

## Plant Symposia

### P-1

The Vision of Biofuels and Biorefining in the US: Opportunities for Biomass. AL DARZINS. National Bioenergy Center, National Renewable Energy Laboratory, 1617 Cole Blvd. Golden, CO 80401. Email: al\_darzins@nrel.gov

Biomass resources run the gamut from corn kernels to corn stalks and cobs, from soybean and canola oils to animal fats, from prairie grasses to hardwoods, and even include microalgae. In the long run, we will need diverse technologies to make use of these different energy sources. Today, the most common technologies involve biochemical, chemical and thermochemical conversion processes. The long-term vision for biofuels at DOE involves integrating a number of conversion technologies into a biomass-based refinery, or “biorefinery.” Biorefineries could draw on a variety of biomass feedstocks and employ several conversion technologies to produce fuels, chemicals and other products. Current sources for commodity sugars, sugar-based chemicals and fuels production, for example, include, primarily, sugar cane, sugar beet, and cereal grains, especially corn and wheat. The potential volumes of commodity liquid transportation fuels (e.g., ethanol) that can be produced from these feedstocks, however, are rather limited and are not sufficient to have a significant impact on fuel markets. Therefore, expanding the existing sugars platform to include lignocellulosic biomass feedstocks - cellulosic residues from agricultural processing operations and forest products and management industries - is essential to be able to supply large enough volumes of renewable liquid transportation fuels to substantially lower dependence on imported petroleum. As with ethanol production from corn grain, biodiesel production from terrestrial vegetable oil crops has the potential of replacing only a small fraction of the roughly 60 billion gallons of petroleum diesel used annually in the US. Microalgae have emerged as another possible source of biomass (lipids or carbohydrates) that could substantially increase our nation’s ability to produce domestic biofuels in the long term. This talk will outline USDOE goals and strategies for bioethanol production and review the most recent lignocellulosic platform technology advances. In addition, it will also highlight the potential but also the challenges associated with algae.

### P-2

Breaking Barriers to Cellulose Ethanol: Role of Aqueous Pretreatments in Enzyme Modifications of Plant Cell Walls. M. LADISCH, N. Mosier and Mira Sedlak. Laboratory of Renewable Resources Engineering, Department of Agricultural and Biological Engineering and Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN 47907. Email: ladisch@purdue.edu

Cellulosic biomass feedstock is necessary for expanding a grain based, 42 million gallon daily production capacity to 170 million gallons per day (i.e., the goal of 30×30). Cellulosic biomass has a tremendous upside potential. Research on pretreatment, conversion and fermentation will improve cellulose ethanol yields. Land is available to grow cellulosic biomass over a widely distributed geographic area. Selection for drought and disease resistance of cellulosic biomass crops is possible and will help to expand the portfolio of consistent and reliable feedstock supplies for ethanol biorefineries. However, cellulosic biomass is more complex than corn because of the relatively large fraction of hemicellulose and lignin that it contains and because of the manner in which glucan, pentosan, and lignin is associated within the cell wall structure itself. Cellulosic biomass contains sugars “locked in a complex polymer composite exquisitely created to resist biological and chemical degradation” (Houghton, Weatherwax, Ferrell, 2006). The impact of cellulose pretreatment on enzyme hydrolysis will be discussed in the context of both cost of enzyme hydrolysis, capital investments required for the bioprocessing of cellulose to ethanol when such bioprocessing includes hemicellulose hydrolysis and fermentation of xylose to ethanol. An overview of the potential of cellulose derived ethanol in Indiana, and the economic and policy drivers that may impact the growth of cellulose ethanol will be discussed.

### P-3

Camelina: An Emerging Crop for Bioenergy. DUANE JOHNSON. Greta Plains Oil and Exploration, LLC, P.O. Box 2691, Bigfork, MN 59911. Email: camelinaguy@juno.com

*Camelina* (*Camelina sativa* L.) is an old world crop native to Scandinavia and north central Europe. Prior to development of oilseeds such as rapeseed, flax and sunflower, camelina was used extensively during the Bronze Age for cooking, cosmetics and as a lamp oil, earning it the name “gold of pleasure”. Camelina fell out of favor in the late 19th century due to the complexity of its oils and comparative low yield when compared to oilseeds receiving selection through plant breeding. That lack of care, however, is now making camelina a very desirable crop because of its low input requirements. The use of low inputs allows producers to increase net income per acre while reducing costs to manufacturers and consumers. Camelina research in Montana has been initiated to compare its potential to eight other species. Across a wide range of environments, camelina has performed well based upon input costs and yield. The emerging biodiesel industry is stimulating the development of camelina in the upper Great Plains as an industrial oilseed. Camelina has production characteristics of high interest: low fertility requirements, excellent cold-tolerance, resistance to weeds and insects and ease of harvest. The oil of camelina is very high in omega 3 fatty acids which typically would make it undesirable as a fuel but it also contains very high levels of effective antioxidants. The result is a durable biodiesel with a strong advantage over soybean and canola biodiesels because of its low cloud point and pour point. By using expeller-pressed oil and meal, the meal retains about 10% oil, high in omega 3 and tocopherols. Livestock trials with cattle, dairy, poultry and farmed fish have shown significant increases in omega 3s from camelina meal as opposed to control diets of soybean and flax.

#### P-4

The ABCs of Polysaccharide Gels. RENGASWAMI CHANDRASEKARAN. Whistler Center for Carbohydrate Research, Purdue University, West Lafayette, IN 47907. Email:chandra@purdue.edu

Polysaccharides are used as gelling agents, thickeners and viscosifiers in pharmaceutical, cosmetics and food applications. Some polysaccharide gels have a unique place in microbial and plant cell culture media. These hydrocolloids prefer to adopt helical structures. Their molecular shapes and assembly govern the macroscopic behavior. While X-ray fiber diffraction data and computer modeling reveal the molecular architecture, electron density maps help in locating the ordered water molecules and cations surrounding the helices. Agarose can exist as a single or double helix; curdlan forms a single or triple helix and derives gelling behavior through micelle formation. Alginate and

pectate (single helices), iota-carrageenan and gellan (double helices) are all ionic; neighboring helices communicate via water molecules, cations and the sulfate groups in carrageenan and carboxylate groups in alginate, pectate and gellan. The resulting junction zones are stronger with divalent than monovalent ions that reflect the observed gel strength. The side groups modify the molecular morphology of welan and rhamsan (in the gellan family) and alter the extent of helix-helix association leading to high viscosity rather than gelation. Guarana, xanthan and acetan are also good viscosifiers. Guarana favors a sheet-like structure stabilized by intrahelical hydrogen bonds between the galactosyl side groups and the main chain. Water molecules cement the sheets which control the rheology as a function of galactose/mannose ratio. However, gelation is observed when guarana complexes with xanthan or acetan; the onset of junction zones involving hybrid double helices causes this synergy.

