In Vitro Cell.Dev.Biol.—Animal
DOI 10.1007/s11626-015-9901-3

Plant Symposia

P-1

Advances in Research Methods for Commercial Micropropagation Systems. B. GYTRI and C. Sluis. Tissue-Grown Corp., 15245 W. Telegraph Road, Santa Paula, CA 93060. Email: brett.gytri@tissuegrown.com

Researchers in universities across the globe have made great strides toward understanding many of the fundamental underlying principals of plant growth regulation, using the model plant *Arabidopsis*. This has resulted in a wealth of avenues to explore for better control of plant development and morphology during propagation *in vitro*. The challenge in the current economic marketplace is to create reliable, consistent, high volume plant production systems using the best research tools and the best scientific breakthroughs, while keeping in mind both the economics and the marketability of the end product. Experimental design and analysis programs have become increasingly accessible to the tissue culture researcher and can help create multifactorial experimental designs which allow evaluation of many interacting factors in a relatively short time frame simultaneously. The steps comprising a tissue culture propagation system can be modeled to produce the maximum number of plants while factoring in the best use of labor and materials. The physical parameters of the culture system: the vessels, gas environment and substrate, play a large role in plant quality. Liquid based systems can be compatible with mechanization. Photoautotrophic systems can improve plant quality and normalcy. Blending the many options into a functional economic propagation system can be challenging. However, most commercial plant tissue culture laboratories subculture their plants in small vessels with gelled, sugar-containing media. Why? We will explore the reasons for this and examine the research tools available for attaining the goal of high volume micropropagation of economically viable crops in novel and sometimes unexpected ways.

P-2

Temporary Immersion Bioreactor & Somatic Embryogenesis in Micropropagation. SERGIO L. FLOREZ[1], Tina S Lai[2], Rachel L Erwin[1], and Wayne R Curtis[1]. [1]Department of Chemical Engineering, The Pennsylvania State University, University Park, PA, 16802 and [2] Department of Plant Biology, The Pennsylvania State University, University Park, PA 16802. Email: Slf5256@psu.edu, WRC@psu.edu

A low-cost temporary immersion bioreactor (TIB) has been established as a platform for developing enhanced technologies for micropropagation with the ability to control the gas and liquid phases. The physical environment can be controlled to compliment the manipulation of the genetic environment through transient gene expression or by initiating signal cascades through the use of "conditioned media". **Completed Studies:** We demonstrated enhanced tissue culture performance: Hairy root cultures - Oxygen transport enhanced heterotrophic growth; Watermelon meristem cultures - Carbon dioxide facilitated photosynthetic growth. Doubling gas O_2 concentration doubled the rate "hairy root" culture of growth; A 5 % CO_2 atmosphere facilitated proliferation of seedless watermelon meristems with higher chlorophyll content. **Ongoing studies:** With the goal of using the TIB for enhanced somatic embryogenesis (SE) and tissue culture propagation of recalcitrant cacao varieties (chocolate tree), we are developing: Transient expression of BABY BOOM transcription factor; Glucocorticoid receptor (GR) inducible expression system of LEAFY COTYLEDON 2 transcription factor;Ectopic treatment with PLA protein derived from conditioned media. The cacao homolog of the BABY BOOM transcription factor was identified and shown to improve yield of somatic embryos. Inducible cacao LEAFY COTYLEDON 2 expression initiated hormone-independent somatic embryo formation. The cacao homolog of a cotton arabinogalactan protein containing a PLA domain that improves SE has also been identified and cloned. Expression of the PLA domain in *E. coli* is underway and is being tested as an alternative means of improving SE through external protein signaling in a TIB. The combined work for somatic embryo enhancement and our bioreactor design has promise towards enabling enhanced propagation of elite plant varieties and improved genetic transformations. With an economical scale-up in mind, our novel temporary immersion bioreactor (TIB) design is based on principles that focus on achieving reliability at a minimal cost.

P-3

Progress on Scale-up Somatic Embryogenesis and Manufacture Seed Technology of Conifer Species at Weyerhaeuser. P. GUPTA and J. Hartle. Weyerhaeuser Technology Center-1B10, 32901 Weyerhaeuser Way S., Federal Way, WA 98001. Email: pramod.gupta@weyerhaeuser.com

Published online: 13 May 2015

Mass clonal propagation via somatic embryogenesis has been used for large number of horticultural and forestry species. Embryogenic cultures have also been used for mutation and genetic transformation. A large number of papers have been published on somatic embryo development, maturation, cryopreservation, germination and automation for several conifer species. Several patents have been granted to forest industries and universities on conifer somatic embryogenesis, automation and delivery system. Weyerhaeuser NR Company also has several patents on this technology. The implementation of this technology has already begun at Weyerhaeuser and several other forestry organizations. Companies are testing the clones in the field and have been storing corresponding embryonal suspensor mass (ESM) in cryostorage. However, plantlets production via somatic embryogenesis is still expensive. At Weyerhaeuser, somatic embryo productions in a bioreactors and manufactures seed delivery have been developed for Douglas-fir and loblolly pine. However, full commercialization is still limited due inability to produce zygotic like somatic embryos quality. In this presentation we will discuss somatic embryo quality improvement and manufactures seed delivery system using Weyerhaeuser patented technology.

P-4

Photoautotrophic (Sugar-free Medium) Micropropagation – Its Principle and Application. TOYOKI KOZAI. Japan Plant Factory Association, Kashiwa-no-ha 6-2-1, Kashiwa City, Chiba, 277-0882, JAPAN. Email: kozai@faculty.chiba-u.jp

Principle, concept and method of photoautotrophic (sugar-free medium) micropropagation are presented together with their practical applications. Procedures of photoautotrophic micropropagation are described in order of : 1) explant preparation, 2) culture vessels with natural or forced ventilation, 3) control of CO2 concentration and humidity (water vapor deficit), 4) substrate (supports) and nutrient composition, 5) light intensity (photosynthetic photon flux) and its quality, and 6) pathogen-free conditions. Methods for measuring the ventilation rate of culture vessel and net photosynthetic rate of in vitro plants are also presented. Commercial applications of photoautotrophic micropropagation method to the vegetative propagation and seedling production as well as leaf vegetable production (plant factory) under artificial lighting in closed systems with controlled environments are also presented.

