In Vitro Cell.Dev.Biol.—Animal
DOI 10.1007/s11626-011-9416-5

Plant Symposia and Workshops

P-1

Cotton Transformation in Plant Transformation Lab – Ambition or Reality. SERGEI KRASNYANSKI, George Allen, Jerson Dominguez, and Michael Stiff. Plant Transformation Lab, North Carolina State University, Campus Box 7550, Partners II Bldg., Room 1208, Raleigh, NC 27695-7550. Email: sfkrasny@ncsu.edu

Significant economic importance of cotton as a fiber crop that has been grown for over 3000 yr requires constant effort to improve its agronomic traits. Biotechnological approach to solve this problem through genetic transformation remains very promising. However, there are particular obstacles and constrains on the path of successful production of transgenic cotton. One of the main problems is an efficient regeneration/transformation system that allows producing transformed fertile cotton plants. Another serious obstacle is genotype dependency in cotton regeneration system. Alternative solutions to these problems have been attempted in Plant Transformation Lab. Many research groups are working on cotton plant and fiber improvements through molecular biology (functional genomics, proteomics), biotechnology, and breeding. One problem faced by cotton breeders and other researchers is the comparatively long time required for plant growth from seed to flower. One possible strategy that could help to solve this inconvenience is to reduce the time required for flowering by over expressing the *FLOWERING LOCUS T* (*FT*) gene. Current status of these and other ongoing experiments will be discussed.

P-2

Cotton Transgenics Derived from Embryogenic Cell Lines. KENT CHAPMAN, Shanmukh Salimath, and Purnima Neogi. University of North Texas, Department of Biological Sciences, 1155 Union Circle, #305220, Denton, TX 76203-5017. Email: chapman@unt.edu

Conventional genetic transformation methodologies for cotton have mostly centered on the regeneration of transgenic plants from hypocotyl and/or cotyledonary tissue explants via somatic embryogenesis, often after a lengthy callus phase. As an alternative, we have been exploring procedures for Agrobacterium-mediated cotton transformation beginning with embryogeneic cell lines and have examined this method with a range of transgenes. Generally, starting with embryogenic cell lines, the recovery of flowering transgenic plants in the greenhouse occurred as early as six months after co-cultivation. Advantages, disadvantages and several examples of the cell-line-based methodology will be presented.

P-3

Genetic Engineering of a Cotton Plant: Progress and the Remaining Bottlenecks, a Quarter of a Century Later. KEERTI S. RATHORE, LeAnne M. Campbell, Lauren Tollack, G. Sunilkumar, and Emily Lopata-Finch. Institute for Plant Genomics & Biotechnology, Texas A&M University, College Station, TX, 77843. Email: rathore@tamu.edu

Although a handful of genetically engineered (GE) crop plants, including cotton, have found tremendous commercial success, these have largely come out of industrial laboratories. Despite a continuing stream of research publications from public institutions, their progress towards releasing crop plants with commercially useful, GE traits has been negligible. Many factors account for this situation,

Published online: 21 April 2011

however, a major reason for this are the difficulties encountered in generating a transgenic plant. This is true particularly for cotton that requires an enormous investment in manpower, resources, and time that only the large agricultural biotechnology companies can afford. Continuing efforts in my laboratory to overcome technical hurdles have resulted in significant improvements in obtaining a transgenic cotton plant, however, many bottlenecks remain. The presentation will detail some of the progress made in generating a transgenic cotton plant. Also, an account will be provided of the obstacles that remain. In addition, results from our efforts to use two different gene-silencing technologies, antisense and RNAi, to metabolically engineer the cottonseed will be presented.

P-4

Silencing of the Pests. C. L. NIBLETT and Ana M. Bailey. Venganza, Inc., 840 Main Campus Drive, Raleigh, NC 27606. Email: niblett@venganzainc.com

Venganza Inc. has developed a disease control technology called Host-Mediated Silencing of Pest Genes (HMSPG) that utilizes essential gene sequences of fungal pathogens to control those pathogens via a gene silencing or RNA interference (RNAi) mechanism. Since cotton is genetically transformable, HMSPG is a promising approach to control damaging pests of this important crop. Potential major targets are Fusarium and Verticillium wilts, cotton root rot, Lygus bugs and the green stink bug.

P-5

Biotechnological Strategies to Test Cotton Gene Function and Improve Fiber Quality. CANDACE H. HAIGLER, John Rich Tuttle, and Dominique Robertson. North Carolina State University, Dept. of Crop Science and Dept. of Plant Biology, 4405 Williams Hall, Campus Box 7620, Raleigh, NC 27695-7620. Email: Candace_haigler@ncsu.edu

The speed and efficiency of cotton (Gossypium) research and biotechnological improvement strategies has been hampered by technological barriers in testing gene function and producing stably transformed germplasm. In light of the first cotton genome sequence appearing soon (diploid G. raimondii) as well as the rapidly increasing transcriptome profiles of commercial tetraploid cotton (G. hirsutum and G. barbadense), it is increasingly important that more efficient pipelines be generated for trait testing and improvement. This talk will describe our strategy for comparing fiber cellular and molecular developmental processes in G. hirsutum and G. barbadense in order to develop leads for regulatory genes that can be tested first through virus-induced-gene silencing in cotton, followed by stable transformation when warranted.

P-6

Biological, Biochemical and Molecular Dissection of Tissue Browning and Death During *Agrobacterium*-mediated Plant Transformation. YINGHUI DAN[1,2]. [1]Institute for Advanced Learning and Research, 150 Slayton Avenue, Danville, VA 24540 and [2]Departments of Horticulture and Forestry, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061. Email: Yinghui.dan@ialr.org, ydan@vt.edu

The biotech crop market provides significant and consistent economic, environmental, health and social benefits to our society, with a global market value of US$ 10.5 billion in 2009. Plant transformation has played a critical role in the production of biotech crops as well as the advancement of the underlying fundamental research that enhances agricultural productivity. However, plant transformation technology is only moderately or marginally successful in many agriculturally important cultivars of crops. One of the major challenges in *Agrobacterium*-meditaed plant transformation is the browning and death of *Agrobacterium*-transformed tissues (BDATT), which severely limits transgenic plant production. A major focus to address this problem is to utilize antioxidants in culture media during *Agrobacterium*-mediated plant transformation. Hydrogen peroxide (H_2O_2) plays an important role in oxidative stress. However, little is known about the biological, biochemical and molecular mechanisms causing and regulating BDATT; the regulation of BDATT by antioxidants; and the signaling pathway leading from H_2O_2 to BDATT. In this presentation I will discuss the current advances in the respective areas.

