first to successfully demonstrate commercial profitability of micropropagation. More than 700 million foliage plantlets including 250 million bromeliads and orchids are produced annually in the world. Micropropagation helps to eliminate systemic diseases in starting materials, reduces greenhouse space normally required for maintaining stock plants, and provides growers with healthy and uniform liners on a year-round schedule. Micropropagation has also become an important means for obtaining new cultivars through the selection of somaclonal variants; more than 100 commercial foliage plant cultivars have been selected from somaclonal variation. Furthermore, micropropagation speeds the introduction of hybrid cultivars. New cultivars can reach sufficient numbers to become commercially available in 2 to 3 years using micropropagation compared to the 5 to 10 years needed via traditional asexual propagation methods. Currently, conservation of foliage plant germplasm is becoming a critical issue. There is an increasing erosion of genetic resources in tropical regions where many foliage plants originate. However, thus far little effort has been placed on preserving these valuable genetic resources. The role of tissue culture in preserving foliage plant resources will be discussed.

P-33

Cryopreservation of Woody Tropical Plants. ANA ABDELNOUR-ESQUIVEL. Costa Rica Institute of Tecnology, Cartago, COSTA RICA. Email: aabdelnour@itcr.ac.cr

Tropical trees of commercial value are currently threatened as consequence of deforestation and unsustainable exploitation. Therefore, there is an urgent need to develop strategies for sustainable management and conservation of the remaining populations. Most seeds of tropical trees cannot be stored at conventional seed banks since they do not survive cold temperatures (4ºC to -20ºC) or drying. Cryopreservation is the storage of plant material in liquid nitrogen (NL, -196ºC) and its major advantages are the possibility to conserve not only seeds, but also materials produced by tissue culture for long time under genetic stability conditions. This study showed the results obtained with the cryopreservation of seeds and/or shoots of various tropical tree species: *Swietenia macrophylla*, *Cedrela odorata*, *Hyeronima alchorneoides*, *Jatropha curcas*, *Uncaria tomentosa*, *Gmelina arborea*, *Tectona grandis*, *Pithecellobium saman* and *Gliricidia sepium*. Drying of fruits and seeds between 3 and 12% moisture content and the rapid freezing in liquid nitrogen (NL) allowed, depending on the species, from 3 to 100% germination. On the other hand, regeneration percentages

greater than 50% were observed on shoots of *Cedrela odorata* and *Hyeronima alchorneoides*, using vitrification solutions PVS2 and PVS3. Encapsulation-dehydration was evaluated on shoots of *Uncaria tomentosa* and highest survival was observed when shoots were cultivated on a medium with 0.3M sucrose for 24 hours, followed by a day on 0.4M sucrose, and capsules dehydrated to 20% moisture content, before freezing (50% survival). In addition, 70% survival of cell suspensions of this species was observed when incubated in 0.15 M sucrose during 24 hours, followed by incubation in 5% DMSO for 1 hour before the fast freezing in NL.

P-34

Cryopreservation of Orchid Seeds, Protocorms and Pollen. WAGNER A.VENDRAME[1], R. F. Galdiano Jr.[2], R. T. Faria[3], and V. S. Carvalho[4]. [1]University of Florida, Tropical Research and Education Center, 18905 SW 280th St, Homestead, FL 33031-3314; [2]Universidade Estadual Paulista "Julio de Mesquita Filho", Via de Acesso Prof. Paulo Donato Castellane, s/n, Jaboticabal, SP 14884-900, BRAZIL; [3]Universidade Estadual de Londrina, Depto. de Fitotecnia, Centro de Ciências Agrárias, CP 6001, Londrina, PR 86051-990, BRAZIL; and [4]Universidade Estadual do Norte Fluminense, Laboratório de Fitotecnia, Centro de Ciências e Tecnologias Agropecuárias, Av. Alberto Lamego, 2000, Campos do Goytacazes, RJ 28013-602 BRAZIL. Email: vendrame@ufl.edu

Orchids are very popular flowering plants worldwide, occupying top position in the international market as cut flowers and potted plants. In the United States, orchids rank second as flowering potted plants, just behind poinsettias, with an estimated wholesale value of US$200 million in 2011 (USDA, 2012). Over collection and habitat destruction have been reported as the main factors for the reduction in wild orchid natural populations (Swarts and Dixon, 2009). Therefore, conservation strategies for orchids need to be addressed. Cryopreservation has been widely regarded as an efficient tool for long-term storage of plant material and several cryopreservation methods have been developed for orchids. In addition to preservation of endangered orchids, cryopreservation also offers a suitable alternative for breeders, allowing long-term storage of orchid genetic material for breeding and genetic improvement programs (Vendrame et al., 2007). In this presentation we will summarize a number of orchid cryopreservation studies performed in our laboratory at the University of Florida's Tropical Research Center for the past seven years. Our first studies with Dendrobium allowed the development of an efficient protocol for cryopreservation of hybrid mature seeds using vitrification, a simple and reliable method (Vendrame et al., 2007). Cryopreservation of Dendrobium pollen also showed to be an effeicient means for long-term storage of orchid pollen (Vendrame et al., 2008). Subsequent studies aimed at improved cryopreservation protocol development with the addition of phloroglucinol, which provided enhanced recovery of cryopreserved *Dendrobium nobile* protocorms (Vendrame and Faria, 2011) and of Dendrobium hybrid seeds and protocorms (Galdiano Jr. et al., 2012). The genetic stability of recovered seedlings from cryopreserved *Oncidium flexuosum* seeds has also been evaluated (Galdiano Jr. et al, 2013). Results from these studies will be presented and discussed.

