

## Plant Symposia and Workshops

### P-1

Automating Plant Transformation. T. MICHAEL SPENCER<sup>1</sup>, Brandon J. Davis<sup>1</sup>, Mary Lou Mangano<sup>1</sup>, Rebecca A. Kelly<sup>1</sup>, Douglas C. Boyes<sup>2</sup>, Michael. W. Petersen<sup>2</sup>, Brian J. Martinell<sup>2</sup>, and David A. Somers<sup>1</sup>. <sup>1</sup>Monsanto Company, 62 Maritime Dr., Mystic, CT 06355 and <sup>2</sup>Monsanto Company, 8520 University Green, Middleton, WI 53562–0999. Email: michael.spencer@monsanto.com

Crop plant transformation systems are typically labor intensive and involve highly repetitive tasks. This presentation will cover strategies for and experiences with applying automation to transgenic plant pipelines with the aim of increasing system throughput and capacity while reducing labor and costs associated with production of transgenic plants.

### P-2

Molecular Approaches Improve Crop Transformation Efficiency. XUDONG YE<sup>1</sup>, Zarir Vagchhipawala<sup>2</sup>, Yurong Chen<sup>2</sup>, Ed Williams<sup>2</sup>, and Larry Gilbertson<sup>1</sup>. <sup>1</sup>Monsanto Company, 700 Chesterfield Pkwy West, Chesterfield, MO 63017 and <sup>2</sup>Monsanto Company, 8520 University Green, Middleton, WI 53562. Email: xudong.ye@monsanto.com

During 30 years of plant transformation technology development, a great deal of focus has been on expanding transformable species and increasing transformation frequency through tissue culture optimization. Transgene integration quality has not been widely addressed although single-copy transgene events lacking vector backbone are desired for most biotechnology applications. We have explored molecular approaches to improve transformation frequency and transgene integration quality in *Agrobacterium*-mediated crop transformation. Through transformation vector modification, such as the addition of a growth retarding gene in the vector backbone, a significantly higher frequency of single-copy, backbone-free transgenic events can be achieved. We have also demonstrated that a vector with a low copy origin of replication in *Agrobacterium* can significantly increase single copy, backbone-free event frequency in transformation of

several crops species. Through site-directed mutation analysis of an ori pRi backbone, we identified a higher copy ori pRi vector, which abolishes the advantage of single copy transgene frequency improvement in crop transformation. In another approach, we have shown that transformation frequency in cotton can be significantly improved by using modified *Agrobacterium* strains with a constitutive active *virG* allele.

### P-3

The EXZACT™ Precision Transformation & Gene Stacking Platform: Design, Development, Deployment and Implications on New Plant Product Discovery and Development. STEVEN R. WEBB, W. Michael Ainley, Lakshmi Sastry-Dent, Pon Samuel Jayakumar, Steven L. Evans, Paul Lewer, Patrick Westfall, David R. Corbin, Gary Rudgers, and Joseph F. Petolino. Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN, 46268. Email: srwebb@dow.com

The EXZACT™ Precision Transformation and Gene Stacking platform represents a suite of technologies that enable precise genomic editing in plants. Although targeted double stranded DNA breaks are a necessary prerequisite to enable precise genomic editing we have found that for many desired genome editing applications the creation of a precise double stranded break by itself was insufficient to efficiently achieve the desired outcome. Our research demonstrates that several additional technologies, including delivery, donor design and structure as well as novel analytical methodologies are required along with engineered zinc finger nucleases to realize the potential of precise genome editing in plants. The present talk describes the design, development and deployment of the precision transformation and gene stacking platform as well as the implications on new plant product development processes.

### P-4

Nanocarrier-cargo Technology for Engineering Nucleus and Organelle Genomes. FRANÇOIS EUDES. Agriculture and Agri-Food Canada, Lethbridge Research Centre, PO Box 3000, Lethbridge, AB T1J 4B1, CANADA. Email: francois.eudes@agr.gc.ca

Short peptides with property to translocate across cell barriers and target specifically subcellular localizations have been discovered. These peptides also have capacity to form hydrogen bonds with cargo molecules such as nucleic acid and protein, and transport them specifically to one of three subcellular destinations: the nucleus, the chloroplast and the mitochondria of plant and animal cells. Three distinct classes of nanocarrier can be described based on the charge and hydrophilicity of their peptide sequences. Complementary to the nanocarrier, the cargo can be designed using nucleic acid and proteins, forming blocks that conjugate in a relatively predictable manner. Various cargo translocations across the plasma membrane, energy dependent, have been studied in crop cell systems such as wheat, triticale, and canola microspores. ssDNA binding proteins such as RecA, Rad51, SSB and VirD2 were used to form cargoes with properties to either increase the integrity of stable integration of donor ssDNA or target site specific integration in the haploid plant genome of microspore. The distinct ability of these short peptides to deliver functional macromolecules specifically in one of the three organelles has led to development of a novel nanocarrier-mediated gene and protein delivery methods in somatic cells and microspores. Nanocarrier mediated transfection in plant microspore opens new possibilities for precision genetic engineering of the three organelles of plant and algae, as well as animal mitochondria, specifically genome editing.

#### P-5

Innovative Approaches to Optimize the Growth of Protoplasts, Cells and Tissues. MICHAEL R. DAVEY and Paul Anthony. Plant and Crop Sciences Division, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UNITED KINGDOM. Email: mike.davey@nottingham.ac.uk

Optimization of the growth of cells in culture is essential to maximize shoot regeneration during micropropagation and to facilitate the generation of novel, genetically manipulated plants through transformation and somatic hybridization. Innovative approaches to the culture of isolated protoplasts and cells include the use of actively growing or irradiated nurse cells of the same or different species to promote growth, and electrostimulation involving exposure to prolonged weak electrical currents, or short-term exposure to high voltage electric fields. Electrostimulation has been shown to stimulate DNA synthesis, the latter inducing mitotic division. Several studies have demonstrated that non-ionic surfactants, such as Pluronic F-68, exert growth-enhancing effects through cell multiplication and differentiation when incorporated at low concentrations into culture media. These responses may be associated with increased

nutrient uptake. Gassing of the culture medium and the headspace above the medium is a simple procedure to increase oxygen availability, while perfluorochemicals (perfluorocarbons; PFCs) are high density, artificial gas-carrying liquids capable of releasing gradually oxygen to cells during culture. Although expensive, PFCs are immiscible with aqueous culture media, enabling these compounds to be recycled. In general, the stimulation of mitosis using the above approaches is a long-term effect, resulting not only in cell proliferation, but also improving shoot regeneration from cultured cells and tissues. Surfactants and PFCs have also been used extensively in the culture of animal and microbial cells, and the more recent exploitation of these compounds in plant cell culture provides an excellent example of technology transfer.

#### P-6

Recalcitrant Radish (*Raphanus sativus*): Bottlenecks and Successes in Culture. I. S. CURTIS. Chromatin Inc., 2109 S. Oak St., Suite 101, Champaign, IL 61820. Email: icurtis@chromatininc.com

For more than a century, scientists have been able to culture isolated plant cells in the laboratory. Despite the substantial progress made in cell-to-plant systems, there remain some commercially important dicotyledons and monocotyledons that are recalcitrant to regeneration, especially under selection conditions for isolating transgenic events. Radish, a dicotyledonous member of the Brassicaceae, cultivated in all continents, has become a challenge to plant cell culturists. Despite improvements in the regeneration of seedling explants in response to the supplementation of ethylene-inhibitors in culture media, the inability to produce shoots from transformed plant tissues greatly delayed the success of producing transgenic events. However, the application of *in planta* transformation techniques to radish, originally developed in the model plant *Arabidopsis*, allowed the development of novel and important germplasms such as late-flowering and also drought and salt tolerance. This presentation will outline how advances in plant cell culture and gene technology techniques enabled transgenic radish to be finally generated in the laboratory.

#### P-7

Phase Change: a Source of Recalcitrance for the Initiation of Yellow Yam (*Dioscorea cayenensis*) and Yampie (*D. trifida*), Two Non-woody Monocotyledonous Tropical Vines. S. A. MITCHELL. The Biotechnology Centre, University of the West Indies, Mona Campus, Kingston 7, Jamaica, WEST INDIES. Email: sylvia.mitchell@uwimona.edu.jm

Herbaceous plants are more amenable to tissue culture than are woody tree species, many of which tend to exhibit degrees of recalcitrance to *in vitro* conditions. A large cause of this recalcitrance is phase-change due mainly to the long juvenile vegetative period trees experience. Initiating adult vegetative shoots to rejuvenate them in culture remains very difficult. Most researchers resort to using somatic embryogenesis as the method of choice to circumvent this problem. Yams, specifically yellow yam (*Dioscorea cayenensis*) and yampie (*D. trifida*), are monocotyledonous non-woody vines, for which the juvenile vegetative period is very short, and seeds are non-existent or in short supply. Thus, it is often only the adult vegetative vine which is available for initiation. This adult vegetative vine of *D. cayenensis* and less often for *D. trifida* and rarely for *D. alata*, is also often recalcitrant in tissue culture, with the nodal explants being unresponsive under unfavorable conditions. Successful initiation has required an understanding of the phase-change in this plant so as to minimize its effect. These have included: minimizing the size of tuber piece from which the mother vine regrows, limiting the time of year for initiation to the early season of growth and long days, initiating nodal explants from vines that are young and actively growing, and initiating on media with relative high BAP levels (0.5–2.0 mg/L). In culture, it has been necessary, especially for the *D. cayenensis* yellow yam variety, to regularly reculture the shoot tips on media with a lower BAP concentration of 0.1 mg/L until the shoot rejuvenates and begins to grow in culture. A possible biochemical explanation of these results and their relevance to the initiation of nodal explants from trees will be given.