#### P-5

Gelling Agent Modification for Large-Scale Axillary Shoot Multiplication and Somatic Embryogenesis of *Pinus radiata* for Commercial Forestry. DALE R. SMITH. MetaGenetics, 93 State Highway 30 Tikitere, Rotorua 3074, New Zealand. Email: dalesmith@xtra.co.nz

*Pinus radiata* is an important commercial plantation species in the Southern Hemisphere. Gelling agents are used for both in-vitro axillary shoot multiplication and somatic embryogenesis in tree improvement. Much research and development carried out is in the form of patents or industrial ‘trade secret’ and has not previously been published in the peer-reviewed scientific literature. Early micropropagation scale-up research for *P. radiata* at the New Zealand Forest Research Institute using Bacto Agar, purchased in 1 kilogram lots, revealed batch-to batch variations of in vitro growth. Research solved some of the problems with this product by altering laboratory practises. Later, gellan gums (for example Gelrite) and less expensive locally produced agars became available. Adapting to these products gave new insights into the mode of action of gelling agents in micropropagation. Open dishes of medium gelled with gellan gum or various brands of agar do not differ significantly in the rates of evaporative water loss to the atmosphere. Different gelling agents do vary significantly in the rate at which liquid is taken up into stacks of dried filter disks in a closed system. They also differed significantly in gel strength. Both of these properties were used to optimise plant growth on a range of gelled medium without induction of vitrification (hyperhydricity). Gellan gums and very pure agars will give optimal growth of *Pinus* sp shoots with the

addition of ‘anti-vitrification’ agents. We determined that alginate is a much more cost-effective anti-vitrification agent than patented products available on the market. For some species, adjustment of the sodium levels of the medium is necessary. For development and maturation for somatic embryos of all *Pinus* species, the optimal free liquid status of the medium must be attained through the use of water-vapour-permeable films since this fine level of control cannot be achieved by manipulation of gelling agent concentration alone.

#### P-6

A Review of Gelling Agents Used for Plant Tissue Culture: Their Sources and Characteristics. K. C. TORRES, G. R. Seckinger, and E. D. Burdick. PhytoTechnology Laboratories, PO Box 12205, Shawnee Mission, KS 66282-2205. Email: tech@phytotechlab.com

Historically, agar has been the gelling agent of choice since the early days of plant tissue culture. Agar is derived from two marine seaweed genera, *Gelidium* and *Gracilaria*. Typically agar is only replaced by another agent when a problem is encountered. Approximately 20 years ago, gellan gum was introduced into the plant tissue culture market by CP Kelco with their product, Gelrite. Since then several manufacturers have started producing comparable products. Gellan gum quickly became a popular alternative due to its clarity and lack of “growth inhibitors” associated with agars. However, the use of gellan gum quickly revealed its own problems with some plants, that of hyperhydricity (which was originally termed vitrification). Carrageenan is another gelling agent that has gained popularity and is also derived from marine algae, primarily various species of red seaweeds. These and other gelling agents will be discussed in terms of their sources and characteristics.

#### P-7

Ecdysone Receptor Gene Switch Technology for Inducible Gene Expression in Plants. S. R. PALLI, V. S. Tavva, A. K. Singh, R. D. Dinkins, and G. B. Collins. Departments of Entomology, and Plant and Soil Sciences, University of Kentucky, Lexington, KY and USDA-ARS-FAPRU, Lexington, KY. Email: rpalli@uky.edu

Inducible gene regulation systems based on specific chemicals have many potential applications in agriculture and in the basic understanding of gene function. As a result several gene switches have been developed. However, the properties of the chemicals used in most of these switches

make their use limited to research purposes. An ecdysone receptor gene switch is one of the best inducible gene regulation systems available, because the chemical, methoxyfenozide required for its regulation is registered for field use. An EcR gene switch with a potential for use in large-scale field applications and its applicability to a variety of plant species has been developed by adopting a two-hybrid format. In a two-hybrid switch format, the GAL4 DNA binding domain (GAL4 DBD) was fused to the ligand binding domain (LBD) of the *Choristoneura fumiferana* ecdysone receptor (CfEcR); and, the VP16 activation domain (VP16 AD) was fused to LBD of *Locust migratoria* retinoid X receptor (LmRXR) or *Homo sapiens* retinoid X receptor (HsRXR). Upon application of methoxyfenozide, the heterodimer of these two fusion proteins transactivates the luciferase reporter gene placed under the control of multiple copies of cis acting elements and a minimal 35S promoter. The sensitivity of the CfEcR gene switch was improved from micromolar to nanomolar concentrations of ligand by using the CfEcR:LmRXR two-hybrid combination and a reduction in the background expression levels was achieved by using the CfEcR:HsRXR two-hybrid combination. The performance of EcR gene switch was improved further using Hs-LmRXR chimeras and/or CfEcR mutants. The efficiency of EcR gene switches in inducing the target gene expression was also tested in functional genomic studies by regulating the expression of a *Superman*-like single zinc finger protein 11 (ZFP11) gene in both *Arabidopsis* and tobacco plants. We have also carried out microarray analysis of gene expression in *Arabidopsis* plants containing switch components and determines that neither gene switch components nor chemical ligands cause significant changes in gene expression.