P-5

Double-strand Break Induced Genome Engineering in Plants. HOLGER PUCHTA. Bontany II, Karlsruhe Institute of Technology, Karlsruhe, GERMANY. Email: holger.puchta@kit.edu

Site-specific nucleases can be used to induce site-specific double-strand breaks (DSBs) in plant genomes. Using the meganuclease I-SceI we could show over the years that homologous recombination (HR) can be enhanced and targeted mutagenesis can be achieved by error-prone non-homologous end joining (NHEJ) by site-specific DSB induction. Moreover by inducing several breaks sequences can be deleted out of the genome and chromosome arms exchanged. Recently, we developed a strategy by which by DSB induction gene targeting can be performed *in planta* during plant development. The strategy does not rely on efficient transformation and regeneration procedures, making it attractive for application in crop plants to improve elite cultivars. The CRISPR/Cas system is becoming the major tool for targeted mutagenesis in eukaryotes to induce either double-strand breaks (DSBs) or single-strand breaks at preselected genomic sites. Thus, homologous recombination (HR) can be enhanced and targeted mutagenesis can be achieved by error-prone non-homologous end joining (NHEJ). Before, we were able to demonstrate heritable targeted mutagenesis in *Arabidopsis thaliana* as well as the first application of a Cas9 nickase in plants. Now, we are able to demonstrate that the *in planta* GT strategy can be applied for targeting natural genes by Cas9-mediated DSB induction. We could integrate a resistance cassette into the *ADH1* locus of *A. thaliana* via HR. Heritable events were identified using PCR-based genotyping, characterized by Southern blotting and confirmed on the sequence level. Moreover, a major concern is the specificity of the CRISPR/Cas system. Off-target effects might be avoided using two adjacent sgRNA target sequences to guide the Cas9 nickase to each of the two DNA strands, resulting in the formation of a DSB. By amplicon deep sequencing, we demonstrate that this Cas9 paired nickase strategy has a mutagenic potential comparable to that of the nuclease. We also demonstrate the stable inheritance of such mutations in *A. thaliana*.

P-6

Developing Toolkits for Plant Genome Editing and Transcriptional Regulation. YIPING QI[1], Levi G. Lowder[1], Nicholas J. Baltes[2], Yong Zhang[3], and Daniel F. Voytas[2]. [1]Department of Biology, East Carolina University, Greenville, NC 27858;

[2]Department of Genetics, Cell Biology & Development and Center for Genome Engineering, University of Minnesota, Minneapolis, MN 55455; and [3]Department of Biotechnology, School of Life Sciences and Technology, University of Electronic Science and Technology of China, Chengdu 610054, CHINA. Email: qiy@ecu.edu

Recent advances in genome editing tools have promised a bright future for plant research and biotechnology. These tools are based on customizable site-specific nucleases (SSNs), such as Zinc Finger Nucleases (ZFNs), TAL Effector Nucleases (TALENs) and the CRISPR-Cas9 system. All these SSNs can be engineered for recognizing specific DNA sequences, and this feature allows them to be repurposed for engineering artificial transcription factors. We have recently developed two toolkits for genome editing and gene regulation in plants. By working on plant species such as Arabidopsis, tobacco and rice, we have demonstrated multifaceted applications of these toolkits in reverse genetics and synthetic biology. We believe these new tools will contribute to future breakthroughs in basic research and agriculture biotechnology.

P-7

TALEN and CRISPR-Cas9 in Rice and Disease Control. BING YANG. Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011. Email: byang@iastate.edu

Modified CRISPR (clustered regularly interspaced short palindromic repeats) systems, comprising single guide RNAs (sgRNAs) and Cas9 endonucleases, and TALENs (TAL effector nucleases) have emerged as potent biotechnological tools for both basic and applied research. The most promising utilization of both Cas9/sgRNA and TALENs is for targeted genome editing, precise genetic alterations within any genome of interest, as demonstrated in a plethora of organisms including several crop plants. My presentation describes development and application of these two technologies to generate heritable genome modifications in rice. TALENs were employed to generate stable, heritable mutations in dozens of rice genes. The frequency of mutagenesis varied from species and constructs targeting different genes. The highest rate in rice reached about 65% of transgenic lines carry desired mutations. Phenotypic changes associated with mutated genes were observed in some mutant lines in rice. We also modified the Cas9/sgRNA system suitable for targeted gene mutagenesis in rice. The two systems have been successfully applied to rice for targeted mutagenesis of many genes. Transgenic lines of T0 generation carrying site-specific mutations were produced at frequency as high as 100% in rice. I will also present examples that TALEN-mediated promoter mutations lead to disease resistance to bacterial blight in the otherwise susceptible rice cultivars. Our results demonstrate that TALENs and Cas9/sgRNA are effective toolboxes for genome editing in rice, empowering the discovery of gene function and the trait improvement.

P-9

Support of Nestlé's Nescafé and Cocoa Plants Using Plant Tissue Culture. J. P. DUCOS, C. Guillou, A. Fillodeau, A. Buchwalder, C Lambot, P. Broun. Nestlé R&D Centre Tours, 101 Avenue Gustave Eiffel, Notre Dame D'Oé, BP 49716, 37097 Tours Cedex 2, FRANCE. Email: Jean-paul.ducos@rdto.nestle.com

Coffee and cocoa are threatened by many factors such as aging trees and increasing exposure to biotic and abiotic stresses. To tackle this situation, Nestlé launched two large sustainability initiatives, the Cocoa Plan (2009) and the Nescafé Plan (2010). One of Nestlé's interventions within these initiatives is the large-scale distribution to farmers of selected cocoa and coffee varieties propagated *in vitro* . The Nestlé R&D Center-Tours (France) has developed expertise in coffee and cocoa somatic embryogenesis. The process that we have implemented for the mass propagation of selected genotypes is mainly based on the multiplication of embryogenic callus and on the use of liquid medium for the production of the embryos. For Robusta coffee (*Coffea canephora*), a batch takes 6 to 8 months to complete and consists in three phases. The development of torpedo embryos is achieved using Erlenmeyer flasks. The pregermination is conducted in a 10-L temporary immersion bioreactor made of glass or flexible disposable bags. The latter type, so-called "Box-in-Bags", ensures a higher light transmittance to the biomass due to its horizontal design. The germination step, i.e. the conversion of embryos at the cotyledonary stage into complete plantlets is performed in the greenhouse. A closer confinement, obtained by placing a transparent plastic cover 2 to 3 cm above the embryos, significantly improves the development of leaves and roots. This positive effect is due to the CO2 released from the horticultural media. For cocoa (*Theobroma cacao*), the process is more complex. The reactivity is highly genotype dependent and there is a large and still unexplained batch-to-batch variability in the aptitude of the embryos to develop into plantlets.

P-10

A Simplified Technique for the Propagation of Shoots from Nodes of Switchgrass (*Panicum virgatum* L.) Genotypes.

JOSHUA M. WEAVER, Laura Sofia Montes Sujo, and Kenneth A. Feldmann. School of Plant Sciences, University of Arizona, 303 Forbes Building, P. O. Box 210036, Tucson, AZ, 85721-0036. Email: jweaver2011@email.arizona.edu

Nodal propagation of switchgrass (Panicum virgatum L.) is an important technique for multiplying a variant plant, with a potentially valuable agronomic trait, for replicated field trials. To develop an improved propagation system, shoot induction from three node positions (Low, Mid, and High) was tested in ten genotypes of swithgrass cv. Alamo. Nodal segments were incubated in a custom hydroponics system with 1/4 strength Murashige and Skoog liquid medium for six weeks. In all genotypes, shoot formation was highest at the Low node position, the largest nodes, ranging from 60 - 98%. From the ten genotypes tested, three genotypes (4,5,and 6) had a significantly higher shoot formation at Mid and High nodes with a shoot formation greater than 80% and 50%, respectively. Shoot formation for all other genotyes at the same two positions was less than 40% and 10%, respectively. In a single replication, 30 shoots from the lowest node position of each genotype were rooted for four weeks in each of two rooting conditions (RC1-Roottone dip and soilless media and RC2 - IBA in a half strength liquid MS media). Four genotypes showed greater root formation in RC1 while one genotype showed greater root formation in RC2. In this paper, we decribe a procedure for generating a population of genetically identical plants from switchgrass, in as little as six-weeks, that is relatively inexpensive, efficient and does not require specialized equipment needed for tissue culture.

