

## Plant Symposia

### P-1

Improved Yield Stability of Maize Under Drought Stress. MICHAEL LUETHY and Jacqueline Heard. Monsanto, 700 Chesterfield Parkway, Chesterfield, MO 63017. Email: michael.luethy@monsanto.com

Efficient use of water in agricultural production will be one of the great challenges during the twenty-first century, with agriculture currently being responsible for ~70% of freshwater withdrawal. As such, yield improvement through tolerance to water deficits that occur routinely in the Central Corn Belt and frequently in western states are an important challenge in the coming decade. Benefits of improving water utilization efficiency, in addition to higher yield, are expected to include reduced water consumption and environmental sustainability. Genetic approaches using model systems are adding to our understanding of plant pathways that are important to water stress tolerance. Transgenic approaches in model systems such as *Arabidopsis* have identified genes that effectively confer drought tolerance in both *Arabidopsis* and crops such as soybean and corn. This presentation will illustrate our ability to uncover novel drought protection mechanisms in crop plants, leveraging data from model systems and highlighting data from corn transgenics that demonstrate the enormous opportunity that exists for the application of genomics to product development in crops.

### P-2

Genetic and Chemical Approaches to Delivering Abiotic Stress Tolerance in Crops. T. LYNNE REUBER. Mendel Biotechnology, 3935 Point Eden Way, Hayward, CA 94545-3720. Email: lreuber@mendelbio.com

Mendel Biotechnology conducted a large-scale, high throughput screening program in *Arabidopsis thaliana* to identify transcription factors that regulate important plant traits. A number of transcription factors identified in these screens produce tolerance to abiotic stresses such as drought, cold, and freezing when overexpressed, in many cases through novel genetic pathways. Many of these transcription factors have been shown to translate from the model plant system to produce effective stress tolerance in crop plants. Information

from genetic pathways regulated by these transcription factors has also guided the development of plant reporter lines for use in fluorescence-based screens that report on pathway activation. High-throughput chemical screens using these lines have led to the identification of structurally distinct compounds that induce drought tolerance.

### P-3

Transgenic Approaches Towards Improved Drought Tolerance in Turf and Forage Grass (*Paspalum notatum* Flugge). FREDY ALTPETER<sup>1</sup>, Xi Xiong<sup>1</sup>, Victoria James<sup>1</sup> and A. Blount<sup>2</sup>. <sup>1</sup>Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida - IFAS, Gainesville, FL-32611 and <sup>2</sup>Agronomy Department, University of Florida-IFAS, North Florida Research and Education Center, Marianna, FL 32446-7906. Email: faltpeter@ifas.ufl.edu

Transcription factors play a critical role in the regulation of plant stress response. Overexpression of stress-responsive transcription factors is a promising strategy for improving abiotic stress tolerance in crops. *DREB* stress-responsive transcription factors are well characterized examples. We isolated *DREB1a* from a Negev desert accession of *Hordeum spontaneum*. Molecular and physiological data on overexpression of *HsDREB1a* in a commercially important, apomictic turf and forage cultivar of bahiagrass will be presented. In contrast to *DREB* transcription factors, very little is known about the function of most other major transcription factor families in abiotic stress tolerance, including *WRKY* proteins. Inducibility of *HvWRKY38* by dehydration treatments suggested that it may have a potential role in abiotic stress responsive signaling and, possibly, in conferring drought tolerance to plants. To address this question, we overexpressed *HvWRKY 38* in bahiagrass. Molecular and physiological data on dehydration tolerance of transgenic and wild-type plants including photosynthetic efficiency during and after stress, whole plant water retention, and biomass production will be presented.

### P-4

Stable Transformation of Freshwater Wetland Monocots and its Ecological Implications. SUZANNE M. D. ROGERS.

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The long-term objectives of this research are to introduce genes into wetland monocot plants targeting enhanced phytoremediation ability. *Typha*, *Juncus*, *Scirpus*, and *Carex* are commonly planted in constructed wetlands for the purposes of wastewater and polluted soil treatment and the bioaccumulation of chemicals. Heavy metal contamination of wetland ecosystems is an increasing problem due to the immutability of metals, global industrialization, and lack of suitable control practices. Phytoremediation, the use of plants for decontamination, offers the opportunity to recycle metal extracted from metal-containing plant residue. Recently, the use of genetically modified plant species with specific genes encoding enzymes that catalyze various biochemical reactions in the process of phytoremediation has emerged as a cost-effective and promising technology. Although wetland plants are exploited for decontamination activities, their usage is limited because of their recalcitrant nature in vitro and the nonavailability of gene transfer systems. Transient transformation studies showed that the cells of these plants did possess the potential to be transformed. A highly reproducible *Agrobacterium*-mediated plant model for stable transformation of *Juncus* was developed. Gene expression, integration, and inheritance were confirmed. The ecological implications of this research will be discussed.