#### P-8

Grass Is Greener on the Other Side – Biotechnology for Turfgrass Genetic Improvement. HONG LUO. Department of Genetics and Biochemistry, Clemson University, 110 Biosystems Research Complex, Clemson, SC 29634–0318. Email: hluo@clemson.edu

Turfgrass provides numerous environmental and societal benefits and contributes significantly to agricultural economy and environmental protection. Trait improvement of turfgrass is important to the turf industry and the environment. With the advance of recombinant DNA and transgenic technologies, biotechnology approaches that have been successfully adopted for row crop improvement can also be applied to turf species for trait modification. Based on a plethora of biochemical and physiological mechanisms determining plant development and plant response to environmental cues, we have designed various molecular strategies to genetically engineer creeping bentgrass for improved turf

quality and enhanced performance under biotic and abiotic stresses. Genomics approaches have also been employed to identify key pathways and candidate genes for use in turfgrass improvement. When genetically engineering perennials for environmentally friendly plants, gene containment is critical for commercialization of transgenic products. We have developed various molecular strategies producing controlled male sterility or total sterility in transgenic creeping bentgrass with improved agronomic traits, providing strong evidence for effective gene containment in transgenic turfgrass. Our data demonstrate the great potential and effectiveness of biotechnology approaches in perennial genetic improvement. With the implementation of effective transgene containment strategies, it is foreseeable that genetic engineering of turf species would play increasingly important roles in turfgrass improvement, significantly impacting turf industry and agriculture production.

#### P-9

Development of Sustainable Turfgrasses. LISA LEE, Laticia Saunders, Becky Torisky, Irina Orlova, Matt Koch, Eric Nelson, and Bob Harriman. The Scotts Miracle-Gro Company, 14111 Scottslawn Road, Marysville, OH 43041. Email: lisa.lee@scotts.com

Turfgrasses play an important role in maintaining a healthy environment and enriching our lives. Not only does turf provide an aesthetically pleasing landscape feature or a functional surface for sporting events, turfgrass helps reduce soil erosion and agricultural runoff, and it absorbs carbon dioxide and ozone while releasing life-sustaining oxygen. While advances in breeding and cultural practices are continuously increasing the positive environmental impact of turf, biotechnology has the potential to dramatically enhance our ability to maintain a healthy turfgrass stand with even fewer inputs. The Scotts Company is demonstrating biotechnology can indeed reduce maintenance inputs. We will present progress on the development of non-plant pest Kentucky bluegrass and St. Augustinegrass events that are glyphosate tolerant and produce a thicker and greener turfgrass stand that requires less mowing.

#### P-10

Improved *Agrobacterium*-mediated Perennial Ryegrass Transformation Using Novel Approaches. R. QU<sup>1</sup>, W. Zhang<sup>1,2</sup>, M. Patel<sup>1</sup>, and R. Dewey<sup>1</sup>. <sup>1</sup>Department of Crop Science, North Carolina State University, Raleigh, NC 27695 and <sup>2</sup>Department of Grassland Science, China Agricultural University, Beijing 100193, CHINA. Email: rongda\_qu@ncsu.edu

Turfgrasses are generally considered recalcitrant grass species for *Agrobacterium*-mediated genetic transformation. In the past few years, we explored various approaches and developed two protocols that remarkably improved transformation efficiency of perennial ryegrass by *Agrobacterium*. The newly developed protocols can also be successfully used to rice transformation, indicating they may also apply to transformation of other turfgrasses. Both approaches use selected embryogenic callus of 3 to 4 month-old on N6-based media, and apply vacuum during infection. One protocol removed myo-inositol from the culture medium, applied a cold shock to the calluses before infection, and treated the calluses with L-glutamine before and during the infection. Another one used a brief heat shock (42°C, 3 min) during infection, and higher maltose (6%) at co-cultivation stage. In both cases, around 80% stable transformation efficiency was achieved as judged by GFP<sup>+</sup> cell clusters a month after infection. Regenerated plants looked normal and were able to set seeds after crossing.

#### P-11

Development and Commercialization of Genetically Engineered Alfalfa Products with Enhanced Performance. STEPHEN J. TEMPLE. Forage Genetics International, N5292 South Gills Coulee Road, West Salem, WI 54669. Email: stemple@foragegenetics.com

Genetic engineering (GE) offers opportunities to introduce novel traits into crops that were not previously available using conventional plant breeding tools. The majority of trait/crop combinations fully deregulated and available for commercial sale in the United States are traits associated with three commercial crops: corn, soybean and cotton. The commercialization of Roundup Ready alfalfa represents the first perennial forage crop commercialized with a GE trait. Data will be presented discussing some of the challenges and opportunities Forage Genetics encountered commercializing Roundup Ready alfalfa as a case study. With the successful deregulation and commercial launch of Roundup Ready alfalfa the opportunity now exists for development and commercialization of other traits in alfalfa. Lignification of secondary cell walls during plant development is a major factor limiting forage digestibility. Lignins are complex phenolic polymers which are associated with the polysaccharides of the cell wall in specific plant cells primarily in mature stems. Several recent studies have demonstrated that transgenic plants which are down regulated in one of several key lignin biosynthetic pathway genes have reduced lignin content and altered lignin subunit composition.

Based on findings of these studies Forage Genetics in collaboration with our technology partners have down regulated caffeoyl CoA 3-*O*-methyltransferase (CCOMT) in alfalfa. Agronomic and molecular event selection allowed us to identify a commercial candidate event. An update of the event selection process, the numerous proof of concept studies that have been carried out to demonstrate grower benefits and early testing of reduced lignin alfalfa varieties will be presented.

#### P-12

New Strategies for Strawberry. RICHARD E. VEILLEUX. Department of Horticulture, 544 Latham Hall, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. Email: potato@vt.edu

Adoption of *Fragaria vesca* as a model rosaceous fruit crop depends upon the development of robust genomic tools to facilitate forward and reverse genetics. The transformation efficiency of strawberry, although adequate for the purpose of generating transgenic plants with single gene alterations, falls short of what would be required to obtain a knockout population of plants with the possibility of saturating gene sequences. Transposon tagging represents another possibility to generate knockout or overexpression lines. We have used three different *AcDs* constructs to attempt to generate transposon tagged lines. The first, pAc-DsN-EG, was able to launch the *Ds* element in several transgenic plants; however, its *GFP* selectable marker was deficient and it had a propensity to somatically transpose prior to flowering, such that multiple seedlings bore the same *Ds* insertion site. The second, *AcDsATag-Bar\_gosGFP*, exhibited good expression of selectable marker genes, *Bar* and *GFP*; however, the transposition rates were low. The jury is still out on the third, which we developed using gametophyte specific promoters to drive expression of the maize transposase gene; the transgenic plants carrying this construct are still under development. A catalog of the transposon tagged lines is available at: <http://hortmutants.vbi.vt.edu/HortMutants/strawberry.html>. Among the plants regenerated after transformation with the knockout and all three transposon-tagging constructs, some of the most interesting phenotypic mutants were somaclonal variants where the phenotype segregated independently of the insertion. However, in other mutants, some gene expression changes could be attributed to the insertion. New technologies, including TALEN and CRISPR offer possibilities of specific gene knockouts without needing large populations.

**P-13**

Towards Cisgenics to Improve Citrus Health and Nutritional Quality Through Metabolic Engineering. L. PEÑA, E. Pons, A. Rodriguez, and B. Alquezar. Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA), Ctra. Moncada-Naquera km. 4,5, Moncada, Valencia, SPAIN. Email: lpenya@ivia.es

Citrus is the most important fruit tree crop in the world. The relative lack of success of traditional breeding programs in citrus is due at least in part to their long juvenile periods and to their complex reproductive biology. The juvenile period of sweet orange lasts for 6 to 10 years until trees begin to flower, and several years more to achieve fully mature characteristics. Due to their high heterozygosity, oranges and grapefruits cannot be improved through breeding. Genetic transformation of mature material allows the introduction of specific traits into known genotypes without altering their elite genetic background, and thus overcoming incompatibility, sterility, heterozygosity and inbreeding depression barriers associated to hybridization. Moreover, it permits to overcome the juvenility constraint of citrus breeding. Additionally, using citrus-derived genes and regulatory sequences may open new biotechnological possibilities related to increasing the level of accumulation of health-promoting compounds in orange fruits through metabolic engineering. Once we have been able to characterize a strong and citrus fruit-specific promoter, we are increasing the content of health promoting phytonutrients such as vitamins and well-known antioxidants in orange fruits and evaluating their biological activities in an animal model. Although GM foods are not being currently accepted by many potential consumers, we believe that public perception may drastically change if health-promoting effects of GM oranges could be demonstrated.