#### P-8

Small RNA Pathways in Plants. ALLISON MALLORY, Taline Elmayan, and Hervé Vaucheret. Laboratoire de Biologie Cellulaire, Institut Jean-Pierre Bourgin, INRA, 78026 Versailles Cedex, FRANCE. Email: amallory@versailles.inra.fr

MicroRNAs (miRNAs) and short interfering RNAs (siRNAs) are involved in a variety of phenomena that are essential for genome stability, development and adaptive responses to biotic and abiotic stresses. Their mode of action also is diverse. They guide DNA elimination during the formation of the macronucleus in protists and heterochromatin assembly in fungi and plants. They target endogenous mRNAs for cleavage and translational repression in plants and animals, and protect both plant and animal cells against virus infection through an RNA-based immune system. They also control the movement of transposable elements

at the transcriptional and posttranscriptional level in plants and animals. Plants contain more ARGONAUTE (AGO), DICER-LIKE (DCL), DOUBLE-STRANDED RNA BINDING (DRB) and RNA-DEPENDENT RNA-POLYMERASE (RDR) proteins than other eukaryotes, resulting in increased small RNA network complexities. The role of these proteins in the different plant small RNA pathways will be presented.

#### P-9

A Genome-Wide View of Small RNAs in *Arabidopsis thaliana*. E. J. CHAPMAN, K. D. Kasschau, N. Fahlgren, M. D. Howell, C. M. Sullivan, J. S. Cumbie, S. A. Givan, S. R. Grant, J. L. Dangl, and J. C. Carrington. Center for Genome Research and Biocomputing and Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331 and Department of Biology, University of North Carolina, Chapel Hill, NC 27599. Email: chapmael@cgrb.oregonstate.edu

Eukaryotes contain a diversified set of small non-coding RNA-guided pathways that control the expression of genes, repeated sequences and viruses at the transcriptional and posttranscriptional levels. MicroRNAs (miRNAs) generally function in trans as negative regulators through base-pair interactions with mRNAs. Trans-acting siRNAs (tasiRNAs) occur in plants, and function like miRNAs as posttranscriptional negative regulators of target transcripts, but form through an RNA-dependent RNA polymerase (RDR)-based mechanism in which precursor transcripts are converted to dsRNA. siRNAs originating from dsRNA by bidirectional transcription, extended foldbacks with perfect complementarity or RDR-based mechanisms can also guide transcriptional silencing in which a locus adopts heterochromatic features, including dense cytosine methylation and histone modifications associated with silent chromatin. We aim to understand the functional roles of these classes of small RNAs in *Arabidopsis*. Using deep sequencing technology and computational approaches, genome-wide distribution, phasing patterns, and evolutionary conservation of *Arabidopsis* small RNAs were analyzed. These data revealed evidence for the existence of nonconserved miRNAs in *Arabidopsis* and for the relatively frequent birth and death of *MIRNA* genes. Results also suggested regulation of highly repeated sequences and rapidly expanding gene families through deployment of siRNAs and tasiRNAs, respectively. We propose that plants may use a variety of RNA-based silencing mechanisms to suppress invasive elements and tame the effects of rapid gene expansion.

#### P-10

Micropropagation as a Tool in Domestication of Medicinal Crops. RITA M. MORAES<sup>1</sup>, Ana M.S. Pereira<sup>2</sup>, Bianca Bertoni<sup>2</sup>, Antonio L. Cerdeira<sup>3</sup>, and Ikhlas Khan<sup>1</sup>. <sup>1</sup>National Center for Natural Products Research, The University of Mississippi, University, MS, 38655; <sup>2</sup>University of Ribeirao Preto, Av. Costabile Romano, 2201, Ribeirao Preto, SP, 14.096-380, BRAZIL; and <sup>3</sup>Brazilian Department of Agriculture, Embrapa/Environment, C.P. 69, Jaguariuna, SP, 13820-000, BRAZIL. Email: rmoraes@olemiss.edu

Micropropagation of medicinal species with broad applications varying from metabolite production to breeding for high yielding (elite) plants has been described by many researchers. During the past decade, we at the National Center for Natural Products Research (NCNPR) have engaged in a program called Medicinal Crops for Small Farmers. Tissue culture has been used as tool to select and maintain elite plants with anticancer and immune stimulatory properties. *Podophyllum peltatum* and *Echinacea* are examples of American species that are being developed as crops for US farmers. In the pursuit of our goal, NCNPR has collaborated with other institutions broadening our experiences. Together with two Brazilian institutions, The University of Ribeirao Preto and EMBRAPA (Brazilian Department of Agriculture Research Service/Environment), micropropagation protocols were developed for producing elite plants. In addition, a germplasm bank of micropropagated medicinal plants native to the Brazilian savanna (Cerrado) was established. Cerrado is a highly depleted biome and has been considered a global hot spot of biodiversity. This discussion will focus on the importance of micropropagation as an important technique for aiding research programs on *ex situ* conservation, drug discovery and drug development.

#### P-11

Micropropagation of Novel Crops for Phytochemical Properties. HSIN-SHENG TSAY. Institute of Biotechnology, Chaoyang University of Technology, Wufong, Taichung 41349, TAIWAN. Email: hstsay@cyut.edu.tw

The plant kingdom provides a wide variety of natural products with diverse chemical structures and a vast array of biological activities. Over the years, many of these bioactive compounds have found applications in the health

sciences and today medicinal plants constitute an important part of the global economy. During the past decade, many investigations into the biosynthetic capabilities of various cell cultures have been conducted by plant scientists and microbiologists worldwide. Different strategies, involving in vitro systems, have been extensively studied to improve the production of biologically active secondary metabolites. At present, advances in tissue culture, such as improvement in genetic engineering, specifically, transformation technology, have opened new avenues for the high-volume production of pharmaceuticals, nutraceuticals and other beneficial substances. This presentation will focus on the importance of cell culture methods in the production of some of these biologically active secondary metabolites. In addition, micropropagation and cell culture protocols to produce biologically important secondary metabolites for a few medicinal plant species from Taiwan will be outlined. Taiwan's abundant botanical resources have provided the country with a reputation as a natural botanical garden. We have successfully established micropropagation procedures, and cell culture methods to produce imperatorin from *Angelica dahurica*, corydaline and tetrahydropalmatine from in-vitro-grown tubers of *Corydalis yanhusuo*, alkyl ferulates from *Dendrobium tosaense*, diosgenin from *Dioscorea doryophora*, gentipicro-12.2 and swertiamarin from *Gentiana*, anthraquinones from *Polygonum multiflorum*, cryptotanshinone from *Salvia miltiorrhiza*, harpagoside from *Scrophularia yoshimurae*, anthocyanins from *Solanum melongena*, and paclitaxel from *Taxus mairei*.