P-35

Can Genetic Engineering Help Save the American Chestnut? WILLIAM POWELL[1], Andy Newhouse[1], Kathleen Baier[1], Linda McGuigan[1], Allison Oakes[1], Kristen Stewart[1],Timothy Tschaplinski[2], and Charles Maynard[1]. [1]SUNY College of Environmental Science & Forestry, Syracuse, NY and [2]Oak Ridge National Lab, Oak Ridge, TN. Email: wapowell@esf.edu

The American chestnut (*Castanea dentata*) and chestnut blight is the classic example of what happens when our forests succumb to exotic pests and pathogens. Because of its environmental, economic, and social importance, many tools have been brought to bear on the chestnut blight problem. We have focused on enhancing blight resistance by producing both transgenic and cisgenic American chestnut trees, adding only 2-5 genes to the approximately 40,000 genes in chestnut genome. The most promising of these transgenes encodes an oxalate oxidase (OxO) from bread wheat (*Triticum aestivum*). According to leaf and small stem assays that predict the level of blight resistance, this OxO has raised resistance levels at least as high as those found in the blight-resistant Chinese chestnut (C. mollissima). This enzyme functions the same way as in its native wheat by detoxifying the oxalate produced by the pathogenic fungus. In preliminary metabolomics studies, transgenic American chestnut trees have fewer metabolite changes than trees produced from traditional hybrid breeding; therefore this tool appears to produce fewer unintended metabolic consequences than traditional methods. Lastly, people eat this gene and its enzyme whenever they consume wheat products. So, as a transgene, its use in chestnut appears to be ideal: it is effective against the harmful fungus, but harmless to people and wildlife. We have also discovered two promising cisgenes from the closely related Chinese chestnut: a laccase-like gene and a proline-rich protein gene, both of which can partially enhance blight resistance when overexpressed in American chestnut. This means the trees have significantly less necrosis in leaf assays than the wild type American controls, but significantly more necrosis than the Chinese

chestnut controls. The next step will be to stack these two genes to determine if they can work synergistically and enhance blight resistance even higher. These results show that genetic engineering is a useful tool that can significantly enhance pathogen resistance while otherwise making very small changes in a tree.

P-36

Conservation and Development of *Fraxinus* spp. for Resistance to the Emerald Ash Borer. PAULA M. PIJUT. USDA Forest Service, Northern Research Station, Hardwood Tree Improvement and Regeneration Center, Purdue University, 715 West State St., West Lafayette, IN 47907. Email: ppijut@purdue.edu, ppijut@fs.fed.us

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a wood-boring beetle indigenous to China, Japan, Korea, Mongolia, the Russian Far East, and Taiwan. In 2002, it was identified as the cause of extensive ash (*Fraxinus* spp.) mortality in Southeastern Michigan and nearby Ontario, Canada. EAB has killed millions of ash trees since its arrival in North America, and infestations have been found in 21 states in the U.S. and into Canada. The risk EAB poses to the ash resource in North America is substantial. Sixteen species are native, most are susceptible to EAB, and highly valued species include white (*F. americana*), green (*F. pennsylvanica*), and black ash (*F. nigra*) which are major components of the forest, and blue (*F. quadrangulata*) and pumpkin ash (*F. profunda*) which are less common species. Ash timber is valued for applications requiring strong, hard wood. White ash is the primary commercial hardwood used in production of baseball bats, furniture, flooring, doors, and cabinets. Green ash is also used for both solid wood applications, crating, boxes, and tool handles. Black ash is typically used for cabinets, paneling, veneer, and Native Americans require this species for the art of basketry. Beyond manufacturing, ash trees play an important role in the urban landscape because of their historical resistance to pests and tolerance of adverse growing conditions. Ash trees are an important component of forests and urban landscapes, providing aesthetic value and shelter and food for wildlife. Without management, EAB threatens these resources and will permanently alter the forest ecosystems throughout North America. *Bacillus thuringiensis* (*Bt*) is a naturally occurring bacterium that produces spores with proteinaceous parasporal inclusion bodies composed of delta endotoxins. Its spores and inclusion bodies express insecticidal activity. Expression of an effective *Bt* gene in transgenic ash trees would impart resistance to the aggressive EAB. Research will be presented on the conservation and development of *Fraxinus* spp. for resistance to EAB.