**P-14**

New Breeding Technologies Through Genetic Engineering in Apple (*Malus × domestica*). M.-V. HANKE<sup>1</sup>, H. Flachowsky<sup>1</sup>, A. Patocchi<sup>2</sup>, and C. Gessler<sup>3</sup>. <sup>1</sup>Pillnitzer Platz 3a, 01326 Dresden, Institute for Breeding Research on Horticultural and Fruit Crops, Julius Kühn-Institute, GERMANY; <sup>2</sup>Schloss 1, 8820 Wädenswil, Department of Plant Protection and Fruit and Vegetable Extension, Research Station Agroscope Changins-Wädenswil, SWITZERLAND; and <sup>3</sup>Universitätstrasse 2, 8092 Zürich, Plant Pathology, Institute of Integrative Biology, ETH Zürich, SWITZERLAND. Email: viola.hanke@jki.bund.de

The cultivated apple is one of the economically most important fruit crops worldwide. Breeding of a new apple cultivar is a protracted and expensive process. The most time-consuming factor is the long juvenile non-flowering phase of seedlings obtained from crosses. Shortened juvenility and precocious flowering are, therefore, important breeding goals. Regardless of the ontogenetic differences between annual and perennial plants, basic floral regulatory mechanisms in plants seem to be conserved. Studies on heterolog expression of known flowering genes of *Arabidopsis* as well as overexpression and silencing of their respective homologs in apple will be presented which resulted in a breakdown of the juvenile phase in apple. Based on this, an innovative breeding approach combining the advantages of transgenic early flowering trees and molecular markers as an efficient tool to speed up the selection process was developed (fast track breeding). Apple has been also one of the prime targets for genetic manipulation in fruit tree species to improve resistance to diseases and other agronomically important traits. The introduction of the natural occurring resistance genes from *Malus* species by classical breeding is long-lasting, because of self-incompatibility and heterozygosity. Cisgenic approaches are a realistic alternative to classical breeding in respect of targeted trait improvement. The introduction of *Malus* own genes into the genome of existing cultivars via *Agrobacterium*-mediated plant transformation based on a site-specific excision of all unwanted DNA sequences after selection will be presented.

**P-15**

A New Strategy to Improve Tomato—development and Characterization of New Tomato Germplasm with Enhanced Phytonutrients, Using Native Plant DNA. YINGHUI DAN<sup>1,2,3</sup>, Faith Campbell<sup>1</sup>, Gad Yousef<sup>4</sup>, Xiaozeng Yang<sup>5</sup>, Mary Ann Lila<sup>4</sup>, and Lei Li<sup>5</sup>. <sup>1</sup>Institute for Advanced Learning and Research, Danville, VA 24540; <sup>2</sup>Department of Horticulture and <sup>3</sup>Department of Forest Resources and Environmental Conservation, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061; <sup>4</sup>Plants for Human Health Institute, North Carolina State University, Kannapolis, NC 28081; and <sup>5</sup>Department of Biology, University of Virginia, Charlottesville, VA 22903. Email: Yinghui.dan@ialr.org

Tomato (*Solanum lycopersicum*) is the second most consumed vegetable crop in the world, and most economically important, with a total farm value of \$2.4 B in the US. A key tomato breeding target is improving nutrient composition. Lycopene (constituting 80-90% of tomato carotenoid content) and  $\beta$ -carotene are believed to reduce the risk of human

cancers and to prevent cardiovascular and eye diseases. Conventional breeding methods are inefficient mainly due to tomato's narrow genetic diversity, and given public concerns regarding transgenic crops, researchers face tremendous challenges in improving tomato. With a new strategy of targeted mutant approaches combining genetic, nutrient, genomics and transcriptome technologies we have developed germline mutants with significantly ( $P < 0.05$ ) enhanced and deficient carotenoid levels, including lycopene (up to 46% increase) and  $\beta$ -carotene (up to 45% increase), in a single genetic background of tomato cv. MicroTom. Our genetic segregation analysis showed that the lycopene reduction caused fruit color change in the mutants and the lycopene reduction was controlled by a single, homozygous gene. Lycopene and  $\beta$ -carotene increases in other two mutants were also associated with fruit color change, controlled by two genes in one mutant. Though our global transcriptome analysis of the carotenoid deficient mutant (CDM) using RNA-Seq we identified a total of 19,682 unigenes. Among these, 2,408 new unigenes were discovered, and 1,895 with significantly differential expression, which were annotated with 55 gene ontology functional categories. No expression of all key genes involved in lycopene and  $\beta$ -carotene biosynthesis was detected in leaf tissues of CDM and control lines, indicating that these genes were developmentally regulated. However, nine genes involved in the first step of carotenoid biosynthesis were significantly down-expressed (up to 5.4-fold) in CDM compared to the control line. Detailed information of genetic, nutrient, genomics and transcriptome analysis of the mutants will be presented.

#### P-16

Haploids in Plant Breeding and Genomic Research. TATIANA BOLUARTE-MEDINA and Richard E. Veilleux. Horticulture Department, Virginia Tech, Blacksburg, VA 24061. Email: [tboluart@vt.edu](mailto:tboluart@vt.edu), [potato@vt.edu](mailto:potato@vt.edu)

Haploid cells are produced from reduced microspores or female gametophytic cells within the embryo sac. A doubled haploid (DH) is formed by chromosome doubling of a haploid plant and may be homozygous if the source species is diploid or heterozygous if the source species is polyploid. A monoploid plant has the basic rather than the haploid chromosome number, so that doubled monoploids (DM) are necessarily completely homozygous. In plants where self-pollination is possible, conventional breeding programs in diploid species require at least six generations to achieve approximate complete homozygosity, whereas DHs can be produced in a single generation. In self-incompatible species, doubled haploidy may be the only possible route to homozygosity. Twin seedlings, anther culture, microspore

culture, ovule culture, wide hybridization by chromosome elimination, centromere-mediated genome elimination, pollination with irradiated pollen or in-vivo haploid induction technology, all followed by chromosome doubling may result in DH production. DH technology, mostly using anther or microspore culture, has resulted in new cultivar releases for barley, wheat, rice, asparagus, canola, tobacco and apple, among others. DHs have been used to develop mapping populations in barley and potato and to analyze linkage disequilibrium and haplotype/trait associations. In the current genomic era, haploids have been critical to facilitate genome sequencing efforts in potato and apple where heterozygosity hampered efforts to assemble scaffolds from short nucleotide reads. Potato mapping populations developed from crosses with the sequenced DM have also been used to verify the assembly of the published genome sequence.

#### P-17

Isolated Microspore Culture in Cereals by Mediating Stresses and Nursing. RAKESH KUMAR SINHA and François Eudes. Agriculture and Agri-Food Canada, Lethbridge, T1J 4B1, AB, CANADA. Email: [rakesh.sinha@agr.gc.ca](mailto:rakesh.sinha@agr.gc.ca), [Francois.Eudes@agr.gc.ca](mailto:Francois.Eudes@agr.gc.ca)

Isolated microspore culture is a double haploid production platform instrumental in breeding programs. Development of microspore into embryo and green plant is dependent on a series of factors. An estimated 50% of the isolated microspore undergoes programme cell death within 24 hours of culture, and few microspores succeed to form scutellar stage embryo in wheat and triticale. Studies were conducted to reduce the frequency of microspore cell death during the early stage of culture, to nurse their embryogenic development and enhance the production of green plants while minimising albinism. Various groups of antioxidants, including reactive oxygen species scavenger dimethyl tyrosine group, and Phytosulfokine-alpha (PSK- $\alpha$ ) were evaluated in triticale and spring wheat genotypes. We report the number of embryo like structure and green plants were enhanced when induction medium was supplemented with proline (10 mM) or Glutathione (2 mM). The use of dimethyl tyrosine labelled organelle targeting peptides, allowing mitochondrial and chloroplast targeted delivery, greatly enhanced frequency of microspore going through embryo like formation and plant production. Complementary to these treatments, we report a dose effect of the nursing PSK- $\alpha$  on the number of embryos and the rate of green to albino plant formations, which resulted in an efficient doubled haploid production platform in wheat and triticale.