#### P-12

Spearmint Plantlet Culture System as a Means to Study Secondary Metabolism In Vitro. B. TISSERAT, M. Berhow and S. F. Vaughn. New Crops Processing Unit, U.S. Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, Peoria, IL 61604. Email: tisserbh@ncaur.usda.gov

The production of phytochemicals in vitro has been obtained from a variety of tissue types and organs. A plantlet culture system offers a means to study whole plant growth and development in a miniature scale and their corresponding phytochemical production. Plantlets resemble their in vivo counterparts both physically and chemically. A plantlet system that semi-isolates the shoot from the root offers a means to study exogenous media and atmospheric effects more critically. In such systems, the roots act indispensably

in the transport of exogenous nutrients to the foliage. The foliage in turn is now free from any immediate contact with media and displays more "typical foliage behavior". Such systems should be considered valuable aids in understanding the mechanisms of how to achieve higher secondary metabolism in vitro. Spearmint plantlets readily manifest terpenoids and phenolics in vitro. Although these compounds are produced from different pathways they respond similarly to the same physical and nutritional treatments. Carbon, particularly, whether added atmospherically or through exogenous media additions, plays a significant role in secondary metabolic production. Plantlet culture may be considered an intermediate step between shoot cultures and seedlings germinated in soil. Information gained from these study systems can then be employed toward enhancing of secondary metabolism both in vitro and in vivo.

#### P-13

Regulatory Perspectives: Comparing Traditional Breeding vs Genetic Improvement. SUSAN C. MACINTOSH. MacIntosh & Associates, Inc., Saint Paul, MN 55116. Email: macintosh-associated@comcast.net

During the 1940s, hybrid corn replacing open pollinated varieties in one of the most sudden technology shifts ever observed in agriculture. Hybrid corn was vastly superior to open pollinated varieties, demonstrating greater than 20% yield enhancement and the one of the first examples of the benefits of hybrid vigor. Despite the large changes in genetic composition, no one was concerned about the safety of hybrid corn and no safety studies or registration were required. That same type of technology shift was recorded 50 years later, with the introduction of crops improved through biotechnology. In this case, only one or a few genes were introduced, relatively minor genetic changes as compared to hybrid corn. Yet regulatory oversight was extensive, requiring large safety data packages and reviews by Food & Drug Administration, U.S. Department of Agriculture and in some cases, for Bt crops, the U.S. Environmental Protection Agency. International regulatory requirements were even more burdensome. New crop plants are produced in a number of different ways; by traditional crop breeding, by chemical or mechanical mutation, and by introducing new traits through genetic improvement using rDNA techniques. The contrasting regulatory oversight will be discussed related to these traditional and more modern genetic improvement techniques.

**P-14**

Comparative Case Studies - Differential Hurdles to the Same Trait Produced Conventionally vs Transgenically. J. E. MAYER. Campus Technologies, Stefan-Meier-Str. 8, Freiburg 79104 GERMANY. Email: jorge.mayer@goldenrice.org

Industrial-scale utilization of transgenic crops has successfully passed the 10-year and 100m-ha marks, following the steepest adoption curve of a new agricultural technology in history. But, at the same time, regulatory requirements have not gone through a learning curve that allows public institutions and developing countries to fully enjoy a technology that has the potential to address many problems in agriculture and health in a custom-tailored fashion. Agriculture as such is one of the most environment-intrusive activities carried out by man, with conventional crop cultivation causing known environmental damages. A technology capable of reducing insecticide use by 90 percent, as with Bt cotton in Australia, or leading to better soil conservation through no-tillage farming, deserves the attention necessary to adapt regulatory regimes to the accumulated knowledge base, taking into consideration the beneficial aspects for society. The development of transgenic crops has been accompanied by intensive risk assessment, especially concerning their interaction with the environment. One outcome of these studies shows that Bt crops have fewer side effects on non-target organisms than most insecticides being currently used. Notwithstanding such clear-cut findings, the costs attached to fulfilling cumbersome regulatory requirements are prohibitive, thus penalizing a method, and not a product. More than two-thousand new crop varieties generated over the years by radiation have been approved based simply on phenotypic evaluation, fully ignoring the fact that radiation causes highly unexpected chromosomal rearrangements. Meanwhile, deployment of a transgenic product like Golden Rice, with the potential to notably help reduce mortality and morbidity in micronutrient-deficient developing countries, may be delayed by 5-10 years or completely blocked, because of the special treatment given to transgenic crops, based on a wrong interpretation of the precautionary principle. This presentation is an appeal to policy makers to promote an experience-based regulatory system.

**P-15**

Impact of Conventional Modification the Plant Genome: How Regulations Ignore the Largest Changes. WAYNE PARROT. University of Georgia, Center for Applied Genetic Technologies, 111 Riverbend Road, Athens, GA 30602. Email: wparrott@uga.edu

When regulations covering transgenic plants were written, plant genomes were viewed as stable and fixed, and transgenes as new and novel. Since then, the view and understanding of the plant genome has changed dramatically based on genomic information that is rapidly accumulating, and which will be highlighted during the seminar. The plant genome is now understood to be fluid and dynamic, undergoing small and large-scale rearrangements- including the creation of new genes- on a human time scale. Many assumptions, such as collinearity (all members of the same species have the same genes), are no longer considered true for some species. It is now evident that conventional plant breeding can create far larger changes in the genome than a transgene can. Yet, conventional breeding is a safe technology, with long history of safe use, and rightfully therefore, almost devoid of regulations. When viewed in this context, many regulations governing transgenic crops are scientifically indefensible.