P-11

Sugarcane Technology Center – Development of Innovative Technologies to Increase Agricultural Efficiency of the Sugarcane Industry. D. M. MELOTTO-PASSARIN, C. E. Faroni, M. Monteiro, A. B. Marchesini, W. J. Ferreira, R. Z. Fernandes, M. I. B. Andia, D. O. Campos, J. H. M. Pontes, A. S. Marchi, and D. H. S. Ferrés. Centro de Tecnologia Canavieira – CTC (Sugarcane Technology Center), Piracicaba, São Paulo, BRAZIL. Email: danila.passarin@ctc.com.br

The perspective for Brazil to consolidate itself as the world's leading supplier of renewable fuels, mostly derived from sugarcane, is feasible thanks to its continental dimensions, abundant natural resources and rational use of its vast areas. Many environmental factors influence sugarcane production and the success of this industry is strongly anchored on the use of varieties adapted to the various soil and climatic conditions of each producing region to ensure their good performance and productivity. The Sugarcane Technology Center (CTC) has the largest program of sugarcane varieties development in the world. The center uses traditional breeding with molecular markers and biotechnology, delivering increasingly productive varieties adapted to different regions of Brazil, and transgenic varieties that enhance sugarcane productivity in the field. This increases biofuel and sugar yields without increasing areas cultivated with sugarcane. To increase ethanol annual production through sugarcane productivity, CTC invests in the development of technology for cellulosic ethanol, produced from cellulosic materials from sugarcane biomass: bagasse and straw. Therefore, to ensure the availability of new sugarcane varieties on a large scale to meet the industry demand, the micropropagation technology is an excellent alternative to the conventional method of vegetative propagation by means of stems and buds. Micropropagation provides high sugarcane multiplication rates, with numerous advantages over conventional multiplication in the field, such as the production of large numbers of high quality seedlings in reduced time and space. Seedlings of varieties produced *in vitro*, with high genetic and phytosanitary quality, allow even higher production and greater competitiveness of cane growers, without the need to advance into environmental preservation areas.

P-12

Fully Sequence Indexed Collections of Chemically Mutagenized Plants: A Tool for Understanding Gene Function. C. WEIL[1], M. Tuinstra[1], B. Dilkes[2], C. Addo-Quaye[2], J. Backlund[1], E. Danquah[3], H. Traore[4], M. Massafaro[1], M. McKneight[1], E. Azu[3], N. Babcock[1], and A. Linville[1]. [1]Purdue Univ., Dept of Agronomy, W. Lafayette, IN, 47907; [2]Purdue Univ., Dept of Horticulture and Landscape Architecture, W. Lafayette, IN, 47907; [3]Univ of Ghana, West Africa Center for Crop Improvement, Legon, Accra, Ghana; and [4]INERA, Ougadougou, BURKINA FASO. Email: cweil@purdue.edu

Although we sequence and resequence genomes on a regular basis, and compare gene annotations among species, the functions of most genes in higher organisms remain unclear. Mutations that alter gene function and phenotype remain the most powerful tools in addressing this gap. Many collections of plant mutants take advantage of transposon insertion or radiation-induced deletion to try and eliminate gene function because they can be screened initially using inexpensive, PCRbased methods. However, the ever-decreasing cost of NextGen

sequencing now permit analysis of the full array of point mutations created in an individual by chemical mutagenesis. These mutants include knockout alleles and a wider range of more subtle mutant phenotypes, including potentially novel, gain-of-function alleles, generating valuable allelic series for every gene in a genome with just a few thousand individuals. We have created a population of 12,000 EMS-induced mutants in sorghum and have completely resequenced 600 of these mutagenized genomes thus far, finding ~1.3 million unique, homozygous mutations compared to the progenitor (~70,000 in protein coding sequences), and nearly 300,000 unique heterozygous SNPs within coding sequence. While recent studies show the utility of exome capture to reduce overall cost and to focus on protein coding mutations, fully resequencing also captures mutations in conserved noncoding sequences, promoter mutations altering transcription and many dominant alleles. The data are now available publically as a searchable database and we are establishing public domain seed distribution so that the resource can be used in both forward and reverse genetic approaches to connect mutation to phenotype and gene function. Genes of interest then become targets for further study, for example in the naturally diverse germplasm that is the core of improving sorghum as a food, feed and fuel crop. Similar sequence-indexed collections projects are now underway for pearl millet and for cowpea, exploring the effectiveness in this context of combining multiple mutagens.

P-13

Sugarcane Mutation Breeding in South Africa: Progress and Prospects. R. S. RUTHERFORD[1,2], K. Z. Maphalala[1], T. Mahlanza[1,2], S. J. Snyman[1,2], and M. P. Watt[2]. [1]South African Sugarcane Research Institute, Private Bag X02, Mount Edgecombe 4300, SOUTH AFRICA and [2]School of Life Sciences, University of Kwa-Zulu Natal, Private Bag X54001, Westville Campus, Durban 4000, SOUTH AFRICA. Email: stuart.rutherford@sugar.org.za

Modern sugarcane cultivars (*Saccharum* spp hybrids) are highly polyploid. Their genetic base is considered narrow due to the hybridisation of only a few genotypes of the autopolyploid species *S. officinarum* (2n = 80) and *S. spontaneum* (2n = 40 - 128) over a century ago. Due to interspecific origin and high ploidy, crosses between sugarcane genotypes can produce aneuploid progeny that make classical breeding studies difficult to interpret. Another major drawback in sugarcane breeding is that it can take up to 15 years to develop a new cultivar. Homoeologous sugarcane genes are characterised by a high level of DNA sequence conservation - the highest level of genetic redundancy of all polyploid plants studied thus far (Garsmeur *et al* 2011, New Phytol. 189: 629–642). Sugarcane can therefore be expected to be tolerant of a mutation breeding approach to increasing genetic and epigenetic diversity. Our approach combines *in vitro* culture with mutagenesis using ethyl methane-sulfonate and epi-mutagenesis using 5-azacytidine. These treatments are likely to affect gene sequence and gene expression, the latter through altering methylation patterns of homoeologous genes, and through re-insertion effects of activated transposons. *In vitro* selection regimes are applied at somatic embryo maturation and germination stages, through to plantlet hardening-off. Several target site and non-target site imidazolinone herbicide resistant mutants are in the process of being characterised in the field. Improved stalk rot resistance has been generated by incorporation of *Fusarium sacchari* mycotoxins into selection media. Since certain fusarium isolates have been found to be beneficial to the stalk boring insect pest *Eldana saccharina*, ongoing research aims to confirm mutant resistance to both fusarium and pest. A new direction involves the exposure of *in vitro* material to priming chemistries before, during and after mutagenesis. Selection pressures (mycotoxins, heat and osmotic), similarly applied, aim to fix epi-genetic priming for tolerance, or to increase responsiveness to priming *ex vitro*.