P-7

Physiological Disorders of Pear Shoot Cultures. BARBARA M. REED[1], Sugae Wada[2], Jeanine DeNoma[1], Terrence J. Evens[3], and Randall P. Niedz[3]. [1]USDA ARS, National Clonal Germplasm Repository, 33447 Peoria Rd., Corvallis, OR 97333-2521; [2]Dept. of Horticulture, Oregon State University, Corvallis, OR 97331; and [3]Horticulture and Breeding Unit, U.S. Horticultural Research Laboratory, 2001 South Rock Road, Ft. Pierce, FL 34945-3030. Email: Barbara.Reed@ars.usda.gov

Physiological disorders are some of the most difficult challenges in micropropagation. Little is known of the causes of plant growth disorders which include callus formation, hyperhydricity, shoot tip necrosis, leaf lesions, epinasty, fasciation and hypertrophy. During our study of mineral nutrition to improve growth of pear shoot cultures we also noted the effect of mineral stock solutions on these disorders. Five mineral nutrient factors from Murashige and Skoog (MS) salts, NH_4NO_3, KNO_3, meso elements ($CaCl_2$, KH_2PO_4 and MgSO4), minor elements (Zn-Mn-Cu-Co-Mo-B-I), and Fe-EDTA, were tested over a range of concentrations in a 5-dimensional experimental design. Five pears (Old Home x Farmingdale 87, *Pyrus dimorphophylla*, Horner 51, Hang Pa Li, and Winter Nelis) were grown on each treatment combination. Analysis for all genotypes identified the factors that contributed to these disorders and also the factors that remedied them. Many of the factors affecting overall shoot quality also contributed to specific physiological disorders. Leaf disorders (spotting and edge burn) were prominent when the mesos concentrations were ≤ MS concentrations, but were also influenced by interactions with either low iron or high NH_4NO_3. Shoot tip necrosis varied with the genotype and either mesos or low nitrogen concentrations contributed to the necrosis. Hyperhydricity was more prominent with low mesos for some genotypes or low NH_4NO_3 for others. Epinasty, fasciation and hypertrophy were seen infrequently and were due either to interactions between mesos and KNO_3 or to low NH_4NO_3. Callus was common on MS medium and appears to be increased by low NH_4NO_3 concentrations.

P-8

Mineral Nutrient Effects on Hyperhydricity. M. J. BOSELA. Department of Biology, Lindsey Wilson College, 210 Lindsey Wilson Street, Columbia, KY 42728. Email: boselam@lindsey.edu

Hyperhydricity is one of the most common disorders observed during micropropagation. Hyperhydric tissues tend to have a higher water content than normal plant tissues, at least extracellularly, and thus are glassy or translucent in appearance, and also are often brittle in texture. Hyperhydric shoots may exhibit high rates of proliferation, but they are generally short lived and difficult to root and establish outside of tissue culture. In the context of performing research evaluating the feasibility of using tissue cultures, in place of more conventional hydroponics techniques for the demonstration mineral nutrient deficiency symptoms, hyperhydricity was noted for some of the treatments. Because the experiments employed whole plants, primarily seedlings, cultured on hormone free, 'low salt' medium (Hoagland's #2 Solution) hyperhydricity was not expected. Nonetheless, for *Arabidopsis thaliana* a severe hyperhydric phenotype was observed for more than half of the experimental media including the calcium, iron, and potassium deficiency media. Hyperhydricity was also apparent on the calcium deficiency media for aspen and tomato, but was ephemeral. This work has been expanded to study mineral nutrient effects during micropropagation, using MS medium and proliferating shoot cultures. The results of these experiments will be presented as part of a review of mineral effects on hyperhydricity in plant tissue culture.

P-9

Chloroplast Vector Systems for Various Biotechnology Applications. HENRY DANIELL. Department of Molecular Biology & Microbiology, College of Medicine, University of Central Florida, Orlando FL 32816-2364. Email: daniell@mail.ucf.edu

Chloroplasts are ideal bioreactors for hyper-expression (up to 72% of total leaf protein, Plant Physiology 152: 2088, 2010) of foreign proteins. Site specific integration, multigene engineering in a single transformation event, lack of gene silencing, minimal effects of toxic proteins due to compartmentalization and transgene containment due to maternal inheritance or harvest of tissues before appearance of reproductive structures are unique advantages of chloroplast transformation. Chloroplast genomes of >35 major crop species and trees have been sequenced recently (Molecular Biology and Evolution 28: 835, 2011) >20 crop species have been transformed, including cotton and soybean (Nature Protocols 3: 739, 2008). Highest levels of resistance or tolerance to insetcs, herbicides, drought, salt or toxic metals reported in the literature were achieved via chloroplast transformation (Plant Physiology 145: 1129, 2007). Vaccine antigens against several infectious diseases and biopharmaceuticals expressed in chloroplasts are properly folded with post-translational modifications (disulfide bonds) are fully functional. (Trends in Plant Science 14: 669–679, 2009). Dehydrated lettuce leaves preserved therapeutic proteins for several months. Orally delivered vaccine antigens develop both mucosal and systemic immunity (Plant biotechnology Journal 8: 223–242, 2010) and confer greater protection than injectable vaccines (Infection & Immunity 76: 3640–3650). Oral delivery of plant cells expressing blood clotting factor IX in chloroplasts prevented fatal anaphylaxis in hemophilia B mice (PNAS 107: 7101–7106, 2010), thereby advancing cure for several autoimmune disorders. Several biomass hydrolysis

enzymes have been expressed in tobacco chloroplasts. Chloroplast-derived crude-extract enzyme cocktails yielded more glucose from pine wood or citrus peel than commercial cocktails produced via fermentation and 1000-fold less expensive (Plant Biotechnology Journal 8: 332–350, 2010). One of these enzymes, B-glucosidase, released active hormones from their inactive conjugates stored within chloroplasts and doubled plant biomass (Plant Physiology 155, Jan 2011). Chloroplast vector systems and transformation techniques used in various biotechnology applications will be presented.

P-10

Virus-induced Gene Silencing as a Tool for Functional Gene Studies in Plants. D. ROBERTSON[1], J. R. Tuttle[1], and C. H. Haigler[1,2]. Depts. of [1]Plant Biology and [2]Crop Science, North Carolina State University, Raleigh, NC 27695. Email: niki_robertson@ncsu.edu

Virus-induced gene silencing (VIGS) uses modified viruses to trigger RNAi of host genes, bypassing the need for stable plant transformation. The diffusible nature of the silencing signal allows uniform silencing even in non-infected cells. We have developed three different geminivirus-based VIGS vectors; two for the model plants Arabidopsis and *Nicotiana benthamiana* and one for the crop plant cotton. Geminivirus-mediated silencing in each of these species is stable; allowing, for example, VIGS to extend into cotton fibers in cotyledon-inoculated plants. We found that VIGS of the 35S:GFP transgene in cotton plants (from K. Rathore) showed a sectored pattern, unlike the systemic silencing seen in 35S:GFP *N. benthamiana* (from D. Baulcombe). Evidence will be presented to suggest that diffusible silencing pathways may differ in polyploid species. Experiments using GFP silencing as a marker for endogenous gene silencing in cotton will also be presented. *Supported by Cotton Incorporated.*

P-11

Non-transgenic Approach for Genome Modifications in Plants. I. Marton[1,2], A. Zuker[1], T. Tzfira[3], and A. VAINSTEIN[2]. [1]Danziger Innovations Ltd., Mishmar Hashiva Village, P.O. Box 24, Beit Dagan 50297, ISRAEL; [2]The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, ISRAEL; and [3]Department of Life Sciences, Ben-Gurion University of the Negev, Beer Sheva, ISRAEL. Email: vain@agri.huji.ac.il