**P-18**

Improving Camelina Doubled Haploidy and Microspore Transformation Using Nanocarriers. ALISON M. R. FERRIE, Pankaj K. Bhowmik, Joan Dirpaul, Jennifer Brost, Maureen Troesch, and Patricia L. Polowick. National Research Council - Saskatoon, 110 Gymnasium Place, Saskatoon, SK, S7N 0W9, CANADA. Email: Alison.Ferrie@nrc-cmc.gc.ca

The importance of an efficient doubled haploidy methodology for crop species is well-recognized, as the ability to produce true-breeding lines in one generation saves valuable time in the development of new cultivars. The conditions leading to the induction and development of microspore-derived embryos vary depending on the species, and therefore doubled haploidy methods have to be determined for each species. A number of factors influence microspore embryogenesis including genotype, stage of microspore development, donor plant growing conditions, media composition, and culture conditions. Camelina [*Camelina sativa* (L.) Crantz] is a member of the Brassicaceae family having a unique oil profile that has potential both for biofuels and as a food crop. A doubled haploidy protocol for Camelina would enhance the breeding and improvement of the crop. As well, the ability to elicit genetic transformation at the microspore phase is most desirable. Previous microspore transformation methods have been inefficient, as well as confined by freedom-to-operate issues. We have developed a novel system of delivering DNA to microspores which involves macroinjection of DNA complexed with a nanocarrier into immature donor floral buds resulting in the regeneration of transgenic doubled haploid (DH) plants. For *B. napus*, we have molecular confirmation of gene integration in both the primary regenerants and their offspring. Positive results have also been observed in Camelina. The unique combination of efficient microspore transformation and successful regeneration of transgenic DH plants has the potential to have a significant impact on many basic and applied research areas, including gene editing for the development of new traits. This presentation will discuss improvement in Camelina doubled haploidy and microspore transformation using nanocarriers.

**P-19**

Use of Metabolomics to Characterize a Dwarf Mutant Line of Rice. P. P. KUMAR<sup>1,2</sup> R. Ramamoorthy<sup>1</sup>, A. Rai<sup>1</sup>, and S. Ramachandran<sup>2</sup>. <sup>1</sup>Department of Biological Sciences, Faculty of Science, National University of Singapore, Singapore 117543, REPUBLIC OF SINGAPORE and <sup>2</sup>Temasek Life Sciences Laboratory, 1 Research Link, National University of Singapore, SINGAPORE 117604. Email: dbskumar@nus.edu.sg

We identified a dwarf mutant rice *Oscyp96B4* from a pool of *Ds* insertion lines in a phenotype screen. In addition to dwarfness, homozygous mutant plants exhibited reduction in panicle size, seeding rates and pollen germination when compared with wild type (WT). Southern blot analysis revealed that *Oscyp96B4* mutant had a single copy of *Ds* insertion. BLAST searches of *Ds* flanking sequences revealed that the *Ds* had transposed into *OSCYP96B4* gene which encodes a cytochrome P450 protein. Cytochrome P450s are monooxygenases, a family of membrane-bound heme-containing proteins and play various roles such as biosynthesis of plant hormones, secondary metabolites and breakdown of endogenous and exogenous toxic compounds. Expression studies using quantitative Real-Time -PCR and Northern blot analysis showed that *OsCYP96B4* expressed in all the WT tissues tested with high level of expression in the reproductive organs and minimal level in the roots. The *OSCYP96B4* gene was able to partially complement the dwarf phenotype. Functions of *CYP96* gene family in rice are unknown. Hence, we have employed metabolomics approach to identify probable function of *OSCYP96B4* gene. The total metabolite profiling and comparison was done in WT, mutant, dsRNAi, complementation line, and ectopic expression line of *OSCYP96B4* gene. The findings from metabolomics analysis will be discussed.

**P-20**

A Field Validated and Versatile QTL for Drought Tolerance in Rice. AJAY KOHLI. International Rice Research Institute, Plant Breeding, Genetics and Biotechnology, DAPO 7777, Metro Makati, PHILIPPINES. Email: a.kohli@irri.org

Over the years numerous genes have been implicated in drought tolerance in plants. The clear message reiterated through this cumulative knowledge is that genes belonging to various functional categories affect drought tolerance and that it is a complex trait. In cereals, despite occasionally well-documented positive effects of a gene on drought tolerance, no single gene was sufficient to engineer stable yields under drought. It is also becoming increasingly clear that there are different pathways and mechanisms for drought tolerance. Thus, the route to adopt may be to, conventionally or biotechnologically, bring together multiple genes of a pathway/mechanism. Exactly this seems to have happened recently by a paradigm shift in selection for drought tolerance in rice by screening for yield under drought. Such selection procedure led to the identification of a large effect QTL for rice yield under severe drought. The QTL has been shown to be valid in the field conditions in different countries and eco-geographies. This presentation

provides evidence that the success and versatility of this QTL is due to multiple effective genes within the said locus. The molecular determinants of this QTL go beyond the novelty of intra-QTL multi-gene effect and implicate an important role for protein post-translational modification. There are indications from another similar large effect QTL that its mechanistic basis for tolerance may be different. Pyramiding of such QTLs to capture their synergistic or additive value has recently proven successful. Thus a comprehensive team effort of breeders, physiologists and molecular biologists is the call of the day to obtain drought tolerant crop plants. This will lead to the desirable drought tolerant product much quicker – the need of the hour under the climatic and demographic pressures. Also, it will lead to a "systems level understanding" of the process, which goes beyond integrating the "omics" and "matics" for an individual and sets it within its environment as well.

### P-21

Biotechnological Approaches to Boost Rice Innate Immunity for Broad-spectrum and Durable Disease Resistance. Y. YANG. Department of Plant Pathology and Environmental Microbiology and Huck Institutes of Life Sciences, Pennsylvania State University, University Park, PA 16802. Email: yuy3@psu.edu

Recent advances in molecular biology and functional genomics of the host-pathogen interaction have led to important novel insights into the underlying mechanisms involved in rice innate immunity against microbial infection. Novel molecular tools such as RNA interference and TALE-based genome editing have greatly enhanced our ability to manipulate rice plants for improving major agronomic traits such as disease resistance. As a result, various biotechnological approaches have been developed to enhance broad-spectrum and durable disease resistance based on rice's own defense mechanisms. Such molecular manipulation and genetic modification may be carried out with rice genes and components involved in pathogen recognition (e.g., pattern recognition receptors and resistance proteins), disease susceptibility (e.g., host proteins targeted by microbial effectors), signal transduction (e.g., reactive oxygen species, hormones, protein kinases and transcription factors), or defense responses (e.g., pathogenesis-related proteins and phytoalexins). Since plant defense response often antagonizes growth and development due to crosstalk among various signaling pathways, increasing attention is being paid to fine tune molecular strategies in order to improve rice disease resistance without negatively impacting crop yield and abiotic stress tolerance. As an alternative to chemical control, biotechnological approach to boost host immunity represents an

important and promising strategy for managing major rice diseases such as blast (*Magnaporthe oryzae*), sheath blight (*Rhizoctonia solani*) and bacterial blight (*Xanthomonas oryzae* pv. *oryzae*).

### P-22

Pesticidal Gene Discovery and Development of Traits from Bacterial Genomes. KURT BOUDONCK. Bayer CropScience, 2 TW Alexander Drive, RTP, NC. Email: kurt.boudonck@bayer.com

Weeds, insects and nematodes cost farmers billions of dollars in crop loss every year. Small molecules, biologicals, breeding and agronomic practices are all employed to help combat crop loss from pest damage. However, new pests and development of resistance have prompted an increased interest in novel pest control solutions. Pesticidal proteins from the bacterium *Bacillus thuringiensis* (*Bt*) have primarily been used in the past decade to combat various pests in crops. At Bayer CropScience we have built a unique microbial strain collection and a proprietary sequence-based trait discovery platform to identify new traits against pests for farmers. We have sequenced over 1,000 bacterial genomes and identified over 450 novel genes in insecticidal and nematocidal families. Each discovered pesticidal protein is tested against a full panel of agriculturally relevant pests, using bioassays. Our pest panel includes species from the Lepidopteran, Coleopteran, Hemipteran, and Nematode families relevant for soy, cotton, corn, rice, canola, and sugarcane. Proteins that show initial efficacy in bioassays are further tested in transgenic crop plants. Multiple insecticidal and nematocidal genes are currently in commercial development in multiple crops.