P-14

Tissue Culture Mutagenesis as a Tool for Developing Herbicide Tolerant Traits in Crops. JILL STEVENSON-PAULIK, Dale Carlson, Luke Mankin, Haiping Hong, and Lou Ann Batts. BASF Plant Science, LP, 26 Davis Drive, Durham, NC 27709. Email: jill.stevenson@basf.com

The Clearfield® Production Systems are currently marketed, non-transgenic herbicide tolerant systems that are comprised of high-quality seed varieties and excellent weed control through imidazolinone herbicides. Clearfield® crops contain single point mutations in the acetohydroxyacid synthase gene involved in branched chain amino acid synthesis. These single base pair changes render the enzyme insensitive to herbicidal inhibition, which confers whole plant herbicide tolerance. As one of the few internationally recognized non-GM weed control systems, the Clearfield® system is valued for its global market acceptance. To achieve this broad approval, there is a preference for the use of conventional breeding techniques to generate the trait, such as through random mutagenesis via seed or tissue culture mutagenesis (TCM). TCM has been used to successfully develop commercial products in Clearfield® Canola and in a new weed control system, The

ProvisaTM Rice System, in which the herbicide tolerance is also based on a single base pair change. The ProvisiaTM rice system is expected to enter the market in the latter part of this decade. Conventional breeding techniques can continue to be utilized to bring additional novel non-GM HT systems to the market and offer superior weed control to farmers.

P-15

Versatile and High Throughput System of Expression Vector Assembly for Plant Genetic Engineering. P. J. WESTFALL, R. Blue, J. Huenemann, N. Jayasuriya, T. Campbell, S. Webb, and O. Folkerts. Dow AgroSciences, Advanced Technology Development; 9330 Zionsville Rd. Indianapolis, IN 46268. Email:pjwestfall@dow.com

The ability to rapidly engage the Design-Build-Test-Analyze cycle of Biological Engineering is built on a foundation of standardized practices that allow for predictable assembly of multipart DNA complexes. Although these practices are widely used for engineering microbial systems for production of natural products, pharmaceuticals and biofuels, they are less common in more complex plant model systems. At Dow AgroSciences we have developed a Rapid Assembly Platform for DNA (RAPD) that leverages a set of standardized DNA "parts" to allow for combinatorial assembly of hundreds of large, multi-genes stacks in as little as 2-3 weeks. Due to its highly robust and predictive assembly rules, the DNA construction process has been fully automated and only requires minimal human interaction throughout the entire assembly. This allows for a shift in resources away from laborious molecular biology liquid handling tasks and towards the development of high-capacity, predictive model systems that can provide contextual hypothesis testing for crops of interest.

P-16

A High Throughput Cell-based System for Screening Synthetic Promoters in Crop Plants. T. WAIBEL, K. Brzezek, J. H. Critchley, and M. L. Roberts. Synpromics Ltd, NINE, Edinburgh Bioquarter, 9 Little France Road, Edinburgh EH16 4UX, UK. Email: Thomas.Waibel@synpromics.com

Plant synthetic promoters have been identified as important tools for manipulation of gene expression. Transgenic crop plants are invaluable assets to modern agriculture, but precise regulation of transgene expression is crucial. Synthetic promoters make it possible to tightly regulate gene expression and offer several advantages compared to natural promoters. Among these are reduced risk of homology dependent gene silencing, differential expression strength and a variety of specific expression characteristics e.g. constitutive, inducible, tissue-specific. The rational design of a synthetic promoter is difficult and functional promoter architecture is challenging to predict. Therefore identification of well performing synthetic promoters requires the screening of a large number of promoter candidates. This, in-turn, necessitates the availability of a high-throughput screening method capable of screening large custom-built libraries of synthetic promoter candidates. The libraries are typically constructed using Cis regulatory elements (CREs), which are identified by computational analysis of transcriptomic data. These CREs are selected based on the synthetic promoter requirements, e.g. inducible, constitutive or tissue-specific. Transient expression assays in protoplasts are the most suitable method for promoter analysis, as assays can be performed rapidly and at a much larger scale than in stably transformed tissue. Automation and the use of multi-well plates are favourable as they decrease human error and allow for high-throughput, respectively. Using an automated protoplast high-throughput screening method we have successfully screened a library of more than 1500 unique promoter candidates for constitutive expression in *Zea mays*. We identified functional synthetic promoters with varying expression strengths, length and number of randomly arranged CREs. Promoters, identified through this protoplast high-throughput assay are synthetic and contain novel sequence combinations, which has favourable functional and licencing implications.

P-17

A Novel Cell-based Bioassay for Allelopathy and Salt Tolerance Using Protoplasts. H. SASAMOTO. Research Institute for Integrated Science, Kanagawa University, Hiratsuka, Kanagawa, 259-1293, JAPAN and Yokohama, Yokohama National University, 240-8501, JAPAN. Email: sasamoto@ynu.ac.jp

'Protoplasts Co-culture Method' was developed to study allelopathic activities of plants using tissues and their cultured cells. This method is based on the idea of co-culturing of callus of a test plant to determine its allelopathic effects on the growthof another callus. However, quantitative evaluation of experimental results is difficult using the callus co-culturing method. We developed a protoplast co-culture method, as this method is better for quantitative determinations of results. The response of cultured protoplasts can be expressed as numbers of enlarged, divided protoplasts, in a 50 μL of medium in a well of a 96-well culture plate. For example, inhibitory effects of protoplasts of a test plant, leguminous *Mucuna pruriens*, on growth of a recipient

protoplasts of lettuce or rice were determined in a Murashige and Skoog's medium containing 2,4-dichlorophenoxyacetic acid and benzyladenine, sucrose and osmoticum mannitol. Protoplasts densities were varied. Putative allelochemical, l-DOPA, was applied to the same system and this compound showed strong inhibitory effects on the growth of lettuce protoplasts. The results were in agreement with those obtained by the conventional *in vitro* bioassay method and field tests. We applied such method to determine the allelopathic activities of salt-tolerant *Sonneratia* mangrove suspension cells on lettuce. And an inverse relationship between allelopathic activity and salt tolerance was found. Several other plants and allelochemicals have been studied. Our studies offer an *in vitro* assay method, which can be used to study allelopathy between various test plants and recipient plants and quantitative evaluations are possible under different culture conditions in order to simulate the possible future environmental risk.