P-37

Somatic Embryogenesis, Cryostorage and Conventional Tree Breeding: A Powerful Combination for Conservation and Restoration of Threatened Forest Trees. SCOTT MERKLE. Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602. Email: smerkle@uga.edu

Two conventional approaches to dealing with forest health threats, selection of naturally occurring resistant or tolerant genotypes and hybrid breeding with resistant related species, could be greatly enhanced by combining them with in vitro clonal mass propagation systems. Somatic embryogenesis (SE), in particular, is well-suited for this purpose, due to the high multiplication rates and the amenability of embryogenic cultures to cryostorage. Potentially blight-resistant American chestnut x Chinese chestnut hybrid backcross B3F3 seeds were used to initiate embryogenic cultures and the first B3F3 somatic seedlings were deployed in clonal field tests in 2013. We are also using SE to clonally propagate hybrids between Carolina and eastern hemlocks, which are under threat of extinction by hemlock woolly adelgid (HWA), and HWA-resistant Asian hemlock species. As part of the effort to restore green ash and white ash populations that are being devastated by the emerald ash borer (EAB), we have developed embryogenic cultures of both of these species. We initiated several embryogenic cultures from seeds collected from "lingering" white ash trees in Michigan, which are potentially resistant to EAB. Cryostoring the embryogenic cultures allows for the clones to be held indefinitely while somatic seedlings from them are tested for pathogen and pest resistance.

P-38

Synthetic Promoters: Targeted Design for High Level Transgene Expression. MAURITZ VENTER. AzarGen Biotechnologies, Natural Science Building, Room 2066, Merriman Avenue, Stellenbosch 7600, SOUTH AFRICA. Email: mauritz@azargen.com

In recent years, plant-based biopharmaceutical production platforms have gained considerable momentum. Several expression vectors combining novel enhancer, signalling and purification components have been designed and tested for commercial feasibility. However, in transgenic plants, reliable high level transgene expression remains a challenge and the choice of strong constitutive promoters is limited. We have designed 21 synthetic promoters by combining specific cis-regulatory modules and/or selected cis-motif arrangements. Rapid 1st round screening in tobacco plants, using an YPET fluorescence reporter gene, showed that several of these synthetic promoters confer superior activity compared to the 35S

and double enhancer 35S promoters respectively. Some of the synthetic promoters highlighted here may serve as promising molecular tools for plant-based biotechnology applications, specifically bio-pharming.

P-39

Synthetic Promoters for Targeted Transgene Activation in Plants. WUSHENG LIU[1], David A. Schmidt[1], Reginald J. Millwood[1], Mary R. Rudis[1], Mitra Mazarei[1], Jonathan D. Chesnut[2], Christopher J. Potter[3], and C. Neal Stewart, Jr.[1]. [1]Department of Plant Sciences, The University of Tennessee, Knoxville, TN, 37996; [2]Synthetic Biology Research and Development, Life Technologies, CA, 92008; and [3]Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, 21205. Email: wliu2@utk.edu; nealstewart@utk.edu

The ability to regulate (trans)gene expression within a plant system is a highly sought after technique. Targeted transgene activation can be achieved by using inducible or tissue-specific promoters in transgenic plants, or by using endogenous or synthetic transcription factors that specifically bind to the promoters of genes of interest in its original genetic context and activate gene expression. We used synthetic promoters for targeted transgene activation by transcription activator-like effectors (TALEs) in tobacco, and found that synthetic TALEs can activate the reporter gene expression by 2 times, irrespective of the strength of the synthetic promoters. We also used synthetic promoters for targeted transgene activation in tobacco by a transcription factor QF, which is derived from the *qa* gene cluster in the fungus *Neurospora crassa*, and found that QF can activate the reporter gene expression driven by each synthetic promoter to be two times the expression rendered by the full-length 35S promoter alone. These studies demonstrate the effectiveness of synthetic promoters together with synthetic transcription factors for targeted transgene activation in plants.

P-40

What Have We Learned About Synthetic Promoter Construction? PAUL J. RUSHTON. Texas A&M AgriLife Research & Extension Center at Dallas. Email: paul.rushton@tamu.edu

Any successful strategy to make improved crop plants using transgenic methods requires two components: A transgene and a promoter to drive expression of the transgene. Much thought is given to the choice of transgene but many potentially promising projects fail because the choice of promoter is poor. Synthetic promoters offer the possibility of designing the expression requirements of the project to the needs of the investigator. Data showing how promoter strength and inducibility can be altered based on the number of copies of a *cis*-acting element in a synthetic promoter will be presented. The range of individual *cis*-acting elements that may be used together or in combination to make promoters that direct biotic and/or abiotic stress-inducible expression will be discussed. Finally, our recent work has centered on a tripartite element (the GAG fragment) that directs wound and JA inducibility in roots. I will show how the three individual *cis*-acting elements that constitute the GAG fragment contribute to the activity of the promoter. The resulting synthetic promoters direct expression at a distance. Wounding or JA treatment of leaves results in expression from the synthetic promoter in the roots. Finally, ideas for the best synthetic promoters will be discussed.