### P-23

The *Pseudomonas Syringae* Type III Effector HopU1, its RNA-binding Protein Target, and Its Effect on Plant Immunity. JAMES R. ALFANO. Center for Plant Science Innovation and the Department of Plant Pathology, University of Nebraska, Lincoln, NE 68588–0660. Email: jalfano2@unl.edu

The bacterial pathogen *Pseudomonas syringae* uses a type III secretion system to inject type III effectors into plant cells and suppress plant immunity. The *P. syringae* pv. *tomato* DC3000 type III effector HopU1 was determined to be a mono-ADP-ribosyltransferase that can use several RNA-binding proteins as substrates. One of these proteins, GRP7 was shown to be involved in innate immunity and Arabidopsis mutants lacking GRP7 were more susceptible



to *P. syringae*. HopU1 ADP-ribosylates an arginine residue in position 49 of GRP7, which is within its RNA-recognition motif. We found that ADP-ribosylated GRP7 was reduced in its ability to bind RNA. Complementation of the *grp7* mutant with wild type GRP7 but not the GRP7<sub>R49K</sub> mutant restored normal immune response. This indicates the amino acid that is the site of ADP-ribosylation is critical for GRP7's function. GRP7 associates with translational components and GRP7 binds to innate immunity-related RNA including those encoding pattern recognition immune receptors. HopU1 blocks the interactions between GRP7 and immunity-related RNA both in vitro and in vivo by ADP-ribosylation of GRP7. Recently, we found that plants over-expressing GRP7 were more resistant to *P. syringae* and other pathogens demonstrating that GRP7 plays a broadly important role in innate immunity. We are currently investigating the extent that these phenotypes can be reproduced in crop plants such as soybean. Two GRP7 homologs from soybean were chosen for further study. Transgenic soybeans over-expressing these proteins are currently being made and our progress in determining their effects on soybean immunity will be discussed.

#### P-24

Expression of *Arabidopsis thaliana* HB17 Gene in Corn Leads to Improved Sink Potential. ELENA RICE, Abha Khandelwal, Cara Griffith, S. Manju, Lesley Murphy, and Paul Loida. Monsanto Company, 700 Chesterfield Parkway North, Chesterfield, MO. Email: elena.a.rice@monsanto.com

As a result of the large scale screening of candidate genes in transgenic corn, we identified an *Arabidopsis thaliana* gene HB17, a member of homeodomain-leucine zipper II (HD-Zip II) family of the plant transcriptional factors, which affects plant growth and leads to increase in ear size at silking. When expressed in corn, AtHB17 lacks the repression domain due to the corn-specific splicing mechanism and loses the ability to bind the co-repressors and affect transcription of the target genes. The protein still can form homo-dimers as well as hetero-dimers with corn endogenous HD-Zip II proteins and bind to the target DNA sequences due to the presence of the functional leucine-zipper and DNA-binding domains. The mode of action of AtHB17 in corn leading to enhanced sink potential will be discussed.

#### P-25

Regulating GM Crops: What's Risk Got to Do with It? ADRIANNE MASSEY. Biotechnology Industry Organization, 1201 Maryland Ave. SW, Washington, DC 20024. Email: amassey@bio.org

The history of U.S. regulatory oversight of field trials and commercial development of genetically engineered organisms (GEOs) dates back to the mid-80s when a comprehensive policy statement, the "Coordinated Framework," (CF) described how U.S. agencies would use existing government laws, regulations and policies to regulate GEOs. Three U.S. regulatory agencies claim authority over different attributes of GE plants: USDA, FDA and EPA. To understand *what* an agency can regulate, one needs to look at the language of the law that provides it with regulatory authority. To see *how* the agency will exercise its authority, one focuses on the regulations. This presentation will focus primarily on the what, how and why of the regulatory approach of two agencies: USDA and EPA. It will also touch briefly on the law that has been the gateway for litigation against USDA's decisions to deregulate GE plants: the National Environmental Policy Act.

#### P-26

Adoption of Genetically Modified Crops in Developing Nations: Regulatory Considerations. JEFF STEIN. Biosafety Advisor, Donald Danforth Plant Science Center, St. Louis, MO. Email: jstein@danforthcenter.org

Chronic malnutrition affects more than 800 million people, the majority of whom live in developing nations. While improvements have been achieved in some geographies, malnourishment across sub-Saharan Africa continues to rise at a disturbing rate. Contributing factors include overall population growth coupled with rural-urban migration, and reduced agricultural productivity due to drought, increased soil salinity, inadequate plant disease and pest control, and insufficient fertilizer. Agricultural growth is needed for sustained hunger reduction. Germplasm improvement programs utilizing selection and breeding continue to achieve advancements in crop yields for many of the staple crops grown in this region. However, some traits, such as drought tolerance in maize and virus resistance in cassava, among others, have remained recalcitrant. In recent years, research and development efforts utilizing the tools of modern biotechnology have made substantial progress in introducing traits into these crops, complementing improvements introduced in breeding programs. Prior to deployment by farmers, crops improved via biotechnology undergo environmental and food/feed safety risk assessments. The regulatory schemes being adopted in sub-Saharan countries are fundamentally similar to those applied in developed nations, such as Australia, Brazil, Canada, and the United States. In the near future, African regulators will be challenged, for the first time, with conducting risk assessments for products specifically developed for their region, without the benefit

of reference to regulatory review and approvals previously granted by another country. We will examine progress towards meeting this challenge and illustrate with examples how general principles of risk assessment are being applied to meet the complexities posed by the agricultural systems of sub-Saharan Africa.

#### P-27

Plant Genome Editing Technologies and the Global Regulatory Bottleneck. GARY RUDGERS. Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN 46268. Email: GWRudgers@dow.com

As genetic technologies evolve, precise methods have been developed to improve plant genomes. Technologies such as zinc-finger nucleases, meganucleases and oligonucleotide directed mutagenesis are allowing scientists, for the first time, to make precise genomic mutations in plants. Although these technologies offer essential agronomic solutions to a growing world population, regulatory clarity on products developed using these modern technologies remains unclear and uncertain due to differences in global regulatory policies. As a result, scientist, researchers, farmers, and consumers have been unable to fully benefit from these precision mutational technologies. This presentation will explore the regulatory bottlenecks facing mutational technologies and the opportunities that can be gained through a global, harmonized, scientific regulatory approach.

#### P-28

Regulation of the Products of Biotechnology in the United States. SALLY L. MCCAMMON. US Department of Agriculture, Biotechnology Regulatory Services, Animal and Health Inspection Services, 4700 River Road, Riverdale, MD 20737–1231. Email: Sally.L.McCammon@aphis.usda.gov

In 2011, in recognition of the rapid pace of technological implementation, the White House issued an Executive Order (13563) and a Memorandum focusing on balanced regulation of emerging technologies including nanotechnology, synthetic biology and genetic engineering. Since the 1986 Coordinated Framework for the Regulation of Biotechnology was crafted, three major United States regulatory agencies, the Environmental Protection Agency, the Food and Drug Administration and the USDA's Animal and Plant Health Inspection Service, have traditionally focused on product development of organisms using recombinant DNA technologies. However, the combined broad mandate of the agencies in product evaluation is to assure protection

of the environment, plant and animal health, and food safety generally. Each of these agencies works to guide developers in understanding those products that fall under agency purview to achieve that mandate. As the products developed using organisms modified by a variety of techniques and combinations of techniques evolve and as the regulatory system, experience, and approach to risk assessment evolves with these products, developers are encouraged to consult with regulators to determine the appropriate course for the product under development.

#### P-29

Plant Genotype: Who's Ya Daddy and Does It Really Matter When Developing Micropropagation Protocols? M. E. KANE. Environmental Horticulture Department, University of Florida, PO Box 110675, Gainesville, FL 32611–0675. Email: micropro@ufl.edu

The standard approach to optimizing a shoot culture micropropagation protocol for a given species typically involves screening the effects of plant growth regulator type, concentration and combinations for optimal culture establishment (Stage I), shoot proliferation (Stage II), rooting (Stage III) and ex vitro acclimatization (Stage IV). The influence of donor plant genotype is typically overlooked but could be a contributing factor in the difficulties frequently encountered by laboratories attempting to apply published protocols. Applications of native plant in vitro propagation for the purposes of ecological restoration or conservation require reliable production of multiple genotypes for ecological stability. Consequently, these types of studies can provide insight into the influence of genotype on in vitro and ex vitro growth performance and guidance for micropropagation protocol development for a wide range of crops. Strategies for development of reliable in vitro propagation protocols for multiple genotypes of the wetland species *Pontederia cordata* and *Sagittaria latifolia*, the wildflower *Coreopsis floridana* and stabilization of seedling explants of the coastal dune plant *Uniola paniculata* will be described.