P-18

Genome Engineering in Tomato: High Throughput Methods for Mutagenesis. P. BUNDOCK, A. Bonné, R. Sevénier, F. Lhuissier, and M. de Both. Keygene N. V., Agro Business Park 90, 6708 PW Wageningen, THE NETHERLANDS. Email: paul.bundock@keygene.com

The challenge of increased global food production and security requires a good understanding of the genes and pathways underlying important traits in crop plants. Crucial to this is the availability of mutants that can be used to unravel gene function and be tested for novel beneficial phenotypes. While collections of mutants are available in plant model systems, the number of mutants in important crop species is limited. With the advent of site specific nuclease technologies such as ZFN's, TALENs and CRISPRs we now have the tools to create mutations in crop plants on demand. However, the application of these technologies to crop plants to produce commercially viable products remains a challenge. We will discuss the methods that we have developed that enable the use of these site specific nuclease technologies to generate tomato mutants in a high throughput manner and how these can also be applied to a broader range of crop species.

P-19

Recent Advances in Cell-based Technologies for Unleashing the Power of Innovation. JAMES F. LEARY. Birck Nanotechnology Center, Purdue University. Email: jfleary@purdue.edu

The cell and molecular biology of individual cells as a complex networked system of thousands of expressed genes in a typical cell, when studied at the single-cell level, provides powerful new insights into the fundamental biology and the "molecular pathology" of normal and diseased cell states in both plant and animal cells. Such fundamental biology should be studied at the single cell level where the inter-relationships of the expressed genes are intact and inter-connected, not in "bulk" assays where the expressed genes from different cells are mixed. Locational single cell proteomics allows for the study of protein networks within individual cells. Specific single cells can be isolated on the basis of these properties allowing for gene expression profiles to be studied on small numbers of or even single cells. There are a number of quantitative single-cell and high-throughput technologies such as multiparameter flow cytometry/cell sorting and scanning image cytometry, when combined with other cell and molecular technologies that allow for these molecules to be quantitatively studied with respect to each other within the same cell. These technologies can be combined with single-cell delivery of drugs or genes to alter the expression of genes and subsequent cell function in both plant and animal cells. Highly targeted delivery of otherwise labile molecules to specific cells can be done using a combination of antibody- or peptide-targeted nanoparticles containing not only these molecules but also delivery to the relevant specific intracellular locations using a combination of membrane penetrating peptides and intracellular localization peptide sequences. The success of such delivery methods can be immediately evaluated by again using these same single cell technologies.

P-20

Plant Protoplast-based Rapid Testing Systems for Assessing ZFN Mediated Genome Engineering. L. SASTRY DENT, W. M. Ainley, P. Samuel, Z. Cao, C. Dewes, L. Shen, C. Caroll, K. West, S. Sriram, S. Evans, and S. Webb. Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN . Email: Lsastry-Dent@dow.com

Next generation technologies for trait discovery and development are critical for generating solutions to meet the anticipated food demands of the growing global population. Recent advances in genome engineering technologies based on site specific nucleases allow specific and predictable alterations in plant genomes to generate value creating traits. The EXZACT^{TM} Precision technology, based on Zinc Finger Nucleases (ZFN) has been used for introducing transgenes or mutations at specific genomic loci in crop species. To accelerate EXZACT^{TM} mediated genome engineering, novel plant protoplast-based rapid testing systems have been developed that allow assessment of targeting without stable plant generation. New

analytical methods and donor designs that enable rapid testing methods and application of the methods for idea testing will be described. These rapid targeting systems increase testing capacity and reduced cycle times to evaluate ZFN activity in plant cells and will have significant impact on deployment of targeting technologies for trait discovery and development.

P-21

Epitope-tagged Protein-based Artificial miRNA Screen in Protoplasts for Optimized Gene Silencing in Plants. J. F. Li[1], D. Zhang[1], and J. Sheen[2]. [1]Sun Yat-sen University, 135 Xin-Gang Xi Road, Guangzhou 510275, CHINA and [2]Harvard Medical School, 185 Cambridge Street, Boston, MA 02114. Email: lijfeng3@mail.sysu.edu.cn

Artificial miRNA (amiRNA) technology offers more specific gene silencing than hairpin RNA-mediated RNA interference in plants. However, the in vivo performance of amiRNAs is not predictable, which has limited their broad applications in plant basic and applied research . To quickly identify the most active amiRNAs for achieving maximal silencing of the target gene(s), we developed the epitope-tagged protein-based amiRNA (ETPamir) screen, in which target gene encoding epitope-tagged proteins was constitutively or inducibly co-expressed with individual amiRNA candidates in plant mesophyll protoplasts. Accumulation of tagged proteins, as monitored by immunoblotting with commercial tag antibodies, inversely and quantitatively reflects the efficacies of individual amiRNA candidates in plant cells. Optimal amiRNAs determined by the ETPamir screen can faithfully generate loss-of-function mutants for the target gene when transgenically expressed, phenocopying corresponding genetic null mutants. Functional analyses of target genes can also be rapidly conducted in protoplasts constitutively expressing these optimal amiRNAs. The ETPamir system can be readily applied to diverse plant species and the concept can be extended to verify predicted target genes for native plant miRNAs.

P-22

A New System for Identification of Haploids in Maize. W. SCHIPPRACK and A. E. Melchinger. Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, D-70593 Stuttgart, GERMANY. Email: schippi@uni-hohenheim.de

In vivo haploid induction has become a routine tool for rapid line development in maize. However, distinguishing haploid (*H*) from diploid crossing (*C*) seeds is problematic for many germplasm due to poor expression or suppression of the currently used *R1-nj* embryo marker. Xenia effects on oil content (OC) of seeds have been proposed as an alternative for distinction of *H* from *C* seeds (Rotarenco et al., 2007). Melchinger et al. (2014) investigated this proposal and showed, that, using an normal oil inducer with OC=3%, OC distributions of H and C fractions overlap too much for reliable sorting. Therefore Melchinger et al. (2013) proposed a new method for sorting H and C seeds, based on pollination with inducers having high oil (HO) content in the seeds, and presented its theoretical foundation. In a proof-of-concept ten source germplasm of different genetic structure were pollinated by a high-oil (HO) inducer with OC=10.8%. The average difference between the mean OC of *C* and *H* seeds was more than twice the standard deviation within each fraction and sorting of *H* and *C* seeds based on their OC was generally more reliable than based on the *R1-nj* embryo marker. For clarification, whether reliable sorting of H and C seeds could possibly be achieved by measuring oil mass (OM) alone, Melchinger et al. (2015) compared the distribution properties of *H* and *C* seeds from 11 induction crosses with high-oil inducers for seed weight (SW), OM, and OC. Since misclassification rates for sorting of *H* and *C* seeds were generally smaller for OC than for OM and the optimal bivariate discrimination function on the basis of OM and SW yielded similar error rates as a univariate discrimination function on the basis of OC alone, it is recommended to use OC as a simple and robust criterion for sorting of *H* and *C* seeds. Altogether, induction crosses with HO inducers hold great promise for promoting the DH technology in maize, especially for many flint and tropical germplasm, which suppress anthocyanin pigmentation of the embryo. To take full advantage of this novel approach an automated high-throughput platform for sorting seeds is recommended.