**P-16**

Genetic Engineering of Stress Tolerance and Turf Quality in a Low-input Turfgrass (*Paspalum notatum* Flugge). F. ALTPETER<sup>1</sup>, V. James<sup>1</sup>, S. Sandhu<sup>1</sup>, M. Agharkar<sup>1</sup>, H. Zhang<sup>1</sup>, W. Fouad<sup>1</sup>, X. Xiong<sup>1</sup>, P. Lomba<sup>1</sup>, G. Luciani<sup>1</sup>, J. Celedon<sup>1</sup>, A. Blount<sup>2</sup>, M. Gallo<sup>1</sup>, R. Meagher<sup>3</sup>, D. Wofford<sup>1</sup>, K. Kenworthy<sup>1</sup>, T. Sinclair<sup>1</sup>. <sup>1</sup>Agronomy Department, PMCB, Genetics Institute, University of Florida - IFAS, Gainesville, FL 32611; <sup>2</sup>Agronomy Department, North Florida Research and Education Center, University of Florida - IFAS, Marianna, FL 32446; and <sup>3</sup>USDA, ARS, CMAVE, Gainesville, FL 32608. Email: faltpeter@ifas.ufl.edu

Bahiagrass is one of the most important forage and turf grasses in the southeastern United States and in subtropical regions around the world. We recently developed an efficient genetic transformation protocol for the commercially important bahiagrass cultivar 'Argentine'. Biolistic transfer of minimal transgene expression cassettes without vector backbone supported stable expression of multiple transgenes with the potential to enhance turf quality, abiotic or biotic stress tolerance. Risk assessment and risk management research are integral components of this grass biotechnology program. Controlled environment and field data will be provided, describing the performance of transgenic bahiagrass plants in respect of herbicide resistance, drought tolerance, cold tolerance, insect resistance and turf quality in comparison to wildtype. Data describing the frequency and outcome of pollen-mediated intraspecific gene flow from transgenic apomictic 'Argentine' bahiagrass to wild type diploids under field and greenhouse conditions will also be presented.

**P-17**

Transgenic Approaches to Improve Quality and Abiotic Stress Tolerance in Forage Crops. ZENG-YU WANG, Xiaofei Cheng, Xuefeng Ma, Jiyi Zhang, Jeremy Bell, Yaxin Ge, Elane Wright, Yajun Xi and Xirong Xiao. Forage Improvement Division, The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401. Email: zywang@noble.org

Forage grasses and legumes contribute extensively to sustainable agriculture. We have established genetic transformation systems for a number of important forage species including tall fescue (*Festuca arundinacea*), switchgrass (*Panicum virgatum*), bermudagrass (*Cynodon dactylon*), Russian wildrye (*Psathyrostachys juncea*), darnel ryegrass (*Lolium temulentum*), zoysiagrass (*Zoysia japonica*), alfalfa (*Medicago sativa*), white clover (*Trifolium repens*) and *Medicago truncatula*. The target agronomic traits are forage quality, drought tolerance and phosphate uptake. Forage quality, particularly digestibility, is a limiting factor for animal productivity. We cloned cDNAs of major enzymes involved in lignin biosynthesis and produced transgenic tall fescue plants with antisense and RNAi constructs. Greatly reduced mRNA levels and significantly decreased enzymatic activities were found in some transgenic lines. The transgenic tall fescue plants had reduced lignin content, altered lignin composition and increased dry matter digestibility. Drought tolerance is an important trait for improvement in perennial forages. We characterized novel ERF transcription factor genes (*WXP1* and *WXP2*) from the model legume *M. truncatula*. Overexpression of *WXP1* led to a significant increase in cuticular wax loading on leaves of transgenic alfalfa. Transgenic leaves showed decreased water loss and reduced chlorophyll leaching. Transgenic alfalfa plants with increased cuticular waxes showed enhanced drought tolerance. Phosphorus is immobile and often deficient in pasture soils. Improving phosphate uptake in plants is an economic way to increase forage production. We cloned and characterized a constitutive promoter, two root-specific promoters, a novel phytase gene and a purple acid phosphatase gene from *M. truncatula*. Transgenic expression of the phytase gene or the purple acid phosphatase gene in Arabidopsis and white clover led to significant improvement in organic phosphorus uptake and plant growth. The results showed that the transgenic approach has great potential for improving plant organic phosphorus utilization and for phytoremediation.

**P-18**

Field Evaluation of Alfalfa Lines Down Regulated for Key Enzymes in the Lignin Biosynthetic Pathway. STEPHEN J. TEMPLE. Forage Genetics International, N5292 South

Gills Coulee Road, West Salem, WI, 54669. Email: stemple@foragegenetics.com

Lignification of secondary cell walls during plant development is a major factor limiting forage digestibility and thus animal performance. Lignins are complex phenolic polymers which are associated with the polysaccharides of the cell wall in specific plant cells primarily in mature stems. In alfalfa, the lignin polymer comprises guaiacyl (G) units and syringyl (S) units. Several recent studies have demonstrated that transgenic plants with down regulated caffeic acid 3-O-methyltransferase (COMT) and caffeoyl CoA 3-O-methyltransferase (CCOMT) had reduced lignin content and altered lignin subunit composition. These studies utilized antisense mediated technology to achieve gene suppression. While these studies have been successful in down regulating the target gene and producing transgenic alfalfa with significantly reduced lignin levels the efficiency is limited. Typically only 5-10% of the transgenic events in these studies exhibited significant levels of down regulation of the target enzyme and corresponding transcript and under field conditions the phenotype has not always been stable. This limits our ability to identify plants that are suitable for commercial development. RNA interference technology offers a powerful methodology for efficiently down regulating genes in the lignin biosynthetic pathway. RNAi constructs targeting COMT and CCOMT were introduced into alfalfa via agrobacterium-mediated plant transformation and the resulting transgenic plants were evaluated for levels of the target enzymes and their effect on lignin content and composition. Enzyme activities were reduced up to 85% of the corresponding wild type level with the majority of events tested exhibiting at least 50% down regulation. Data on the lignin content and compositional analysis on these plants grown under greenhouse conditions will be presented. In 2005 and 2006 progeny (F1 families) from 20 of the COMT and 20 of the CCOMT down regulated events were evaluated under field conditions at three locations: West Salem WI, Prairie du Sac WI and Ardmore OK. The plants were evaluated for a variety of agronomic and quality traits. Under field conditions the elite COMT and CCOMT down regulated events contained 15-20% less lignin which resulted in a significant increase in digestibility as measured by Near Infrared Spectroscopy (NIRS). The phenotype was found to be stable over environments and over the two years of the study. A dairy feeding proof of concept study is planned for 2007 to see if the increased forage digestibility in this material results in an increase in milk production.