#### P-30

Donor Plant Influence on Clonal Micropropagation of *Veratrum californicum*-a Native Medicinal Species. JEFFREY ADELBERG<sup>1</sup>, Ju Yeon Song<sup>1</sup>, Jacqueline Naylor-Adelberg<sup>1</sup>, Sarah A. White<sup>1</sup>, and Dave Mann<sup>2</sup>. <sup>1</sup>School of Agricultural, Forest, and Environmental Sciences, Clemson University, Clemson, SC 29634 and <sup>2</sup>Infinity Pharmaceuticals, Inc., Cambridge, MA 02139. Email: jadlberg@clemson.edu

A micropropagation system was established for *Veratrum californicum*, a slow-growing species. Stage I plantlets were initiated on Murashige and Skoog (MS) medium with 23  $\mu\text{M}$  benzyladenine (BA), 0.7% (w/v) agar and 3% sucrose (w/v). Distinct clones from each individual donor plant were maintained and divided for 1–2 years. When 25–40 shoots were available, a clone was assigned to experimental conditions and the varying parameters assessed for their effects on multiplication and survival. To study the effect of temperature, 8 clones were divided in chambers at 10, 16, and 24°C for 5 successive subculture periods with 3  $\mu\text{M}$  BA, 20  $\mu\text{M}$  cool white fluorescent light and a 16 h photoperiod. To observe the effect of light, 5 clones were placed at 16°C under 20  $\mu\text{M}$  light emitting diode (LED) monochromatic blue, red, red+blue, and 40  $\mu\text{M}$  red+blue light for 3 subculture cycles. Lastly, to study the effect of certain plant growth regulators (PGR's), 3 clones were placed in 16°C, 40  $\mu\text{M}$  red+blue LED light, and cultured on the MS medium with 3, 6, or 9  $\mu\text{M}$  BA in the presence or absence of 0.5  $\mu\text{M}$   $\alpha$ -naphthaleneacetic acid (NAA) for 4 subculture cycles. Individual clones responded differently to temperature, however 16°C was overall the best with 4 of 8 clones increasing in numbers at 16°C and 2 of 8 clones likely to remain in stable Stage II multiplication at 16°C. None of the clones increased at 24°C during the 5 subculture cycles. Light influenced greenhouse survival but not multiplication ratio. Only 1 of 3 clones exhibited increased multiplication with the PGR combination 9  $\mu\text{M}$  BA and 0.5  $\mu\text{M}$  NAA, and we hypothesize that other untested factors control multiplication of this species. All the plantlets were transferred into the greenhouse for acclimatization and results will be presented.

### P-31

Genetic Influences in Development of an Ecologically Sound Sea Oats Micropropagation Protocol. J. JASINSKI and M. E. Kane. Environmental Horticulture Department, University of Florida, PO Box 110675, Gainesville, FL 32611–0675. Email: beetlebo@ufl.edu

*Uniola paniculata* L. (Poaceae; sea oats), an ecologically important dune species in the southeastern United States, plays an integral role in coastal dune stabilization and dune building. Sea oats are nursery propagated using field collected seed. *U. paniculata* is not a prolific seed producer and seed donor sites in Florida have been diminished due to recent hurricane activity. Micropropagation has the potential to supplement seed propagation of a wide range of sea oats genotypes. A micropropagation protocol was originally optimized using a single sea oats genotype utilizing the cytokinin N6-benzyladenine (2.2  $\mu\text{M}$ ) for Stage II multiplication. Further

studies indicated that in vitro multiplication, rooting, and ex vitro acclimatization varied widely among genotypes when this protocol was used. To assess genotypic responses, 43 genotypes from 13 Florida populations were micropropagated using this “optimized” protocol. Stage II, Stage III, and Stage IV growth responses differed significantly within and between sea oats populations and individual genotypes. Of the 43 genotypes screened, twenty-seven (62.7%) were shown to be moderately-difficult or difficult-to-acclimatize resulting in survival rates of 69.7% or less. Similarities in responses between genotypes within the same population were also observed. Significantly different genotypic responses observed in Stages II-IV reveal the importance of taking into consideration the influence of genotype when designing and optimizing micropropagation protocols.

### P-32

Standardized Approach to Considering Genotypic Influences During Micropropagation Protocol Development – Group Discussion. M. E. KANE. Environmental Horticulture Department, University of Florida, PO Box 110675, Gainesville, FL 32611–0675. Email: micropro@ufl.edu

During this session the influence of genotype on in vitro and ex vitro growth performance of diverse crops will be presented using a number of plant species as examples. An interactive session will be offered during which participants will have the opportunity to share their research experiences and strategies in developing common criteria for reliable micropropagation protocol development which recognizes the influence of genotype.

### P-33

High Efficiency Wheat Transformation Mediated by *Agrobacterium tumefaciens*, YUJI ISHIDA, Yukoh Hiei, and Toshihiko Komari. Plant Innovation Center, Japan Tobacco Inc., 700 Higashibara, Iwata, Shizuoka 438–0802 JAPAN. Email: yuji.a.ishida@jt.com

Creation of the first transgenic wheat mediated by *A. tumefaciens* was published 16 years ago (Cheng et al. 1997, Plant Physiol. 115:971–980), but not much progress has been made in transformation methods since then. The frequency of transformation (independent transgenics / explant), mostly less than 5%, has been much lower than that in other major cereals such as rice and maize and varied from experiment to experiment. A transformation protocol developed by a laboratory often did not work at another. We have now identified and optimized the key factors so that

the frequencies between 50% and 60% were routinely observed and higher than 90% were recorded in the best cases. Both bar and hpt genes were useful for effective selection of transgenic plants. Not surprisingly, the key factors did not differ much from those in other plants, and the use of vigorous immature embryos from healthy wheat grown in well-conditioned greenhouses (25°C at day, 10°C at night) with supplemental light was the most critical. We have been able to obtain transgenic plants from the embryos harvested from the greenhouses throughout the year. Fielder, a spring wheat cultivar, constantly showed high efficiency of transformation by our protocol. Various parameters related to media, handling of embryos, bacterial strains and vectors have been carefully adjusted. For example, centrifugation of embryos before infection resulted in several fold increase in the frequency, and exclusion of auxin from co-cultivation medium also elevated the efficiency of transformation. Most of the transformed plants were normal in morphology and fully fertile. We also confirmed that more than 40% of transformants had a single copy of the transgenes and the transgenes were inherited in a Mendelian fashion. Transgenic wheat has been generated at high frequency by several research groups by our protocol by now. Therefore, wheat has finally joined the list of cereals that can be efficiently transformed.

Cheng et al. (1997) *Plant Physiol.* 115:971–980.

#### P-34

Development of Efficient Transformation Systems in a Commercial Wheat Variety. J. XU, P. Attri, F-M. Lu, K. Thai, J. Berg, J. Layton and C. Armstrong. Monsanto Company, 700 Chesterfield Pkwy West, Chesterfield, MO 63017. Email: jianping.xu@monsanto.com

Wheat is an important staple food worldwide. In recent years, wheat breeding technology development, in particular utilization of biotechnology tools, has fallen behind that of other crops. Genetic transformation systems have been established, however most are based on the model genotype Bob White, using immature embryos and either *Agrobacterium* or particle bombardment. We have developed reproducible and robust *Agrobacterium*-based transformation systems using immature embryos of a commercial hard red spring wheat variety. Large numbers of stable transgenic plants have been generated using the selectable markers *cp4*, *pat* or *nptII*, linked to various genes of interest. Transformation frequencies are dependent on the selection system and selectable marker cassette. Molecular quality of the plants is also dependent on the construct, suggesting that the choice of an appropriate selectable marker cassette is crucial to the development of an efficient wheat transformation pipeline.

#### P-35

The Socioeconomic and Biodiversity Impacts of Currently Commercialized GM Crops. JANET E. CARPENTER. J E Carpenter Consulting LLC, PO Box 968, Boylston, MA 01505. Email: janet.e.carpenter@gmail.com

The benefits and risks of genetically modified (GM) crops continue to be disputed, despite rapid and widespread adoption since their commercial introduction in the mid-1990's. In 2011, an estimated 16.7 million farmers in 29 countries grew GM crops, over 90% of them small farmers in developing countries. Three recently conducted literature reviews shed light on our current understanding of the socioeconomic and biodiversity impacts of currently commercialized GM crops. The first review analyzed the results of peer-reviewed publications presenting farmer surveys from 12 countries that compared yields and other indicators of economic performance for adopters and non-adopters of the technology. The results indicated that with few exceptions, GM crops have benefitted farmers, particularly those in developing countries. The second review was focused on the biodiversity impacts of GM crops, considering the potential impacts of GM crops at three levels: the crop, farm and landscape scales. The review considers potential impacts on crop diversity, non-target soil organisms, weeds, land use, non-target above-ground organisms and area-wide pest suppression. The third review covers literature on the broadly defined socio-economic impacts of GM crops, looking beyond changes in yields and costs to the distribution of impacts across groups, as well as secondary impacts on labor markets, non-pecuniary factors and social welfare. The primary findings include: farmers receive a substantial share of overall benefits, consumers also benefit from lower prices, small farmers in developing countries benefit from GM crops and adopters report improvements in health, education, debt repayment, maternal care services and food security.