P-23

Canola Variety Development using Doubled Haploidy. K. STECKLER. Dow AgroSciences Canada Inc., 101-421 Downey Road, Saskatoon, Saskatchewan, CANADA S7N 4L8. Email: kasteckler@dow.com

Doubled haploid (DH) production accelerates the development of homozygous breeding lines and facilitates the identification of recessive traits and mutations in breeding populations. DH production can be achieved through various technologies including the culture of anthers or microspores, the culture of unfertilized ovules, and interspecific crosses followed by chromosome elimination. The efficiency of each technology depends primarily on the crop species. Microspore culture is

utilized for DH production in canola. Late stage uninucleate canola microspores are isolated from donor plants and cultured with Trifluralin, a mitotic inhibitor. These individual haploid cells respond to the in vitro chromosome doubling agent within 72 hours. The dividing microspores form embryo like structures which are transferred to agar media for regeneration into DH plants. On average, seventy percent of the plants are doubled haploids. Canola DH seed is harvested within 9 months of donor plant initiation. Canola DH production is a high throughput process which incorporates in vitro herbicide selection, automated media preparation, flow cytometry, and gene of interest selection. The presentation will elaborate on these strategies which have optimized canola DH production.

P-24

Recent Advances in In Vivo Haploid-induction Technologies. ANNE B. BRITT[1], Sundaram Kuppu[1], Ek Han Tan[2], Luca Comai[2], and Simon W. L. Chan[1], [1]Dept. Plant Biology, University of California, Davis, CA 95616 and [2]Plant Biology and Genome Center, University of California, Davis, CA 95616. Email: abbritt@ucdavis.edu

The centromeric histone variant histone 3 (CENH3, aka CENP-A) is essential for the segregation of sister chromatids. To better define CENH3 functional constraints we complemented a null allele with a variety of mutant alleles each inducing a single amino acid change in conserved residues of the histone fold domain. Surprisingly, many of these missense lines displayed wild-type growth and fertility on self-pollination, but exhibited frequent post-zygotic death and uniparental inheritance when crossed by wild-type plants. The failure of centromeres marked by the altered CENH3 is consistent with quantitative determination of centromere strength, as well as with a role for CENH3 variation in reproductive isolation, and so as a driver for speciation. Additionally, these findings provide a simple method for the identification of non-transgenic haploid inducers in existing mutagenized collections of crop species. We demonstrate the feasibility of this approach by identifying a haploid-inducing mutant in an existing Arabidopsis TILLING population. *We gratefully acknowledge funding for this project from Rijk Zwaan.*

P-25

Design of Experiments (DOE) and *In Vitro* Culture – History, Concepts, and Relevance. RANDALL P. NIEDZ. USDA-ARS, U. S. Horticultural Research Laboratory, Ft. Pierce, FL. Email: Randall.Niedz@ars.usda.gov

A successful *in vitro* culture system matches the environmental conditions and media components to the requirements of the plant species. Because an *in vitro* system is extremely complex, determining the condition/component types and levels for acceptable *in vitro* growth can be difficult. Many of the DOE principles for understanding complex systems were articulated by Ronald A. Fisher in his book, *The Design of Experiments* (1935). Fisher's basic insight was that nature is complex and inherently multivariate; thus, only multifactor experiments are capable of detecting important interactions. The statistical methods he describes (DOE) were designed to maximize the information obtained from the least amount of data. However, fully applying DOE was difficult because the statistical calculations were extremely cumbersome without a computer/software. Today, the mechanical drudgery of the statistical calculations have been eliminated because computers are sufficiently powerful and software is widely available. Applying these DOE principles has several benefits: 1) *Resource efficient* – Experiments precisely answer the question(s) being asked using the least amount of resources; 2) *Scientific contribution* – The resulting data is information rich and contributes to a basic understanding of the system and; 3) *Practical application* – The types and levels of components for acceptable *in vitro* growth are identified. Key DOE concepts, the role and use of computer/software in DOE, why plant tissue culture is an ideal system for DOE, how implementing DOE concepts in *in vitro* research will compliment other fields of biology such as the various –omics, and how to get started will be discussed.

P-26

Practical Aspects of Running DOE for Improving Growth Media for *In Vitro* Plants. BARBARA M. REED and Jeanine S. DeNoma. USDA-ARS National Clonal Germplasm Repository, 33447 Peoria Rd, Corvallis, OR 97333-2521. Email: Barbara.Reed@ars.usda.gov

Experiments using design of experiment (DOE) software to improve plant tissue culture growth medium are complicated and require complex setups. Once the experimental design is set and the treatment points calculated, media sheets and mixing charts must be developed. Since these experiments require three passages on the same treatment before data is taken, it is very important that the calculations are correct and the procedures clear so they can be repeated accurately. It may be necessary

to develop new stock solutions unique to the experiment. Calculating the amount of each stock solution needed for a treatment is most easily done with a spreadsheet program. The third step is a chart indicating how much of each stock is to be used for the required number of containers of each treatment and any factors that are part of the basal medium (agar, vitamins and growth regulators). All treatments can be run at one time or sequentially in smaller more manageable sets. In addition, one experiment can require 60-80 containers per genotype tested and considerable space in the growth room. Generally test-medium preparation requires one or two days and planting two to three days for each passage. Useful data may include a quality rating, shoot length and multiplication or callus size and fresh/dry weight, leaf or stem color, size or abnormalities and biochemical analyses. Photographs of the shoots or callus at the end of the experiment are useful as a comparison to graphical data and rating scales. Large amounts of data are generated and these can be managed on a spreadsheet. These types of experiments answer questions with fewer treatments and less plant material than the traditional factorials and model the results so that the driving factors can be determined and further explored.

P-27

Using a DOE Approach for Improving Growth of the Endangered *Cycladenia humilis* var. *jonesii* (Apocynaceae) In Vitro. VALERIE C. PENCE[1], Linda Finke[1], and Randall Niedz.[2] [1]Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220 and [2]USDA-ARS-U.S. Horticultural Research Laboratory, 2001 South Rock Road, Ft. Pierce, FL 34945-3030. Email: valerie.pence@cincinnatizoo.org