The ability to modify genome sequences in plant cells is fundamental to modern agriculture. Naturally occurring and artificial rare-cutting restriction enzymes have been used for targeted mutagenesis in model and crop species. In animal and human cells, transient expression of the enzymes is often achieved by direct gene transfer into the target cells. Stable transformation, however, is the preferred method for gene expression in plant species, and nuclease-expressing transgenic plants have been used for recovery of mutants that are likely to be classified as transgenic due to the use of direct gene-transfer methods into the target cells. We developed an alternative, non-transgenic approach for zinc finger nucleases (ZFNs) delivery and production of mutant plants using a novel Tobacco rattle virus (TRV)-based expression system for indirect transient delivery of ZFNs into a variety of tissues and cells of intact plants. TRV systemically infected its hosts and virus ZFN-mediated targeted mutagenesis could be clearly observed in newly developed infected tissues as measured by activation of a mutated reporter transgene in tobacco and petunia plants. The ability of TRV to move to developing buds and regenerating tissues enabled recovery of mutated tobacco and petunia plants. Sequence analysis and transmission of the mutations to the next generation confirmed the stability of the ZFN-induced genetic changes. Because TRV is an RNA virus that can infect a wide range of plant species, it provides a viable alternative to the production of ZFN-mediated mutants while avoiding the use of direct plant-transformation methods.

P-12

Targeted Gene Deletion. JOSEPH F. PETOLINO, Andrew Worden, Krisi Curlee, James Connell, Tonya L. Strange Moynahan, Cory Larsen, and Sean Russell. Dow AgroSciences, 9330 Zionsville Rd., Indianapolis, IN 46268. Email: jfpetolino@dow.com

The ability to delete large, targeted DNA sequences would have value for both basic and applied research. In the present study, a 4.3 kb gene sequence, flanked by zinc finger nuclease (ZFN) cleavage sites, was deleted from a plant genome by crossing with a second plant expressing a corresponding ZFN gene. A target construct containing a reporter gene expression cassette flanked by ZFN binding sites and a second construct containing a ZFN gene expression cassette were transformed separately into tobacco. Reporter gene deletion was carried out by crossing selected transgenic plants, wherein, upon expression, a functional ZFN recognized the ZFN

binding sites flanking the reporter gene cassette, thereby creating 5' and 3' double strand breaks (DSBs) which, following DNA repair, resulted in intervening sequence deletion. Numerous reporter gene-negative plants were observed among the hybrids with one particular cross displaying ~35% reporter gene-negative plants. Evidence for complete deletion of a 4.3 kb sequence comprising the reporter gene cassette was obtained and confirmed via sequence analysis. Selected hybrid plants were allowed to self-pollinate and F_2 progenies were analyzed. Co-segregation of 'truncated' and 'intact' target sequences with expected reporter gene phenotypes were observed. Since ZFNs can be designed to bind and cleave a wide range of DNA sequences, these results constitute a general strategy for creating targeted gene deletions.

P-13

Strawberry Transformation as a Means to Accelerate Functional and Translational Studies in the Rosaceae. KEVIN M. FOLTA[1], Janet P. Slovin[2], M. Fadhli Mad Atari[1], Kaylie Smith[1], Laura Gonzalez[1], and Hannah Needleman[1]. [1]Horticultural Sciences Department and the Graduate Program in Plant Molecular and Cellular Biology, University of Florida, Gainesville, FL 32611 and [2]Small Fruit Laboratory, United States Department of Agriculture, Beltsville, MD 20705. Email: kfolta@ufl.edu

The Rosaceae Family contains a variety of valuable fruit, wood and ornamental crops. Species within this family exhibit a wide range of growth habits, architectures and physiologies, yet gene coding sequences are remarkably well conserved. For this reason strawberry (*Fragaria spp*.) is an outstanding system to dissect critical questions that underlie traits of horticultural interest in the species. Strawberry is an herbaceous perennial that can be grown in a laboratory setting, is easily transformable, maintains a miniscule genome, and can be propagated by flowers or runners. These attributes offer many advantages over other members of the family for in planta validation of function genomics hypotheses. Our laboratory has contributed to the development of multiple systems that are useful in the construction of transgenic lines. An octoploid strawberry (*Fragaria x ananassa*) line, LF9, regenerates rapidly and is easily transformed. Transgenes may be easily mobilized into elite commercial germplasm using this line. A number of diploid (*F. vesca*) lines are also used. 'Hawaii-4' was identified as a rapid and efficient transformation line by scientists at Virginia tech. We have optimized protocols to speed transformation and regeneration in this line. We have optimized the use of a seven-generation diploid called 5AF7. This line offers greater homozygousity, a more stable presentation of phenotypes than Hawaii-4 and it does not runner, making management of thousands of plants possible. A 'Rugen' line has also been inbred seven generations and tissue culture protocols are being devised to utilize this line. The diploid lines offer the advantage of small stature, rapid cycling and the ability to save seeds. Transformation is simple and efficient. Efforts are underway to develop a 'floral dip' transformation techniques to complement in vitro capacities. These attributes make strawberry a powerful translational system, allowing in planta tests of gene function that are begin to approach the utility of Arabidopsis.

P-14

Wheat Genetic Engineering: from the Gene to the Field. F. TORNEY, D. Gerentes, W. Paul, P. Perez, and C. Sallaud. BIOGEMMA, 8 rue des frères lumières, Clermont-Ferrand 63028, FRANCE. Email: francois.torney@biogemma.com

Biogemma is a Europe based Plant Biotechnology company developed and financed by the agricultural world. The company's shareholders consist of crop improvement and financial institutions of the plant production and transformation chain in France. Besides corn, Biogemma has set up its genetic engineering pipeline to handle wheat. The development and use of a proprietary method for wheat transformation has allowed the establishment of a pipeline ranging from gene/trait discovery to thorough agronomical field evaluation of selected transformation events. Genes for traits of interest are identified form a wide range of sources. Biogemma is using its internal data and studies from association genetics, genetic mapping and transcriptomics to identify key genes and alleles. Additionally, Biogemma is actively involved in numerous collaborations with partners from both public and private sectors. Genetic transformation of a proprietary spring wheat line is performed by Biogemma's Clermont-Ferrand based facility in France. Transformation events are selected based on a thorough and standardized molecular analysis platform. Only high quality events are selected, the rest are discarded. Phenotypes of transgenic plants are monitored throughout the process of selection and seed increase to ensure that each event can be brought to Biogemma's US based facility for field evaluation. The entire process is monitored through an integrated LIMS system and punctuated by Quality controls check points to ensure thorough traceability and perfect compliance with GLP and local legal obligations. We will present the entire process of the Biogemma wheat transformation pipeline and its agronomical trait goals.

P-15

High Throughput Transformation and Transgenic Analysis for Maize. J REGISTER. DuPont Agricultural Biotechnology, Pioneer Hi-Bred International, Inc., Johnston, IA 50131. Email: jim.register@pioneer.com

Over the past decade a number of institutions have developed dedicated high-throughput transgenic plant production and analysis systems. Most of these make use of species of relatively small stature and for which facile transformation systems exist, such as Arabidopsis and Japonica rice. Because the intent of such systems is typically facilitation of gene discovery for application in harder-to-transform crop species, an ongoing concern with these systems is their relevance to species like maize or soybean. At Pioneer we have therefore developed FASTCorn (**F**unctional **A**nalysis **S**ystem for **T**raits in **Corn**). This system will be described. As we have used this system over the past 5+ yr and as dramatically improved maize transformation methods have been developed, we have realized that tremendous power exists in using elements of the FASTCorn system in combination with other tools and methods to address questions beyond gene discovery. Examples will be discussed.