**P-19**

Transgenic Tall Fescue for Fungal Disease Resistance and Transgenic Perennial Ryegrass for Induced Self-elimination. RONGDA QU. Dept of Crop Science, North Carolina State

University, Raleigh, NC 27695-7620. Email: rongda\_qu@ncsu.edu

Brown patch (*Rhizoctonia solani*) and gray leaf spot (*Magnaporthe grisea*) are two severe diseases for turf-grasses. Introduction of a bacteriophage T4 lysozyme gene, a truncated frog dermaseptin SI gene, or an alfalfa glucanase *AGLU1* gene into tall fescue conferred significant resistance to both pathogens. Lesion size was reduced by about 70% or more in resistant transgenic plants. Transgenic tall fescue plant with a rice R gene against *Magnaporthe grisea* (rice blast) was also highly resistant to turfgrass isolates of the pathogen. To help solve summer persistence problem, an *E. coli argE* gene was introduced into perennial ryegrass. Transgenic plants expressing the gene converts a non-toxic pro-herbicide, N-acetyl-PPT, to herbicide and can thus be selectively eliminated.

## P-20

Overview of In Vitro Secondary Metabolite Research - Challenges and Opportunities, Case Study of *Catharanthus roseus*. SYLVIA MITCHELL. Lecturer, Biotechnology Centre, 2 St. John's Close, University of the West Indies, Kingston 7, JAMAICA. Email: sylvia.mitchell@uwimona.edu.jm

Plant cell culture technologies were introduced at the end of the 1960s as a possible tool for studying and producing plant secondary metabolites. Sophisticated in vitro systems have since been devised with the objective of improving the production of secondary plant compounds including the use of various plant types (cell culture, callus, hairy roots, shoot cultures), media (including hormones, elicitors) and containers (bioreactors). Commercial successes resulting from this early research include the production of shikonin (a 750 L tank cell suspension in 14 d produced the same amount as obtained from the yield of a 176,000 m<sup>2</sup> field). More recent successes are based on a better biochemical and molecular understanding of the regulatory and metabolic pathways underlying the biosynthesis of secondary metabolites including the effect of elicitors (such as cytokinin, ethylene, salicylates, jasmonates), use of new plant types (eg somatic embryos and direct culture of intact plant leaves), and by improvement of reactor facilities. The emergence of recombinant DNA technology has opened a new field with the possibility of directly modifying the expression of genes related to biosyntheses - plant metabolic engineering. Molecular techniques such as RT-PCR, cDNA RFLP, in situ RNA hybridization, immunolocalization and laser-capture micro-dissection are increasingly being used for pathway analysis, linking

transcript profiling to metabolic profiling, and for the identification of suitable plant and cell types. The challenge is: to devise a system that can consistently and continually produce large quantities of select natural chemicals at a lower cost than can be produced in vivo. There are many opportunities including those that are more well-known (eg *Catharanthus roseus* [vincristine, vinblastine from the leaves, and ajmalicine, serpentine from the roots], *Lithospermum erythrorhizon* [shikonin], *Vanilla planifolia* [vanillin] and *Crocus sativus* [saffron, crocin, crocetin, picrocroan, safranal] and natural chemicals whose bioactivity has recently been elucidated (eg spirit weed [*Erygium foetidum*] and guinea hen weed [*Petiveria alliacea*]) for many different products (eg flavors, pigments, pharmaceutical products, cosmetic products, and biopesticides) and for bioconversions. A case study of *Catharanthus roseus* in light of the above challenges and opportunities will be discussed.

## P-21

Potential for In Vitro Manipulation and Production of Tropical Secondary Metabolites. SYLVIA MITCHELL. Lecturer, Biotechnology Centre, 2 St. John's Close, University of the West Indies, Kingston 7, JAMAICA. Email: sylvia.mitchell@uwimona.edu.jm

Why tropical secondary metabolites? The tropical region is home to most of the world's biodiversity. Seventy per cent of the world's species is found in just 12 countries: Australia, Brazil, China, Colombia, Costa Rica, Ecuador, India, Indonesia, Madagascar, Mexico, Peru and the Democratic Republic of Congo. Tropical regions alone support two-thirds of the estimated 250,000 plant species worldwide. Overall, tropical rainforests are thought to contain 50 to 90% of all species. The Caribbean 'hotspot' alone, has 2.3 per cent of the world's endemic plants in 263,535 km<sup>2</sup> of land area. Of the 12,000-12,500 vascular plants in the Caribbean 'hotspot', 58 per cent are endemic. In Jamaica alone, 366 of these plant species have been identified as medicinal while Chinese herbal medicine, as another example, uses 11,470 documented plant species. While there is still no comprehensive list of the number of secondary metabolites (or associated bioactivity) present in tropical plants, there are several websites that hint at the wide range of natural chemicals, which represent a staggering potential for a wide range of uses, for example, as nutraceuticals, phytochemicals, pharmaceuticals, preservatives, and biopesticides. In vitro systems can be used to screen for such bioactivity. The advantage of in vitro manipulation include: all-year round production of constitutively and elicitor-only produced secondary metabolites, production of sufficient secondary

metabolite for pre-clinical trials, suitability for molecular studies including elucidation of metabolic pathways, possibilities for bioconversion, metabolic engineering, and use of transgenic plants for novel uses. These systems are most useful for complex chemicals that have unique biological activity that occur in difficult-to-grow/difficult-to-multiply plants in low concentrations. Many tropical secondary metabolites have been produced *in vitro* using cells, hairy roots, shoot cultures, biofilms and somatic embryos with manipulation of media and other culture conditions resulting in some cases to 800x the production levels possible *in vivo*. *In vitro* biopharming from tropical plants is especially interesting due to the wide biodiversity and increasing biotechnological knowledge of these plants while *in vitro* systems can be developed using lessons learnt from existing best practices - for single secondary metabolite production but also for multiple secondary metabolite synergistic mixtures. The advantage of working with tropics is that there is plenty of room for research (many plants and many tropical diseases), but also many excellent tropical institutions to work with so we no longer have to work with plants that grow within our borders. In this regard, *in vitro* systems for such biofarming activity is of interest to both temperate and tropical countries, and both have important roles to play if such potential is to be finally realized.