#### P-36

Hydroefficiency – provided by the First Commercially Launched Transgenic Yield and Stress Trait in *DroughtGard* Hybrids. M. STEPHENS<sup>1</sup>, K. Nemali<sup>1</sup>, M. Edge<sup>1</sup>, M. Lawson<sup>1</sup>, and C. Bonin<sup>2</sup>. <sup>1</sup>Monsanto Company, 700 Chesterfield Parkway West, Chesterfield, MO 63017 and <sup>2</sup>Agrivida, Inc., 1392 Storrs Road, Storrs, CT 06268 Email: mike.stephens@monsanto.com

*DroughtGard* corn hybrids are a combination of elite germplasm and traits that includes the MON87460 trait designed to enhance tolerance to drought stress. The MON87460 trait is the first transgenic abiotic stress-tolerance trait to be commercially launched. The opportunities and challenges

in developing the transgenic MON87460 corn trait will be discussed with special focus on the investigation of the Hydroefficiency trait that it provides.

### P-37

Enogen: What if Corn Already New Its Destiny? The Story of Alpha-amylase Corn. TERRY STONE. Syngenta, 11055 Wayzata Boulevard, Minnetonka, MN. Email: terry-1.stone@syngenta.com

Enogen technology is a revolutionary solution from Syngenta, bio-engineered specifically to enhance the productivity and efficiency of dry grind ethanol production. Enogen grain contains alpha amylase enzyme directly in the endosperm of the grain, eliminating the need to use liquid alpha amylase enzyme in the production process and driving dramatic improvements in plant throughput, efficiency and profitability.

### P-38

Developing Consumer Biotech Products. BOB HARRIMAN, Becky Torisky, and Lisa Lee. The Scotts Miracle-Gro Company, 14111 Scottslawn Road, Marysville, OH 43041. Email: bob.harriman@scotts.com

Turfgrasses play an important role in maintaining a healthy environment and enriching our lives. Turfgrass provides both an aesthetically pleasing landscape feature and functional surface for sporting events, helps reduce soil erosion and agricultural runoff, and represents one of the most effective carbon sinks in the world. While advances in breeding and cultural practices are continuously increasing the positive environmental benefits of turf, biotechnology has the potential to dramatically enhance our ability to maintain a healthy turfgrass stand with even fewer inputs. The Scotts Company is demonstrating biotechnology can indeed reduce maintenance inputs. We have developed preliminary data with non-plant pest Kentucky bluegrass events that contain traits for glyphosate tolerance and dwarfing thus resulting in a thicker and greener turfgrass stand requiring less mowing and fertilization. Further advances in sustainability could be achieved with abiotic and biotech stress tolerance.

### P-39

Exploring Desiccation Tolerance Using Comparative Integrative 'Omic Analyses in Resurrection Plants. JOHN C. CUSHMAN<sup>1</sup>, Sangho Kang<sup>1</sup>, Richard L. Tillett<sup>1</sup>, Abou Yobi<sup>2</sup>, Robert E. Sharp<sup>3</sup>, Karen A. Schlauch<sup>1</sup>, and Melvin J. Oliver<sup>2</sup>. <sup>1</sup>Department of Biochemistry & Molecular

Biology, University of Nevada, Reno, NV 89557; <sup>2</sup>USDA-ARS-MWA, Plant Genetics Research Unit, University of Missouri, Columbia, MO 65211; and <sup>3</sup>Division of Plant Sciences, University of Missouri, Columbia, MO 65211. Email: jcushman@unr.edu

In an effort to explore and develop novel strategies to improve drought tolerance in crops, a series of integrative metabolomic, proteomic, and transcriptomic studies have been undertaken in both lycophytic and euphyllophytic resurrection plants. Resurrection plants, which are rare among vascular plants, are defined as species that can withstand and recover from air-drying of their vegetative tissues. Within the ancient lycophyte lineage, a sister group comparison of desiccation sensitive (DS) *Selaginella moellendorffii* and desiccation tolerant (DT) *Selaginella lepidophylla* revealed ~200 compounds with significantly altered abundances with sugars (e.g. glucose, 1-kestose, and sucrose), sugar alcohols (e.g., sorbitol, xylitol, arabinitol), osmoprotectants (e.g., betaine, carnitine), aromatic and gamma-glutamyl amino acids, secondary metabolites and many unnamed compounds being overrepresented in the DT species. In this same sister comparison, Illumina-based RNA-seq analysis revealed ~2,800 differentially expressed genes (DEGs) in the DT species compared with only 360 DEGs in the DS species at 50% relative water content (RWC) relative to 100% RWC. Only 36 responsive genes were common to both species. Thus, desiccation tolerance is associated with a greater than 7.5-fold difference in the dynamics of gene responsiveness. Biological Networks Gene Ontology (BiNGO) analysis revealed that genes encoding early light-induced proteins (ELIPs), late embryogenesis abundant (LEA) proteins, heat shock proteins (HSPs), and reactive oxygen scavenging enzymes (e.g., glutathione-S-transferases, thioredoxins, peroxidases), and ABA metabolism and signaling components were significantly over represented during drying in the DT species, but not in the DS species. In addition, genes encoding protein synthesis and degradation (e.g., proteasome, ubiquitin-related) components were also over represented indicating that protein-remodeling events are likely to be critical to DT. Lastly, functional testing of candidate genes by overexpression in *Arabidopsis thaliana* has revealed that some genes (e.g., LEA4) from *Selaginella lepidophylla* confer improved osmotic stress tolerance. Combinatorial strategies for improved drought tolerance in crop models will be discussed.

### P-40

Analysis of Combined Drought and Ozone Stress in *Medicago truncatula*. RAMAMURTHY MAHALINGAM. Oklahoma State University, Dept. of Biochemistry and Molecular Biology, Stillwater, OK 74078. Email: ramamurthy.mahalingam@okstate.edu

Drought and tropospheric ozone are escalating climate change problems that can co-occur. In this study we observed *Medicago truncatula* cultivar Jemalong that is sensitive to ozone and drought stress when applied singly, showed tolerance when subjected to a combined application of these stresses. Lowered stomatal conductance may be a vital tolerance mechanism to overcome combined ozone and drought. Sustained increases in both reduced ascorbate and glutathione in response to combined stress may play a role in lowering ROS and NO toxicity. Transcriptome analysis indicated genes associated with glucan metabolism, responses to temperature and light signaling may play a role in dampening ozone responses due to drought-induced stomatal closure during combined occurrence of these two stresses. Gene ontologies for jasmonic acid signaling and innate immunity were enriched among the 300 differentially expressed genes unique to combined stress. Differential expression of transcription factors associated with redox, defense signaling, jasmonate responses and chromatin modifications may be important for evoking novel gene networks during combined occurrence of drought and ozone. The alterations in redox milieu and distinct transcriptome changes in response to combined stress could aid in tweaking the metabolome and proteome to annul the detrimental effects of ozone and drought in Jemalong.

#### P-41

Parasitic Plants Add New Perspectives to Plant Stress. JAMES H. WESTWOOD. Virginia Tech, Department of Plant Pathology, Physiology & Weed Science, 401 Latham Hall, Blacksburg, VA 24061. Email: westwood@vt.edu

Parasitic plants live in association with other plants, connecting directly to the vascular system of their hosts to obtain water and nutritional resources. These specialized plant-plant interactions provide fresh perspectives for understanding plant development and response to the environment. With respect to the parasites, they have evolved specialized mechanisms that facilitate their parasitic lifestyle, such as locating suitable hosts by detecting chemical signals, invading host tissues, forming seamless connections to hosts, and accessing host nutrients. From the perspective of host plants, they face the challenge of defending themselves against pathogens that are equipped with essentially the same biochemical and physiological systems as the host. Thus, even as host plants appear to recognize parasitic plants as invaders, relatively few cases of effective resistance to parasitic plants are known. This presentation will

consider two classes of parasites, root parasites of the family Orobanchaceae, and shoot parasites of the genus *Cuscuta*. Parasitism evolved independently in these groups and is reflected by similarities and differences in host-parasite interactions. Although all parasites have haustoria, they differ in function and the degree to which parasites and hosts form symplastic associations. Recent findings will be presented from molecular and genomic studies that are revolutionizing understanding of parasite-host interactions.