P-16

ZmODP2 and ZmWUS: New Developments for Improving Maize Transformation. W. GORDON-KAMM, K. Lowe, E. Wu, N. Wang, C. Scelonge, L. Wang, B. Lenderts, G. Hoerster, L. Ryan, W. Hua, P. Shen, J. Chow-Yiu, C. Sweeney, Z-Y. Zhao, D. Xu, C. Falco, and J. Register. Pioneer Hi-Bred International, Inc. 7300 NW 62nd Ave, Johnston, IA. Email: william.gordon-kamm@pioneer.com

After testing many cell cycle and developmental genes in maize, we have found that ZmODP2 (BBM) alone or in conjunction with ZmWUS can dramatically improve maize inbred transformation efficiencies. Using ODP2 driven by a strong constitutive promoter improved Agrobacterium-mediated transformation frequencies of immature embryos in Pioneer inbreds PH581, PHN46 and PHP38 from baseline levels of less that 1% (vigorously growing callus events relative to the number of starting embryos) to levels ranging from 12% to 32% depending on the genotype. When a weakly expressed WUS was used with the ODP2 expression cassette, frequencies ranged from approximately 20% to 50% depending on the genotype. For another Pioneer inbred PHH5G, no events were recovered in the control (no developmental genes) or with ODP2 alone, but with both ODP2 + WUS, callus events were recovered at above a 40% frequency. For all genotypes, the ODP2 and WUS genes were excised and fertile plants were regenerated. Using both ODP2 and WUS, explants from mature inbred seed and from leaf bases were also directly transformed using Agrobacterium and fertile plants were regenerated. For example, transformation of mature embryo slices from a PHI-Flint inbred produced callus events at a 25% frequency.

P-17

Syngenta's Integrated Program in Renewable Fuels. PAUL OELLER[1], Jason Nichols[1], Jason Geisjkes[2], Stacy Miles[1] and Manuel Sainz[2]. [1]Syngenta Biotechnology Incorporated, 3054 E. Cornwallis Rd, RTP NC 27709 and [2]Syngenta Centre for Sugarcane Biofuels Development, Centre for Tropical Crops and Biocommodities, Queensland University of Technology, PO Box 2434, 2 George Street Bldg H/Level 3/Rm 312, Brisbane QLD 4001 AUSTRALIA. Email: paul.oeller@syngenta.com

Syngenta has pioneered the concept of plant expressed enzymes for biofuel production and is the only company to date to have taken a biofuels trait into and through the US regulatory system. Enogen™, Syngenta's corn-expressed amylase trait for dry grind ethanol production, was developed to enhance the sustainability of first generation biofuel production. Syngenta is continuing to develop crops tailored for next generation biofuel production by expressing optimized enzymes *in planta* for the conversion of cellulose to fermentable sugars. Agricultural waste streams such as corn stover, corn cobs and sugar cane bagasse are attractive potential feed stocks for advanced second generation biofuels due to their comparatively low cost, abundance and availability. Syngenta has demonstrated the production of several classes of cellulases in corn and shown that they are nearly as active as their microbially produced counterparts. Furthermore, corn expressed cellulases have been tested on several industrially relevant substrates including sugar cane bagasse. To make sugarcane production and delivery of cellulases a reality, Syngenta recently formed the Syngenta Center for Sugarcane Biofuel Development (SCSBD) in collaboration with the Queensland University of Technology in Australia to develop efficient cane transformation technologies. By providing technologies like these Syngenta is helping to make second generation biofuels economically viable, while reducing agricultural waste and increasing the value realized from an acre of land.

P-18

Genetic and Genomics Approaches to Overcoming Recalcitrance of Populus Feedstock for Biofuel Production. UDAYA C. KALLURI. BioEnergy Science Center and Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831. Email: kalluriudayc@ornl.gov

Central to developing economically and environmentally sustainable biofuels through the enhancement of yield per acre land is achieving a delicate balance between usable and recalcitrant fractions of biomass. In this presentation a case is made for *Populus* as a model bioenergy crop with excellent resources for research and development towards feedstock improvement. Investigations using a suit of phenotypic and molecular characterization techniques such as –omics, spectroscopy, sugar release assay, imaging and bioinformatics approaches are being used in understanding the stem system properties of *Populus* plants. The genetic to genomics range of approaches incorporate samples collected from four different structured studies including association, QTL, activation tag and expression studies. New insights from these studies are being evaluated in targeted functional genomics experiments to clarify their roles in determining biomass properties.

P-19

Redesigning Lignocellulosic Feedstocks: Genetic Modification of Lignin Biosynthesis Significantly Improves Ethanol Production in Switchgrass. ZENG-YU WANG[1,3], Chunxiang Fu[1], Jonathan Mielenz[2,3], Xirong Xiao[1,3], Yaxin Ge[1], Choo Y. Hamilton[2,3], and Joseph Bouton[1]. [1]Forage Improvement Division, The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401; [2]Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831; and [3]BioEnergy Science Center. Email: zywang@noble.org

Switchgrass (*Panicum virgatum* L.), a perennial C4 warm-season grass native throughout North America, has been developed into an herbaceous bioenergy crop. Recalcitrance to saccharification is one of the major limitations for conversion of lignocellulosic biomass into ethanol. We take a transgenic approach to genetically modify lignin biosynthesis and processing properties of switchgrass. Major lignin biosynthetic genes were isolated from switchgrass and transgenic plants carrying RNAi constructs were produced. Analyses of the transgenic switchgrass plants by RT-PCR and real time PCR revealed that expression levels of the endogenous genes were down-regulated. Reduced enzyme activity, decreased lignin content or alerted ratios of S/G were found in different transgenic lines. The down-regulation of lignin biosynthesis with certain transgenes resulted in improved sugar recovery. Ethanol yield was significantly increased in some of the transgenics. Thus, genetic modification of switchgrass can produce normal plants that have reduced thermochemical and enzymatic recalcitrance. Compared with control, the modified transgenic switchgrass lines should yield significantly more fermentation chemicals per hectare under identical process conditions.

P-20

Environmental Implications of GM Crops – Impact of an Unprecedented Change in Global Agriculture. MICHEAL D. K. OWEN. 3218 Agronomy Hall, Department of Agronomy, Iowa State University, Ames, IA 50011. Email: mdowen@iastate.edu

Global demands to produce more food have increased dramatically and the ever-increasing global population has placed incredible demands on agriculture to produce sufficient yields thus avoiding "Malthusian" disasters in the future. The adoption of genetically-modified crops represents a change in agriculture unprecedented in more than six millennia of agrarian society and is suggested to be a major opportunity to increase global food supplies. Ideally, increased crop yields will be achieved through sustainable but intensive production practices that allow dramatic increases in food production while protecting aquatic and terrestrial ecosystems. A longer term solution to the global demands on agricultural production is to improve crop genetic yield potentials, specifically responses to stress and increased resources utilization efficiency as well as improved crop management decisions by growers. Agriculture has a significant impact on the environment, in part attributable to increased soil erosion and resultant water quality issues, but also with regard to the pervasive use pesticides and other agronomic inputs and the use of petroleum products. The impacts of tillage on the agroecosystem and specifically on the flora and fauna are significant. Genetically-modified crops are suggested to be an important tool that will allow improved yields and more efficient use of resources thus enhancing crop production efficiency while minimizing risks to the environment. The implications of this unprecedented change in global agriculture will be addressed, benefits offered and risks described.