#### P-22

Controlled Environment Production: Key to Consistency and Efficacy of Plant Medicinal Metabolites. PRAVEEN K. SAXENA. Department of Plant Agriculture, University of Guelph, Guelph, Ontario, CANADA, N1G 2W1. Email: psaxena@uoguelph.ca

In their natural environment plants consistently face threat of extinction from microbes, insects, mammals, and other environmental factors. Plants synthesize, accumulate and use a bewildering range of secondary metabolites as part of their overall defense strategy and many of these metabolites have been used as medicines for centuries. The secondary metabolite production of medicinal plants is affected by plant genetics and environmental conditions of cultivation, harvesting, processing and distribution. The widespread occurrence of chemical variability and compromised quality of medicinal plants often produce inconsistent results in clinical trials. Thus, the up-coming legislations requiring consistency and efficacy in many parts of the world would change how plant-based medicines are developed, manufactured, and marketed. This presentation illustrates the importance of chemical profiling of medicinal plants and describes an integrated process referred to as "Optimum Medicine Technology" for safe and effective production of

secondary metabolites of medicinal importance. The Optimum Medicine technology employs *in vitro* cell culture and controlled environment greenhouse production systems for the selection and seasonally independent propagation of elite lines with specific, consistent levels of medicinal metabolites with minimum contamination. Several unique lines of medicinal species such as Saint John's wort, Echinacea, and Scutellaria were chemically profiled using standard and emerging metabolomics techniques and successfully tested using *in vitro* bioassays and animal model systems. The Optimum Medicine technology provides a model for the development of novel medicinal products with consistent chemistry and medicinal efficacy.

#### P-23

Crop Transformation: the Next Ten Years. M. B. SAINZ. Syngenta Biotechnology, Inc., 3054 E. Cornwallis Rd., Research Triangle Park, NC 27709. Email: manuel.sainz@syngenta.com

Advances in crop transformation paved the way for the development of genetically modified (GM) crops that are revolutionizing agriculture. Global acreage of the major GM crops (maize, soybean, cotton, canola) continues to increase at a high rate, most recently at 13% per annum in 2006. Where GM technology becomes available, adoption by farmers is rapid and driven by reduced production costs. GM crops provide environmental benefits, including lower pesticide use and soil erosion. Extensive testing for regulatory approval ensures the safety of current and future GM crops for human consumption. Currently, GM product offerings are limited principally to herbicide tolerant and insect resistant crops. In addition to improved versions of these products, the next ten years will see a host of novel GM crop offerings. Complex agronomic traits such as drought tolerance will be engineered into crop plants. The first GM crops with specific benefits for the consumer or processor will also be introduced. Such crops may have improved characteristics for human food, animal feed and biofuel production applications, extending the benefits of this technology to segments of society beyond the farm.

#### P-24

Molecular Dissection of Embryogenesis in Higher Plants. JOHN J. HARADA<sup>1</sup>, Sandra L. Stone<sup>1</sup>, Siobhan A. Braybrook<sup>1</sup>, Soomin Park<sup>1</sup>, Robert L. Fischer<sup>2</sup>, and Robert B. Goldberg<sup>3</sup>. <sup>1</sup>Section of Plant Biology, College of Biological Sciences, University of California, One Shields Avenue, Davis, CA 95616; <sup>2</sup>Department of Plant and

Microbial Biology, University of California, Berkeley, CA 94720; and <sup>3</sup>Department of Molecular, Cell and Development Biology, University of California, Los Angeles, CA 90024-1606. Email: [jjharada@ucdavis.edu](mailto:jjharada@ucdavis.edu)

Plants make embryos through a number of developmental pathways. The most common is zygotic embryogenesis initiated by the fertilization of the egg cell of the female gametophyte with a sperm cell. The zygote then undergoes a series of differentiation events, leading to the formation of a mature embryo. Somatic cells can be induced to undergo embryogenesis, generally as a result of hormone treatments. Microspore cells can be diverted by heat shock treatments from their normal pathway of pollen development to give rise to haploid embryos through microspore embryogenesis. Apomixis describes a suite of processes in which various cells of the ovule undergo adventitious embryogenesis. All of these different forms of embryos go through similar morphological pathways of development. However, the cellular processes that cause these cells to change their fate and undergo embryonic pathways are not known. To gain insight into the control of embryo development, we are studying transcription factors encoded by Arabidopsis *LEAFY COTYLEDON (LEC)* genes, *LEC1*, *LEC2*, and *FUSCA3 (FUS3)*. These transcription factors are central regulators of embryogenesis. They are required to maintain embryonic cell identity and specify cotyledon development early in embryogenesis and initiate maturation programs during late embryogenesis. Ectopic expression of each of these genes causes vegetative and reproductive organs to acquire embryonic characteristics and initiate somatic embryo formation. In this talk, our studies to understand the roles of the LEC transcription factors in controlling embryo development will be discussed.

#### P-25

A New Generation of GM Plants. T. WEEKS, J. Voorhees, J. Ye, and C. Rommens. Plant Sciences, J.R. Simplot Company, Boise, ID 83706. Email: [troy.weeks@simplot.com](mailto:troy.weeks@simplot.com)

Public concern about the release of transgenic crops centers on the foreign DNA-based alteration of plant processes. Consumers have indicated a much more positive attitude towards GM crops if they would only contain native DNA. We addressed this perception issue by developing a variety of all-native DNA transformation methods. These methods rely on the use of plant-derived transfer DNAs, native promoter and gene sequences and marker-free transformation. They were successfully applied to improve the quality of food crops such as potato and tomato. Alfalfa is another plant system that would greatly benefit from all-native DNA transformation.

Here, we present a novel marker-free in planta transformation method for this crop. Unlike conventional transformation systems, the method is variety-independent, limits the need for tissue culture manipulations and reduces the time needed to obtain seed. It relies on vortex-mediated transformation of the exposed apical meristems of young seedlings and yields marker-free transformation frequencies of ~3%. The vortex-mediated seedling transformation method was employed to introduce an alfalfa-derived transfer DNA containing a silencing construct for the caffeic acid o-methyltransferase (*Comt*) gene into the plant's genome. Reduced levels of syringyl (S-) lignin monomers in the resulting plants enhance the nutritional value of forage feed. The absence of foreign DNA in intragenic alfalfa plants may not only alleviate some of the public concerns regarding genetically modified crops but also limit biosafety risk and facilitate the governmental deregulation process.