Extreme hyperhydricity is a problem in shoot cultures of the endangered species, *Cycladenia humilis* var. *jonesii*. Six potential medium and environmental factors that might affect hyperhydricity were tested using a Design of Experiments (DOE) approach, to determine both main and interaction effects. The six factors included NO_3, NH4:K ratio, $CaCl_2$, BAP concentration, gelling agent (agar vs. gel), and venting (vented vs. nonvented). A 2-level fractional factorial experimental design was used to identify which, if any, of these factors or their 2-way interactions had large effects on hyperhydricity. Four responses were measured including tissue DW, shoot morphology, catalase activity, and protein level. Three factors were associated with a more normal phenotype - venting, agar, and a lower NO_3 concentration. Using this information a second experiment using a 2x2 factorial compared the revised culture medium to the original medium, with and without venting, through three 4-week subculture cycles, using hyperhydric tissue as the starting material. At the time of each subculture, tissue from each treatment was harvested and the 4 responses measured. Tissues grown on the original medium (OM) with venting were tall, robust, and more normal than tissues on OM with no venting, which remained very hyperhydric. Tissues on the revised medium (RM) with venting were also more normal in character than those on RM without venting, although the shoots were smaller than those on OM. However, even without venting, tissues on RM displayed more normal characteristics than tissues on OM without venting (e.g. 9.8% DW, 428 U catalase/mg protein compared with 6.8% DW, 587 U catalase/mg protein, respectively), indicating that media components were driving this change, even in the absence of venting. These results provide insights into the physiological disorder of hyperhydricity and demonstrate that the DOE approach, used as a screening tool, can provide guidance for improving media. This approach has led to improvements in the propagation of *Cycladenia*, as well as other rare species in our laboratory.

P-28

Different Solutions for Optimal Responses in Laboratory and Greenhouse Growth: A Case Study with Turmeric. JEFFREY ADELBERG and Rabia F. El-Hawaz. Clemson University, 277 Poole Ag. Center, Dept. of Agricultural and Environmental Sciences, Clemson SC, 29634. Email: jadlbrg@clemson.edu

A series of multifactor DOE nutrient optimization experiments were conducted on the medicinal herb, turmeric. All tissue culture was done in liquid media and high, low and moderate describe ion concentrations relative to MS solution. The first experiment optimized macronutrients, and the second meso-nutrients for micropropagation. Lower NH_4^+(5mM), high PO_4^3 (6.25mM) and Mg^{+2} (4.5mM) medium produced the greatest number of plants and the largest sized plants. The meso-nutrient multifactor design was repeated in a fed-batch bioreactor where plants were grown for five months by periodic supplementation with

water, sucrose and nutrients. Optimal nutrients to produce the most new plants and highest fresh mass was similar to micropropagation. Dry mass accumulation in the rhizome was driven by high PO_4^{-3}(6.25mM) which required more sucrose (whereas greatest fresh mass was driven by KNO_3 which required more water). The bioreactor grown plants were brought to the greenhouse and grown for another six months. The most massive lab plants from the high meso-nutrient media grew more slowly and had fewer offsets than plants that been grown on low PO_4^{-3}(1.25mM) and low Ca^{+2} (3mM) media in the lab. Curcumin, demethoxy-curcumin, and bisdemethoxycurcumin concentrations in dried rhizomes were greatest from lab plants that had been cultured in the low PO_4^{-3} (1.25mM) and Ca^{+2} (3mM), moderate KNO_3 (60mM) formulations. Another meso- nutrient multifactor micropropagation experiment was conducted on two different clones of turmeric, a greater range of mineral concentrations, with an MS control. During acclimatization in the greenhouse, plants from the multi-factor group of experimental medium grew better than MS control. Low NH_4^+ (5mM), moderate KNO_3 (60mM), and low Ca^{+2} (2mM) yielded plants that grew the fastest in the greenhouse, for both clones. The mineral composition of in vitro medium had a strong impact on subsequent greenhouse growth. There was consensus across experiments of an optimal medium for ex vitro responses that differed from what was optimal in the lab.

P-29

In Vitro Technology at the US Potato Genebank. J. B. BAMBERG and M. W. Martin. US Potato Genebank, 4312 Hwy 42, Sturgeon Bay, WI, 54235. Email: John.Bamberg@ars.usda.gov

The US Potato Genebank at Sturgeon Bay, WI, is the active national germplasm collection for the world's most important vegetable crop. It contains 6,000 accessions of 100 species of tuber-bearing relatives of *Solanum tuberosum*. The potato of commerce is a clonal crop susceptible to many systemic pathogens, so the genebank routinely uses *in vitro* clonal maintenance and distribution for named cultivars. *In vitro* management is also the tool of choice for various breeding and genetic stocks of interest to breeders and researchers. Long-term back-up cryo-preservation of clones is done at the base collection at Ft. Collins, CO. *In vitro* techniques also play an important role in virus elimination of clones. We have recently expanded *in vitro* propagation to the temporary safekeeping of meristem propagules in antibiotic medium during collecting expeditions in the wild in Arizona. The genebank mission includes promoting technology that supports expanded use of the germplasm, particularly finding ways to overcome interspecific hybridization barriers. Thus, *in vitro* techniques like pollen viability testing, ploidy manipulation, protoplast fusion, and embryo rescue have contributed to major advances. Finally, advancing *in vitro* technology holds promise as a new tool for mass bioassay and selection of seeds, pollen, or somaclones for useful traits.

P-30

Pre-Columbian Agaves in Arizona: New Potential for Ancient Domesticates. ANDREW SALYWON and Wendy C. Hodgson. Desert Botanical Garden, 1201 N. Galvin Parkway, Phoenix, AZ, 85008. Email: asalywon@dbg.org

The importance of Agaves (century plants: Agaveaceae) to Native Americans in Mesoamerica has long been recognized, with Mexico being the center of origin and diversity of the genus from the total number of species and from the agricultural perspective. Agaves were, and continue to be, used for numerous purposes including food, fiber and beverage. However, their significance to Mesoamerican cultures has overshadowed and distorted their role in the lives and cultures of indigenous peoples north of the modern international border. Pre-Columbian farmers cultivated/domesticated several species of agave in Arizona from at least 600 to approximately 1400 A.D., including Agave murpheyi, A. delamateri, A. phillipsiana, Agave verdensis and Agave yavapaiensis. Because of their longevity and primarily asexual reproduction (their cultivation is correlated with clonal reproduction and sterility), relict agave clones have persisted in the landscape to the present and provide an opportunity to study pre-Columbian nutrition, trade, migration and agricultural practices. Vegetative reproduction has perpetuated favorable characteristics selected by farmers and allowed agaves to persist for over seven hundred years. Molecular genetic data has revealed at least four separate evolutionary lineages contributing to the domesticated agave diversity in Arizona pointing to a complex pattern of agave use and cultivation. All of these cultivated/domesticated species are extremely rare and in need of conservation. The opportunity exists to rediscover these ancient domesticates as potential 'new crops' for the production of mescal (a distilled alcoholic beverage similar to tequila), while at the same time serving as a form of ex-situ conservation. In order for the successful implementation of this agave conservation and agricultural program, methods will need to be developed for the mass production of propagules – most likely via tissue culture.