P-21

What We Know and What We Don't Know About the Farm-level Economics of Crop Biotechnology. M. C. MARRA. Department of Agricultural and Resource Economics, North Carolina State University, Box 8109, Raleigh, NC 27695-8109. Email: michele_marra@ncsu.edu

This presentation draws heavily from the recent book, "The Impact of Genetically Engineered Crops on Farm Sustainability in the United States", published by the National Research Council of the National Academies of Science and co-authored by the presenter. The presentation summarizes the findings to date and then concentrates on what we don't know about the farm level economics of crop biotechnologies in the United States and why. The final portion of the presentation will be suggestions for future economic and interdisciplinary research to shed some light on what we don't know.

P-22

Sociological Aspects of GM Crop Development: Do They Exist and Do They Matter? R. JUSSAUME. Department of Natural Resource Sciences, Washington State University. Email: rajussaume@wsu.edu

The development and dissemination of technology has long been recognized by the social sciences as central to the process of contemporary human development. More recently, development theorists have begun to argue about the necessity to promote sustainable development. The latter has many definitions, but most are centered around a recognition that any development strategy must maximize economic returns with minimal ecological impact and in a manner that addresses inequities in social structures if that technology is to have a long term positive impact on human development. However, of these three dimensions of sustainable development, the social dimension has been the most ignored. The purpose of this presentation will be to demonstrate the importance of the social dimension through a critical examination of the potential social costs and benefits of GM crop development.

P-23

Environmental Risks and Opportunities of Transgenic Crops: the Role of Science in Regulatory Decision-making. ALAN RAYBOULD. Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, UNITED KINGDOM. Email: alan.raybould@syngenta.com

Herbicide-tolerant and insect-resistant transgenic crops have realised environmental benefits in developed and developing countries. The potential for further environmental benefits is offered by transgenic crops that have, for example, improved water- and nitrogen-use efficiency. If the environmental benefits of transgenic crops are to be enjoyed widely, it is important that environmental regulations do not stifle the development of such crops while managing the risks posed by their cultivation. Evaluating the risks posed by a particular transgenic crop requires scientific knowledge of its likely behaviour when cultivated; however, regulations may become overbearing when risk assessment and management are regarded as purely scientific activities. Risk is a function of the severity and likelihood of harm following an action. What is regarded as harmful is a matter for policy; it is not discovered by scientific research. It follows that the proper role of science in risk assessment and management is to help decision-making by predicting the likelihood of effects that policy has defined as harmful. If harm is not defined, risk assessments may become research to measure or predict the properties of transgenic crops with increasing precision, when what is required is confidence that certain events will or will not occur following cultivation of the crops. Using examples from insect-resistant transgenic crops, this paper will argue that suitable regulation of transgenic crops will come from better risk assessment led by policy. Attempts to make policy through scientific research may lead to onerous regulatory data requirements that increase environmental risks by holding back the introduction of new environmentally beneficial products.

P-24

Developing an *Agrobacterium*-mediated Transformation System for Leafy Spurge (*Euphorbia esula*), a Model System for Weeds. W. S. CHAO[1], B. Xu[2], and M. E. Foley[1]. [1]USDA-Agricultural Research Service, Biosciences Research Lab, 1605 Albrecht Boulevard, Fargo, ND 58102-2765 and [2]Virginia Tech, Horticulture Department, Blacksburg, VA 24060. Email: wun.chao@ars.usda.gov

Leafy spurge is a perennial, invasive weed used as a model to study invasive plant behavior, because molecular tools such as a deep expressed sequence tag database and deoxyribonucleic acid microarrays have been developed for this species. However, the lack of effective in vitro regeneration and genetic transformation systems has hampered molecular approaches to study this weed. Due to the recalcitrant nature of leafy spurge, it is necessity to develop an effective regeneration system prior to the development of transformation systems. Three highly regenerative lines were selected by screening the in vitro regeneration capabilities of stem explants of 162 seedlings based on explant competence to form calluses and shoots.

High rates of shoot regeneration can be obtained using a growth medium containing 1× woody plant basal medium and 1× Murashige and Skoog (MS) basal salts, 1× MS vitamins, 1.11 μM 6-benzylaminopurine, 1.97 μM indole-3-butyric acid, and 3% sucrose, pH 5.6–5.8. To develop a genetic transformation system for leafy spurge, leaf segments or internodes from the in vitro-grown plants of highly regenerative lines are used as explants. In addition, variations in antibiotic selection and levels, *Agrobacterium tumefacien*s concentrations and strains, co-cultivation periods and conditions, and acetosyringone levels are tested to optimize transformation capability. To date, a single transgenic leafy spurge was obtained and confirmed by both Southern blot analysis and β-glucuronidase staining. Efforts are underway to improve the efficiency and consistency of transformation for the high throughput required of a model system.

P-25

Camelina as a Model for Oilseed Biotechnology. J. JAWORSKI and W. Yang. Donald Danforth Plant Science Center, 975 N. Warson Rd., St Louis, MO. Email: jjaworski@danforthcenter.org

Camelina (*Camelina sativa*) is an ancient crop that originated in Europe and whose use declined in medieval times. However, as a result of increased needs for food oils and biofuels, interest in Camelina and acreage of Camelina planted have both increased substantially recently. A member of the Brassicaceae family, it is a cool weather crop presently grown in the upper Midwest of the U. S. Deep sequencing of developing seed cDNA demonstrated that it is very closely related to Arabidopsis. Furthermore, like Arabidopsis, it is transformable by the floral dip method. In addition, its growing season is only 85–100 d. These advantages provide Camelina with the great potential for oilseed biotechnology in the area of biofuels and biomaterials. Several examples of progress we have made will be presented.

P-26

MicroTom—A Model System for Translational Genomics. YINGHUI DAN. Institute for Advanced Learning and Research, 150 Slayton Avenue, Danville, VA 24540 and Departments of Horticulture and Forestry, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061. E-mail: Yinghui.dan@ialr.org, ydan@vt.edu

Plant model systems have played an important role in understanding plant biology. The most highly developed plant model system is *Arabidopsis* because of its small size, rapid life cycle, small genome, and transformability. MicroTom is a miniature dwarf determinate cultivar of tomato (*Solanum lycopersicum*), differing from standard tomato cultivars primarily by two recessive genes, which confer the dwarf phenotype. It shares the major features with *Arabidopsis* that make it successful as a model system, including small size (up to 1357 plants/m^2), short life cycle (70–90 d from sowing to fruit-ripening), relatively small genome (950 Mb) and transformability. Tomato is a member of the family Solanaceae that includes more than 3000 species and is the third most economically important plant taxa, exceeded only by the grasses and legumes. It is an important vegetable crop and a generally acceptable addition to the diet for human cancer prevention. The global annual production of tomatoes is nearly 130 million tons. Because of its significant biological, economic and human health importance, the enormous progresses have been made in its genome sequencing, functional genomics, genetic transformation and improvement of nutrition, flavor and other trait quality. In this presentation, I will provide the current status of the advances, which provides a vision of a model system for translational genomics to enable plant biology discovery and improve the human condition through improved agriculture.