#### P-26

Corn Transformation at Monsanto: Development of an Enabling Technology. T. MICHAEL SPENCER. Monsanto Company, Mystic Research, Mystic, CT 06355. Email: [michael.spencer@monsanto.com](mailto:michael.spencer@monsanto.com)

Transformation technology for introduction of genes into maize has evolved significantly over the last 20 years. Transformation efficiencies have reached the level where large numbers of genes can be directly evaluated in the crop plant itself rather than relying solely on initial determination of transgene function in model systems like Arabidopsis. Effective DNA delivery methods include microprojectile bombardment and Agrobacterium-mediated T-DNA transfer, and a variety of efficient selectable marker systems have been employed. A wide range of maize germplasm is amenable to efficient transformation, enabling evaluation of complex traits directly in elite genetic backgrounds. The evolution of this technology will be discussed, as well as its contribution to gene discovery and product development.

#### P-27

Fine Hardwood Biotechnology: Enhancing Productivity for the Midwest. C. H. MICHLER, P. M. Pijut, D. F. Jacobs, R. Meilan, K. E. Woeste, and M. Ginzel. Hardwood Tree Improvement and Regeneration Center at Purdue University, 715 W. State Street, West Lafayette, IN 47907-2061. Email: [michler@purdue.edu](mailto:michler@purdue.edu)

The Hardwood Tree Improvement and Regeneration Center (HTIRC) at Purdue University is a partnership of the USDA

Forest Service and Purdue University. HTIRC receives additional support from the hardwood forest industry, forest landowner associations, and National Science Foundation. The mission of HTIRC is to enhance the productivity of native and plantation hardwood forests in order to maintain and enhance the forest resource for forest landowners and forest products industry in the Central Hardwoods Region. Some of the hardwood species that are the focus of research include black walnut, northern red oak, butternut, American chestnut, black cherry, and white oak. Conventional breeding and bioengineering research are focused genetic traits for improvement in growth and form; insect, disease and herbicide resistance; and control of flowering. Additional work is being conducted on nursery production and out-planting performance. Results and products are disseminated through various extension and technology transfer activities. Highlights of research on breeding, heartwood regulation, hardwood restoration, sterility, seedling production and quality, and numerous other projects will be presented.

#### P-28

Genomics and Pine Disease. ALISON M. MORSE<sup>1</sup>, Gogce Kayihan<sup>1</sup>, Henrietta Myburg<sup>3</sup>, Katherine E. Smith<sup>1,4</sup>, C. Dana Nelson<sup>4</sup>, and John M. Davis<sup>1,2</sup>. <sup>1</sup>School of Forest Resources and Conservation and <sup>2</sup>Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL 32611; <sup>3</sup>Forest Biotechnology Group, North Carolina State University, Raleigh, NC 27695; and <sup>4</sup>USDA Forest Service, Southern Institute of Forest Genetics, Saucier, MS 39574. Email: ammorse@ufl.edu

The gymnosperm genus *Pinus* is commercially important worldwide but fungal diseases can significantly lower productivity. The economically and ecologically important southern pine species *Pinus taeda* (loblolly pine) shows genetic variation for resistance to two diseases to be discussed in this talk. Fusiform rust disease, incited by the biotrophic fungus *Cronartium quercuum* f. sp. *fusiforme*, is characterized by the presence of branch and stem galls that reduce wood quality and result in breakage. Pitch canker is an episodic disease incited by the necrotrophic fungus *Fusarium circinatum* Nirenberg and O'Donnell with symptoms including pigmented lesions on stems and branches that can result in significant stem and crown damage. Both diseases can lead to mortality of infected trees. Selection, breeding, and genomic mapping for fusiform rust and pitch canker disease resistance has been undertaken to facilitate disease management and reduce economic losses. However, the underlying molecular processes that occur in pine genotypes that are resistant to either disease are largely unknown. To dissect the molecular basis of defense

responses in *Pinus* to these two biologically diverse fungal pathogens, we have used a combination of large-scale disease resistance phenotyping, gene expression analysis, EST sequencing and quantitative genetic analysis. Distinct host genes have been identified that are regulated during each disease state and are candidates for analyses designed to reveal the mechanisms underlying resistance.

#### P-29

Gene Discovery in *Populus* Using Activation Tagging. VICTOR BUSOV and Steven H. Strauss. Michigan Technological University, School of Forest Resources and Environmental Science, 1400 Townsend Drive, 185 Horner Hall, Houghton, MI 49931. Email: vbusov@mtu.edu

*Populus* is emerging as the model tree taxon. An indispensable part of a model organism attributes is mutagenized collections allowing forward and reverse genetics for testing trait-gene associations. Activation tagging is feasible for trees because the dominant mutations circumvent the necessity of multiple rounds of selfing that would be a prohibitively lengthy process in trees. We have generated the first poplar activation tagging population comprising of 627 independent events. The rate of mutant identification after screening in tissue culture, greenhouse, and field was approximately 7% and much higher compared to 1% in *Arabidopsis* using the same vector. Most of the mutants were discovered after 1 year of field growth. Using TAIL PCR, plasmid rescue, and RT-PCR we have identified the insertion position and demonstrated transcription gene activation for a number of genes. For 4 genes we have fully recapitulated the phenotype via retransformation. The identified genes provide insights into the regulation of dormancy, secondary growth, and metabolic pathways in trees. Our studies indicate that activation tagging can be effectively used in poplar for identification of genes of economic, environmental and scientific value.

#### P-30

The Benefits of Biotechnology for Working Forests. NARENDER NEHRA and M. Hinchee. ArborGen, LLC, P.O. Box 840001, Summerville, SC 29484. Email: mahinch@arborgen.com

Highly productive plantation forests are expected to provide more than fifty percent of the world's wood supply in the course of the next fifty years. It is therefore essential that we develop technologies that can be used to maximize the production potential of these working forests to meet the ever

increasing demand for wood and wood products, while preserving natural forests for the benefit and enjoyment of future generations. Improved forest trees developed through the interface of traditional breeding, varietal forestry and the application of biotechnology, together with improved silviculture and plantation management practices, will be required for sustainable wood production. In this context, biotechnology is emerging as an important tool in the technology toolbox for enhancing the productivity of working forests. Research in plant tissue culture, genetic transformation and

genomics technology is expanding the forestry industry's capabilities to identify and utilize new molecular techniques for forest tree improvement. ArborGen, a forest biotechnology company dedicated to improving the sustainable productivity of working forests, is developing tree planting stock with improved growth, wood properties, and stress tolerance. The presentation will provide an overview of the progress and effort ArborGen has made towards the development of better trees. These trees will benefit the forest industry and also have a positive impact on the environment and the world's forests.