P-31

The Desert Legume Program Seed Bank - Conservation of Fabaceae from the World's Dry Regions. M. B. JOHNSON. Desert Legume Program, The University of Arizona/Boyce Thompson Arboretum, 2120 East Allen Road, Tucson, AZ, 85719. Email: mjohnson@ag.arizona.edu

The Fabaceae is one of the largest and most diverse families of flowering plants with over 18,000 species worldwide. It is second only to the cereal grains in terms of importance for human nutrition. Varous species are important for forage and fodder for livestock, cover crops, soil improvment, medicinal uses, industrial compounds, fuel, timber, erosion control, shade, windbreaks, and as amenity and landscape plants among other uses. The Fabaceae is well-represented in the floras of dry regions, and in many hot, dry environments, species of Fabaceae are often keystone species. While much work has been done on crop species, wild species of Fabaceae have recieved comparatively little attention. The Desert Legume Program (DELEP) was established in 1988 ot develop a comprehensive collection of seeds of wild species of Fabaceae from around the world that are native to or adapted to dry climates, to learn more about potential uses of these plants, to provide samples of seeds to individuals and organizatons interested in working with these plants, and to promote the preservation and conservation of the diversity of Fabaceae from dry regions. Working with a wide range of stakeholders, DELEP has accomplished much and continues to seek opportunities to further its mission.

P-32

Cryobanking of Plant Species, Status and Promise. M. M. JENDEREK[1] and B. M. Reed[2]. [1]Plant and Animal Genetic Resources Preservation, NCGRP, USDA-ARS, Fort Collins, CO 80521 and [2]National Clonal Germplasm Repository, USDA-ARS, Corvallis, OR 97333. Email: maria.jenderek@ars.usda.gov

Currently, the PAGRP has over 4,000 unique samples of clonally propagated species and about 49,000 seed samples in long-term liquid nitrogen storage. Cryopreservation of plant genetic resources has several advantages over germplasm maintenance in field or in vitro; the main of the advantages are protection from biotic and abiotic stress factors, small storage space needs and the possibility of longterm storage without a necessity of replanting or culture transfer. Any totipotent plant fragments can be considered for cryopreservation. In applied germplasm cryopreservation, the most commonly used explants are meristem shoots (MS) and winter dormant buds (DB) due to their low risk for somaclonal changes during the storage and regeneration. Each cryo-event requires several pretreatment steps of the processed tissue. The steps are more involved for MS than for DB; however these depend on the plant species and the cryo technique applied. The excision of meristem shoots is more laborious for monocotyledonous than for dicotyledonous species and the pretreatment steps are more involved for tropical and subtropical plant species than for species originating from temperate zones. Costs are trivial compared to the yearly cost of maintaining duplicate field or greenhouse collections. The initial expense of cryopreserving an accession by clonal propagules is high but the cost amortizes in a few years and the yearly maintenance cost of an accession in a liquid nitrogen tank is below $1. Cryopreservation of dormant buds is ca. 10 times less expensive than cryopreservation of meristem shoots. The USDA-ARS, National Plant Germplasm System holds over 570,000 plant germplasm accessions in field collections and under various storage conditions. The maintenance efforts of the genetic resources are enormous. Cryobanking of new plant species, beyond the currently cryopreserved, requires research on developing applicable procedures and may be challenging; however, it carries a great promise for food security in the future.

P-33

Epigenetic Control of Gametophyte Development and Embryo Development. IGOR KOVALCHUK. Dept. of Biological Sciences, University of Lethbridge, Lethbridge, CANADA. Email: igor.kovalchuk@uleth.ca

Angiosperms do not have a discrete germline, but rather develop gametes upon transition of vegetative meristem to flowering meristem. These cells give rise to male and female gametophytes, and later on to endosperm and the embryo. During their lifespan plants accumulate the information about environmental exposures in the form of differentially expressed non-coding RNAs, changes in the pattern of DNA cytosine methylation as well as DNA association with various histone and non-histone proteins. Gametophyte development and subsequent embryo development includes substantial epigenetic modifications, including partial reprogramming of acquired epigenetic changes. Since reprogramming is not as extensive as in animals, plants are able to pass substantial amount of information about environmental exposure from one generation to another. In this talk I will present an overview of epigenetic changes

occurring in male and female gametes and will discuss the role of epigenetic factors in transgenerational inheritance of acquired traits.

P-34

Natural Variation in Epigenetic Pathways Affects the Specification of Female Gamete Precursors in Arabidopsis. DANIEL RODRIGUEZ-LEAL, Gloria León-Martínez, Úrsula Abad-Vivero, and Jean-Phiippe Vielle-Calzada. Grupo de Desarrollo Reproductivo y Apomixis, Laboratorio Nacional de Genómica para la Biodiversidad y Departamento de Ingeniería Genética de Plantas, Cinvestav Irapuato CP36821 Guanajuato, MEXICO. Email: drodriguez@langebio.cinvestav.mx, vielle@ira.cinvestav.mx

In Angiosperms, the transition to the female gametophytic phase relies on the specification of pre-meiotic gamete precursors from sporophytic cells in the ovule. In Arabidopsis, a single diploid cell is specified as the pre-meiotic female gamete precursor. Here we show that ecotypes of Arabidopsis exhibit differences leading to phenotypes reminiscent of defects in dominant mutations that epigenetically affect the specification of female gamete precursors during megasporogenesis. Intraspecific hybridization and polyploidy exacerbate these defects, which segregate quantitatively in F2 populations derived from ecotypic hybrids, suggesting that multiple loci control cell specification at the onset of female meiosis. This variation in cell differentiation is influenced by the functional activity of *ARGONAUTE9 (AGO9)* and *RNA-DEPENDENT RNA POLYMERASE6 (RDR6),* two genes involved in epigenetic silencing that control the specification of female gamete precursors. The pattern of transcriptional regulation and localization of AGO9 varies between ecotypes, and abnormal gamete precursors in ovules defective for *RDR6* share identity with ectopic gamete precursors found in selected ecotypes. Our overall results indicate that differences in the epigenetic control of cell specification lead to natural phenotypic variation during megasporogenesis, a mechanism that could be implicated in the emergence and evolution of the reproductive alternatives that prevail in flowering plants.

P-35

Transgene-induced Female Reproductive Development in the Absence of Egg Cell Fertilization. P. OZIAS-AKINS, J. A. Conner, H. Huo, and K. Chae. Department of Horticulture and Institute of Plant Breeding, Genetics & Genomics, The University of Georgia, Tifton, GA 31793. Email: pozias@uga.edu

During normal sexual reproduction in flowering plants, egg cells are stimulated to develop into embryos upon fertilization by a sperm cell. A gene that induces egg cell division in the absence of fertilization was cloned from a natural apomict, *Pennisetum squamulatum*. The *BABY BOOM-like* gene (*ASGR-BBML*) is predicted to encode an AP2-domain containing transcription factor that is most similar to a rice *BBM* ortholog. When transformed into sexual pearl millet (*P. glaucum*) or rice (*Oryza sativa*), haploid eggs are capable of embryo formation. Seed development is non-uniform, probably due to variation in endosperm formation that may be prevented by precocious embryo growth. Nevertheless, haploid plants have been recovered from transgenic lines, suggesting that expression of *ASGR-BBML* may have utility for haploid induction as well as apomixis when appropriately regulated.