P-27

Setaria: Its Potential as a Model for C4 Grass Species. RICHARD S. NELSON[1], Hema Ramanna[1], and Andrew N. Doust[2]. [1]Plant Biology Division, Samuel Roberts Noble Foundation, Inc., 73401 and [2]Botany Department, Oklahoma State University, Stillwater, OK 74078. Email: rsnelson@noble.org

Species within the Poaceae (grass family) of the monocotyledonous plants include many important agricultural crops. These include rice, wheat, barley and oats that solely use the C3 photosynthesis pathway and maize, sorghum and sugarcane that use the C4 photosynthesis pathway. Multiple species considered for the biofuel industry are C4 grasses (e.g. *Miscanthus, Saccharum* and *Panicum* species, including *Panicum virgatum* or switchgrass). Although sorghum and, to a great extent, maize have been sequenced they are less related to switchgrass than *Setaria italica* (foxtail millet) and *Setaria viridis* (green millet). The sequences of these millets have been completed and will soon be publically released, and, in addition, these millets contain relatively small genomes, are diploid and generally self-pollinate, unlike switchgrass. These factors make foxtail and green millets very tractable model systems for switchgrass. *Setaria italica* has other positive attributes for gene function studies such as a large germplasm resource and the existence of a moderate

throughput, transient gene knockdown system using virus-induced gene silencing to allow the rapid analysis of gene function in reverse and forward genetic studies. Stable transformation has been reported for both *S. italica* and *S. viridis*, allowing greenhouse and field analysis of targeted modifications. Studies utilizing *S. viridis* also will benefit from its rapid generation time (circa 4 wk which is approximately half the time necessary for *S. italica*) and small size. This talk will provide further detail of the traits noted above and the current status of studies with *Setaria* as a model system for C4 grass improvement.

P-28

Geminiviral Vectors for Vaccine Antigen and Antibody Expression in Plants. HUGH S. MASON, Qiang Chen, Zhong Huang, Waranyoo Phoolcharoen, and Sun Hee Park. Biodesign Institute at Arizona State University, Tempe, AZ 85287-5401. Email: Hugh.Mason@asu.edu

Transient expression of recombinant proteins in plants using replicating viral vectors has benefited greatly from a growing body of work in the past decade. Advantages include high yields of recombinant protein obtained rapidly by *Agrobacterium-mediated* delivery of viral vectors to leaves. Bean yellow dwarf virus (BeYDV) is a geminivirus with a 2.7 kb, single stranded DNA genome that replicates in the nucleus of an infected plant cell. The only virus-encoded protein required for replication is Rep, the initiator protein. We developed BeYDV-derived expression vectors that contain one or more expression cassettes for proteins of interest. *Agrobacterium*-mediated delivery into leaves of *Nicotiana benthamiana* results in replication that greatly amplifies the recombinant DNA, which acts as a transcription template to produce high levels of mRNA. We have produced vaccine antigens, including Norwalk virus capsid protein and hepatitis B core antigen, at levels up to 1 mg per g of leaf mass. Moreover, multiple BeYDV replicons can be stacked in the same vector molecule, which enables the production of multi-subunit proteins. For example, monoclonal antibody (mAb) heavy and light chains, including the anti-Ebola virus mAb 6D8 was produced at 0.5 mg per g of leaf mass. The geminiviral replicon system is thus a highly efficient and robust system for protein production in plants.

P-29

Production of a Vaccine Candidate Using a Plant Transient Expression System. E. HIATT. Kentucky BioProcessing, 3700 Airpark Drive, Owensboro, KY, 42301. Email: ehiatt@kbpllc.com

Kentucky BioProcessing (KBP) is a service provider in the biotechnology industry specializing in the expression, extraction, purification and commercial scale production of proteins and other high value products from plants. KBP was awarded an agreement in 2010 from the Defense Advanced Research Projects Agency (DARPA) under the H1N1 Acceleration (Blue Angel) Program. This program funds efforts to produce effective interventions for pandemic influenza. As a participant in the Accelerated Manufacture of Pharmaceuticals (AMP) "live fire test" KBP was specifically challenged to produce 1 kg of a recombinant H1N1 vaccine candidate using a plant transient expression platform in a time period of 30 d. The platform is a plant based system that uses non-genetically modified tobacco plants as a host for the expression of a recombinant protein. A genetically modified tobacco pathogen containing a H1N1 gene was used to infect plants and after a period of incubation plants harvested to extract desired recombinant protein. The goal of the project was to demonstrate that plant based systems were scalable for rapid delivery of purified recombinant protein that meets all FDA requirements for human use. The duration of the total project was 12 mo and all cloning/molecular biology, process development, growth room construction, scaling of process, final production cycles and final release of product needed to be completed in this time frame. For this presentation all aspects of KBP's efforts will be discussed. To achieve the goals of this project 75 different genetic constructs were evaluated, hundreds of small scale purification experiments performed and an automated, climate-controlled growth environment with a capacity of 3 million individual plants was constructed, qualified and used to produce the biomass necessary to result in 1 kg of H1N1 vaccine candidate in 30 d.

P-30

Production of Vaccines and Human Therapuetics in the Chloroplast of *Chlamydomonas reinhardtii*. J. GREGORY, C. Jones, S. Mayfield. University of California - San Diego, Division of Biological Sciences, 2150 Bonner Hall, 9500 Gilman Dr., La Jolla, CA 92093-0322. Email: j1gregor@ucsd.edu, smayfield@ucsd.edu

Fuel, food, and biological products are all forms of chemical energy that are ultimately derived from photosynthesis. Over the last 100 yr we have utilized fossil fuels to drive unprecedented economic and agricultural growth, but in so doing we have released vast quantities of previously sequestered CO2 into the atmosphere, and it is now beginning to

impact our climate. In addition, fossil fuel reserves are finite, and we are starting to see the initial signs of the depletion of these reserves, including the rising cost of fuels and food. Together these factors have provided the impetus behind the development of renewable energy sources that can supplant fossil fuels while greatly reducing carbon emissions. Eukaryotic algae offer tremendous potential for the large scale production of biofuels and bio-products because they are efficient photoautotrophs, thus sequestering CO2 to produce biomass. We are developing the genetic tools to enable algae to produce biomass that can be harvested for bio-fuels, bio-products, and feedstock. To date we have successfully introduced biosynthetic enzymes to modify hydrocarbon biosynthesis, as well as a variety of genes that allow production of valuable protein co-products in micro-algae. The challenges, potential, and some early successes of utilizing the chloroplast of *C. reinhardtii* to produce a malarial transmission blocking vaccine and cancer therapeutics will be discussed.