#### P-42

A DEAD Box RNA Helicase Is Critical for Pre-mRNA Splicing, Cold-responsive Gene Regulation, and Cold Tolerance in *Arabidopsis*. JIANHUA ZHU<sup>1</sup>, Qingmei Guan<sup>1</sup>, Jianmin Wu<sup>1</sup>, Yanyan Zhang<sup>1</sup>, Changhua Jiang<sup>1</sup>, Renyi Liu<sup>2</sup>, and Chenglin Chai<sup>1</sup>. <sup>1</sup>Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD 20742 and <sup>2</sup>Department of Botany and Plant Sciences, University of California, Riverside, CA 92521. jhzh@umd.edu

Cold stress resulting from chilling and freezing temperatures substantially reduces crop production worldwide. To identify genes critical for cold tolerance in plants, we screened *Arabidopsis thaliana* mutants for de-regulated expression of a firefly *luciferase* reporter gene under the control of the *CBF2* (C-repeat binding factor 2) promoter (*CBF2:LUC*). An *rcf1-1* (*regulator of CBF gene expression 1*) mutant that is hypersensitive to cold stress was chosen for in-depth characterization. *RCF1* encodes a cold-inducible DEAD (Asp-Glu-Ala-Asp) box RNA helicase. Unlike a previously reported DEAD box RNA helicase (LOS4) that regulates mRNA export, RCF1 does not play a role in mRNA export. Instead, RCF1 functions to maintain proper splicing of pre-mRNAs; many cold-responsive genes are mis-spliced in *rcf1-1* mutant plants under cold stress. Functional characterization of four genes (*Pseudo-response regulator 5* [*PRR5*], *Shaggy-like serine/threonine kinase* [*SK12*], *MYB family transcription factor circadian 1* [*CIR1*], and *SPFH/PHB domain-containing membrane-associated protein* [*SPFH*]) that are mis-spliced in *rcf1-1* revealed that these genes are cold-inducible, positive (*CIR1* and *SPFH*) and negative (*PRR5* and *SK12*) regulators of cold-responsive genes and cold tolerance. Together, our results suggest that the cold-inducible RNA helicase RCF1 is essential for pre-mRNA splicing and is important for cold-responsive gene regulation and cold tolerance in plants.

## P-43

Overexpression of PvMYB4 in Switchgrass Reduces Cell Wall Recalcitrance and Leads to Very High Cellulosic Ethanol Yields. HUI SHEN<sup>1,5</sup>, Charleson R. Poovaiah<sup>2,5</sup>, Angela Ziebell<sup>3,5</sup>, Timothy J. Tschaplinski<sup>5</sup>, Sivakumar Pattathil<sup>4,5</sup>, Kelsey L. Yee<sup>5</sup>, Miguel Rodriguez, Jr.<sup>5</sup>, Erica Gjersing<sup>3,5</sup>, Nancy Engle<sup>5</sup>, Rui Katahira<sup>3,5</sup>, Yunqiao Pu<sup>5</sup>, Robert Sykes<sup>3,5</sup>, Fang Chen<sup>1,5</sup>, Arthur J. Ragauskas<sup>5</sup>, Jonathan R. Mielenz<sup>5</sup>, Michael G. Hahn<sup>4,5</sup>, Mark Davis<sup>3,5</sup>, C. Neal Stewart, Jr.<sup>2,5</sup> and Richard A. Dixon<sup>1,5</sup>. <sup>1</sup>University of North Texas, Denton, TX 76203; <sup>2</sup>University of Tennessee, Knoxville, TN 37996; <sup>3</sup>National Renewable Energy Laboratory, Golden, CO 80401; <sup>4</sup>University of Georgia, Athens, GA 30602; and <sup>5</sup>BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, TN 37831. Email: hshen@noble.org; corresponding author, Richard.Dixon@unt.edu

Switchgrass is a dedicated lignocellulosic feedstock for bioenergy production in the United States. One strategy for overcoming cell wall recalcitrance in order to increase sugar yield and improve cellulosic ethanol production from plant biomass involves reducing the lignin content and/or modifying lignin-polysaccharides linkages of the lignocellulosic cell wall matrix. Overexpression of the transcription factor PvMYB4 reduces the lignin content of switchgrass by 60-70% and increases sugar release efficiency approximately 3-fold without acid pretreatment. This translates into a 2.6-fold increase in ethanol yield using yeast-based simultaneous saccharification and fermentation (SSF) without pretreatment. The trait of high cellulosic ethanol yield is stable with both greenhouse and field harvested materials. Gel permeation chromatography (GPC) of isolated ball-milled lignin indicates that overexpression of PvMYB4 decreases the average molecular weight of extractable lignin by about 10%. Nuclear magnetic resonance (NMR) spectroscopy reveals parallel reductions in lignin internal linkage levels as well as increased wall-associated fucose levels. Glycome profiling shows reduced levels of pectins and xylans in the lignin-polysaccharides enriched fraction. Metabolite profiling by GC-MS indicates that PvMYB4 overexpressing lines have reduced levels of potential phenolic inhibitors to fermentation. Our data indicate that the levels and nature of lignin embedded in the cell wall, linkages between lignin, xylans and pectins, as well as lignin polymer size all likely contribute to the recalcitrance of switchgrass biomass. Therefore, the genetically engineered PvMYB4 switchgrass provides a new germplasm for developing switchgrass feedstocks for high biofuel production and a novel system for studying the recalcitrance of switchgrass cell walls.

## P-44

Jet Fuel from *Camelina Sativa*: Engineering Plant Primary Metabolism. HEIKE SEDEROFF<sup>1</sup>, Amy Grunden<sup>2</sup>, Rongda Qu<sup>3</sup>, and Deyu Xie<sup>1</sup>. <sup>1</sup>Dept. Plant Biology, <sup>2</sup>Dept. Microbiology, and <sup>3</sup>Crop Science, North Carolina State University, Raleigh, NC 27695-7612. Email: hwsedero@ncsu.edu

Biofuels offer renewable alternatives to petroleum-based fuels that reduce net greenhouse gas emissions to nearly zero. However, traditional biofuel production is limited not only by the small amount of solar energy that plants convert through photosynthesis into biological materials, but also by inefficient processes for converting these biological materials into fuels. Farm-ready, non-food crops are needed that produce fuels or fuel-like precursors at significantly lower costs with significantly higher productivity. To make biofuels cost-competitive with petroleum-based fuels, biofuel production costs must be cut in half or - yield at least doubled. In a large-scale effort to increase productivity of the oil-crop plant *Camelina sativa*, we are genetically engineering more than 18 heterologous genes into one line. The purpose of this effort is to re-engineer carbon flux through a plant from CO<sub>2</sub> uptake to seed oil yield and composition. The transgenes will increase CO<sub>2</sub> uptake, generate a novel RUBISCO-independent, synthetic pathways for CO<sub>2</sub> assimilation, modify allocation of assimilate, increase yield and modify the seed oil composition. These transgenic lines will produce terpenes and modified seed oil that is energetically and economically optimized for thermo-catalytic conversion into energy-dense drop-in transportation fuels. The engineered camelina will be more tolerant to drought and heat, which makes it suitable for farming in warmer and drier climate zones in the United States. The increased productivity of this enhanced camelina and the development of energy-effective harvesting, extraction, and conversion technology could provide an alternative non-petrochemical source of fuel. The principles underlying the genetically modified Camelina will also be used for improvement of other crops. (Funded by DOE ARPA grant DE-AR0000207).

## P-45

Southern Pines the Bioenergy and Renewable Chemical Star of the Southeast US. GARY F. PETER<sup>1</sup>, Chris Dervinis<sup>1</sup>, Jared Westbrook<sup>1</sup>, John M. Davis<sup>1</sup>, James Kirby<sup>2</sup>, Jay Keasling<sup>2</sup>, and William Rottmann<sup>3</sup>. <sup>1</sup>School of Forest Resources and Conservation, Genetics Institute, University of Florida, PO Box 110410, Gainesville, FL; <sup>2</sup>University of California, Berkeley, 5885 Hollis St., Emeryville, CA

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Southern pines are a proven sustainable source of renewable biomass for biomaterials, bioenergy and renewable chemicals. The S.E. forest industry not only produces ~18% of the global supply of industrial roundwood, 25% of the global pulp supply, 40% of the global pine chemical supply, but also generates 77% of all industrial biomass energy in the U.S. by burning wood waste and lignin. The 93 million hectares of standing pines in the S.E. include 15.6 million hectares of genetically improved plantations; all grow with low water, fertilizer and herbicide inputs on land not suitable for cultivation of food crops. This large extant southern pine resource, with its well established supply chain and predictable year round harvests, has drawn substantial commercial interest for new stand alone electrical power generating facilities being constructed in FL, TX and AL; the largest wood pellet mills in the world operating in FL

and GA, and liquid biofuels facilities being constructed in MS and planned in AL, GA and SC. As demand for southern pine continues to grow for renewable chemicals and fuels, pine trees need to be developed that substantially increase energy and renewable chemical yields per acre per year. To achieve this goal, we are developing terpene enhanced pine trees. Terpenes are energy dense hydrocarbons that naturally accumulate in the wood of conifers, primarily for defense of the stem to insect and fungal attack. Increasing the amount of terpene produced by pines will dramatically increase the energy yield per hectare per year. We are mining the wide natural genetic variation in southern pine terpene production and using functional genomics to discover genes that can be used to develop terpene enhanced pines with dramatically increased biosynthetic and storage capacity and efficiencies to boost yields for the existing >\$3 billion global pine oleochemical industry as well as to supply directly extractable advanced drop-in biofuels suitable for jets, ships and cars.