P-31

Site-specific Integration of Transgenes in Soybean via Recombinase Mediated DNA Cassette Exchange. ZHONGSEN LI, Aiqiu Xing, Bryan P. Moon, Richard P. McCardell, Zhan-Bin Liu, Howard G. Damude, and S. Carl Falco. DuPont Agricultural Biotechnology, Experimental Station E353, Wilmington, DE 19880-0353. Email: zhongsen.li@cgr.dupont.com

A targeting method to insert genes at a previously characterized genetic locus to make plant transformation and transgene expression predictable is highly desirable for plant biotechnology. We report the successful targeting of transgenes to predefined soybean genome sites using the yeast FLP-*FRT* recombination system. First a target DNA containing a pair of incompatible *FRT* sites flanking a selection gene was introduced in soybean by standard biolistic transformation. Transgenic events containing a single copy of the target were retransformed with a donor DNA, which contained the same pair of *FRT* sites flanking a different selection gene, and a FLP expression DNA. Precise DNA cassette exchange was achieved between the target and donor DNA via recombinase mediated cassette exchange (RMCE) so that the donor DNA was introduced at the locus previously occupied by the target DNA. Following the same strategy, more transgenes can be stacked to the same site through more rounds of RMCE. A co-suppression gene cassette, designed to simultaneously silence two soybean genes omega-6 desaturase (*FAD2*) and thioesterase 2 (*TE2*) to improve the oleic acid (18:1) content, was first inserted by RMCE at a pre-characterized genomic site in soybean. Selected transgenic events were subsequently retransformed with the second DNA construct containing a *Yarrowia lipolytica* diacylglycerol acyltransferase gene (*DGAT1*) to help cells accumulate oleic acid in oil bodies (high oil), and three other genes, a *Corynebacterium* glutamicum dihydrodipicolinate synthetase gene (*DHPS*), a barley high lysine protein gene (*BHL8*), and a truncated soybean cysteine synthase gene (*CGS*) to improve the contents of essential amino acids lysine and methionine. Molecular characterization confirmed that all the four over-expression cassettes were successfully stacked to the *FAD2-TE2* co-suppression cassette by the second RMCE. Phenotypic analyses confirmed that all the transgenes conferred expected phenotypes

P-32

Design of Complex Multigene Cassettes - Insulator Sequnces for Quelling Promoter Cross-talk. T. M. KLEIN[1], J. Dull[1], A. Finch[1], S. Abbitt[2], and H. Yi[1,3]. [1]DuPont Agricultural Biotechnology, DuPont Experimental Station, Wilmington, DE 19880; [2]Pioneer Hi-Bred Int'l, Johnston, IA 50131; and [3]present address Syngenta, Research Triangle and Park, NC 27709. Email: Ted.M.Klein@Pioneer.com

Interactions between transgenes in a multigene cassettes can be unpredictable. Expression of a transgene can be suppressed by transcriptional interference from upstream genes or activated in an unwanted fashion by nearby enhancer elements. Although there are certain general rules that can help minimize these interactions, there remains a need for tools to increase the fidelity and reliability of transgene expression. Short DNA elements that block transcriptional interference and quell promoter cross-talk have been identified in animal and yeast systems. Although there is some evidence that these elements can function in plants, we attempted to isolate insulator elements from Arabidopsis. The presentation will describe the approach we took for identifying putative insulators from Arabiopsis and preliminary results showing the function of these elements in maize transgenics.

P-33

Multi-gene Constructs. P. CHRISTOU[1,2], G. Farre[1], C. Bai[1], S. M. Rivera[1], G. Sanahuja[1], T. Capell[1], and C. Zhu[1]. [1]Department of Plant Production and Forestry Science, ETSEA, University of Lleida, Av. Alcalde, Rovira, Roure 191, 25198 Lleida, SPAIN and [2]Institució Catalana de Reserca i Estudis Avançats, Passeig Lluís Companys 23, 08010 Barcelona, SPAIN. Email: christou@pvcf.udl.es

Effective methodology to co-introduce multiple transgenes into target crops is vital in order to generate plants with

more complex traits. Such traits might include the simultaneous enhancement of multiple agronomic or nutritional properties as well as a number of traits associated with alternative uses of crops for specific applications, such as molecular pharming, bioenergy and others. Direct DNA transfer allows simultaneous co-transformation of large numbers of unlinked transgenes. As a result of the mechanism of integration of such unlinked transgenes into the plant genome it is relatively straightforward to reconstruct and extend multiple related or unrelated biosynthetic pathways in one experiment. We will exemplify multi gene engineering using the carotenoid pathway in corn and rice to generate transgenic plants with enhanced nutritional qualities. The notion of multi-gene and multi-pathway engineering does not appear to be such a daunting task because of advances in the use of multi-gene constructs and the development of a more in depth understanding of mechanisms of integration of multiple unlinked transgenes through direct DNA transfer.

P-34

Targeted Modification of Plant Genomes. D. VOYTAS. Dept. of Genetics, Cell Biology & Development and Center for Genome Engineering, University of Minnesota, Minneapolis, MN 55455. Email: voytas@umn.edu

Sequence-specific nucleases are powerful tools for the targeted modification of plant genomes. As a founding member of the Zinc Finger Consortium, our group has worked collaboratively to develop efficient methods for the design of zinc finger nucleases (ZFNs) suitable for plant genome engineering. Using our zinc finger reagents, we have demonstrated targeted gene modification by homologous recombination in tobacco at frequencies exceeding 1% of transformed cells. We have also created targeted gene knockouts in Arabidopsis and soybean through targeted DNA cleavage and imprecise repair of the cleaved chromosomes by non-homologous end-joining. Such ZFN-induced mutations are germinally transmitted, enabling the recovery of Arabidopsis and soybean plants with mutations in genes of interest. More recently, we have worked collaboratively to develop methods to engineer the DNA binding domain of Transcription Activator-Like (TAL) effectors for targeted mutagenesis. When fused to FokI nuclease, the TAL effector DNA binding domain creates chromosome breaks at specific DNA sites. Reagents and protocols for the rapid assembly and testing of custom TAL Effector Nucleases (TALENs) have been developed, and we have used our custom TALENs to create mutations in human and Arabidopsis genes. Ongoing work is focused on fully assessing this platform for its potential for plant genome modification.

P-35

Regulation and Modeling of Lignin Biosynthesis. V. CHIANG. Forest Biotechnology Research Group, Department of Forestry and Environmental Resources. 840 Main Campus Drive, 2500 Partners II Bldg., Raleigh, NC 27606. Email: Vincent_Chiang@ncsu.edu

Lignin is a complex phenolic structural component of the secondary cell walls of all vascular plants. It is an irreversible end point of a major metabolic pathway in plant secondary metabolism. Lignin is fundamental to the adaptation of plants to land, the evolution of vascular transport and the resistance of plants to pests and pathogens. Lignin is a major barrier to the utilization of biomass for energy, for papermaking, and for forage digestibility due to the interaction of lignin with cellulosics in the plant cell secondary wall. We are conducting a systems biology study on regulation of lignin biosynthesis in wood formation. We seek to build models to quantitatively illustrate how the entire pathway is organized and regulated and to reveal regulatory and metabolic flux control mechanisms, leading to lignin quantity and structures. We use the model woody plant, *Populus trichocarpa* (Nisqually-1), and the systems approach including advanced quantitative methods of genomics, proteomics, metabolomics, biochemistry and structural chemistry. A perturbation strategy is used to systematically knock down the expression of all pathway and regulatory genes known to be involved in lignin biosynthesis during wood formation, and the effects on lignin biosynthesis (gene transcripts, proteins, metabolites, quantity and structures) analyzed using advanced genomic methods available. This information forms the foundation of statistics-based mechanistic modeling and lignin quantity/structural predictions for a quantitative model of lignin biosynthesis. Substantial data have been generated for gene-specific (amiRNA & RNAi) transgenic *P. trichocarpa*, enzyme kinetics, and stable-isotope-dilution based absolute quantitation of proteins and of metabolites. Details of systems data generation, data analyses and model development will be presented.

P-36

Over-expression of *Corngrass1* in Poplar Affects Morphology and Lignin Content and Composition. R. MEILAN[1], P. M. Rubinelli[1], G. Chuck[2], and X. Li[3]. [1]Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907; [2]Department of Plant Biology, University of California at Berkeley, Berkeley, CA 94720; and [3]Department of Biochemistry, Purdue University, West Lafayette, IN 47907. E-mail: rmeilanr@purdue.edu

The potential roles for microRNAs (miRNAs) in controlling plant development have been well studied in model annual species, but are poorly understood in perennials, particularly trees. The *Corngrass1* (*Cg1*) gene encodes a MIR156-class miRNA. In herbaceous species, *Cg1* is known to control the initiation of meristems and lateral organs. Plants in which it has been expressed constitutively produced multiple axillary branches, grew faster, contained less lignin, and were either sterile or exhibited delayed flowering. We over-expressed *Cg1* in poplar (genus *Populus*) under the control of the cauliflower mosaic virus 35S promoter. These transgenic plants had significantly greater branching and shorter internodes, and up to 30% less lignin. The severity of the phenotype was positively correlated with *Cg1* expression level. In addition, the syringyl to guaiacyl ratio (S/G) was lower in 35S::*Cg1* lines than in wild-type poplar or a control transgenic line lacking *Cg1* expression. We have demonstrated for the first time that over-expression in poplar of a MIR156-class miRNA has dramatic effects on its development, and demonstrated that miRNA over-expression represents a novel approach to altering lignin content and composition in poplar. It is yet to be determined whether MIR156 directly regulates lignin biosynthesis or if the observed lignin changes were indirect consequences of the developmental changes caused by *Cg1* over-expression. Nevertheless, plants expressing *Cg1* may have commercial value as a cellulosic feedstock for biofuel production and in the paper-manufacturing industry.

P-37

Develop Somatic Embryogenesis System for *Larix spp.* in China and the MicroRNAs Differentially Regulated Between Embryogenic and Non-embryogenic Callus Tissues. LIWANG QI, Suying Han, and Dong Wang. Research Institute of Forestry, Chinese Academy of Forestry, Beijing 100091, CHINA. Email: lwqi@caf.ac.cn

Since 1996, we have been conducting studies on somatic embryogenesis for several conifer species including *Larix spp*., *Picea spp*., *Pinus tabulaeformis*, *Pinus bungeana*, *Pinus armandi*, and *Pinus kesiva* var. *langbianensis*. Particularly, we have obtained several government grants for research on multiple aspects of somatic embryogenesis on *Larix*. For example, one of grants funded by government's "National High Technology Research and Development Programs of China (a.k.a. 863 program)" was to establish a mass propagation system based on the techniques developed in a small scale. We have developed a system that can produce 300–500 embryos with one gram of embryogenic callus in a solid culture system and 1,500 embryos with one gram of embryogenic callus in a liquid culture system. Through the process, we have developed the key technologies in the following areas: (1) mass propagation of early-stage embryos, (2) improving embryo quality and somatic embryogenesis frequency of *Larix spp*, (3) maturation and mass propagation of somatic embryos, (4) bioreactor liquid cell culture system, and (5) cryopreservation and utilization of the good embryogenic cell lines. Based on the successes, we believe that the technologies can be scaled up for operational production. In recent years, we studied the molecular signaling or regulation of somatic embryogenesis. By using our established somatic embryo development system in conifer species, for the first time, we have identified the differentially expressed miRNAs at key stages of embryogenic development of different cell lines and studied their biological functions of those differentially expressed miRNAs. We have especially interested in understanding the role of miRNAs on switching from embryogenic cells to non-embryogenic cells. To investigate the miRNA regulation underlying this detrimental transformation in Japanese Larch (*Larix leptolepis*), we compared miRNA expression profiles between embryogenic and non-embryogenic callus at days 3 and 14 after subculture. Four miRNA families dominated the 165 differentially expressed miRNAs between embryogenic and non-embryogenic callus. Of the four, miR171 was up-regulated, and miR159, 169 and 172 were down-regulated in the embryogenic callus. These four families are all abiotic stress induced miRNAs. This study represents the first report on the differential miRNA expression between embryogenic and non-embryogenic callus in plant, and thus provide important clue for further functional investigation.

P-38

Eucalyptus with Improved Pulping and Bioenergy Characteristics. MAUD HINCHEE[1], Will Rottmann[1], Mark Davis[2], and Shujun Chang[1]. [1]ArborGen, LLC, P.O. Box 840001, Summerville, SC 29484 and [2]National Renewable Energy Lab, Golden, CO 80401. Email: mahinch@ArborGen.com

ArborGen is a global leader in developing trees for short rotation, highly productive plantations. Along with the objectives to increase the biomass productivity of trees, we are targeting the wood formation process to improve in the accessibility and yield of cellulose for greater efficiency in pulping for paper production and for greater release of fermentable sugars in biofuel production. A highly productive tropical *Eucalyptus* hybrid, *E. grandis x E. urophylla*, has been engineered using several genes in the lignin biosynthesis pathway, and here we report on the resulting wood characteristics from field grown trees. The results

from 4-yr-old trees overexpressing coniferaldehyde 5-hydroxylase (Cald5H) showed that the ratio of syringyl (S) lignin to guaiacyl (G) lignin could be increased significantly without change in total lignin. Micropulping studies showed the increase in S:G ratio had significant positive effect on pulp yield and reduced chemical use. The promoter driving the Cald5H gene played a significant role in the effectiveness of the construct. Lignin reduction has been achieved via targeted downregulation of genes involved in lignin biosynthesis. Using RNAi constructs that targeted coumarate 4-hydroxylase (C4H) or 4-coumaroyl shikimate 3'-hydroxylase (C3H), transgenic lines were produced with strong lignin reduction as determined by *pyrolysis–molecular beam mass* spectrometry analysis. The C4H lines showed a significant increase in the amount of C6 sugars released during hot water pretreatment. Down regulation of C3H resulted in the greater reduction of lignin but had a modest effect on release of C6 sugars. These results reveal the potential to use biotechnology to not only improve the cellulose yield, but also to reduce the recalcitrance and increase the release of fermentable sugar at the same time. http://www.ArborGen.com