#### P-5

Using In Vitro Techniques as Tools to Differentiate Ecotypes of *Calopogon tuberosus*, a North American Native Orchid. P. J. KAUTH<sup>1</sup>, M. E. Kane<sup>1</sup>, W. A. Vendrame<sup>2</sup>. <sup>1</sup>Department of Environmental Horticulture, University of Florida, P.O. Box 110675, Gainesville, FL 32611 and <sup>2</sup>Tropical Research and Education Center, University of Florida, 18905 SW 280th Street, Homestead, FL 33031-3314. Email: pkauth@ufl.edu

*Calopogon tuberosus* is a terrestrial orchid of eastern North America from Florida to Canada and west to Texas. This orchid colonizes habitats such as alkaline prairies, pine flatwoods, roadsides, and sphagnum bogs. With a wide distribution and diverse habitats, the potential for ecotypic differentiation in *C. tuberosus* exists. Identifying ecotypes is important from both conservation and evolutionary perspectives. Ecotypes may be the beginning of speciation, and transplanting plants from one habitat to another may alter the species' genetic composition. Using local seed for local conservation programs assures species survival. Common garden studies are widely used as efficient

methods for differentiating ecotypes. Orchids, including *C. tuberosus*, are protected by most states, and using wild-collected plants for common garden studies is not feasible. Seed germination studies are also used to identify ecotypes. Since orchid seeds are small, are difficult to work with, require mycorrhizal fungi to germinate in situ, and often take months or years to germinate in nature, using non-orchid seed germination methods is not practical. To circumvent these shortcomings, in vitro techniques have been employed to differentiate *C. tuberosus* ecotypes. The role of three photoperiods and six culture media in promoting seed germination and seedling development of six *C. tuberosus* populations was studied. Germination was similar within each population, but development differed greatly among photoperiod and culture media. However, germination and development among the populations was significantly different. A timecourse in vitro seedling development experiment revealed different growth patterns. Corm biomass accumulation was greater in northern populations, while biomass accumulation was more evenly distributed in southern populations. The effect of seed chilling on in vitro asymbiotic seed germination of several populations is currently being investigated. These methods of differentiating ecotypes may serve as a model for other rare, threatened, or endangered species.

#### P-6

Applications of Underwater Grass Cultures for Bioassays and Chemical Ecology Studies. M. STEPHEN AILSTOCK. Anne Arundel Community College, 101 College Parkway, Arnold, MD 21012. Email: smailstock@aacc.edu

Underwater grasses play important roles in many of the shallow water ecosystems of the world. These grasses stabilize sediments, serve as flood buffers, improve water clarity, are important in geochemical cycling, and provide food and habitat for many kinds of other aquatic organisms. Underwater grasses are secondarily adapted to water. Their evolutionary ancestors were flowering land plants. Unlike their terrestrial ancestors, underwater grasses are surrounded by a nutrient-rich broth that serves as an excellent growth media for many other life forms, both heterotrophic and autotrophic, that often live as epiphytes on their surfaces. As a consequence, in situ and nonaxenic culture systems do not provide sufficiently sensitive experimental control to be used for bioassays, studies of subtle chemical ecology relationships, and many physiological studies because it is difficult to distinguish between the activity of the plants and that of the epiphytes. In contrast, axenic underwater grass culture systems employing clones provides the rigorous controls needed for these types of studies. This paper reports on the development and

applications of axenic underwater grass culture systems for three underwater grasses, *Stuckenia pectinata*, *Potamogeton perfoliatus*, and *Ruppia maritima*. Specific examples are given for how these culture systems have been used to conduct bioassays for studies of contaminant effects, the presence of antibacterial compounds, and for physiological studies used to identify their in situ habitat requirements.

#### P-7

Targeted Integration after *Agrobacterium*-mediated DNA Delivery. P. J. J. HOOYKAAS. Institute of Biology, Leiden University, Clusius lab, Wassenaarseweg 64, 2333AL Leiden, THE NETHERLANDS. Email: p.j.j.hooykaas@biology.leidenuniv.nl

Genetic modification of plants by *Agrobacterium* T-DNA is now routinely performed. It has been observed that the T-DNA with the transgenes integrates at fairly random positions and in variable copy numbers in the plant genome by nonhomologous recombination. This may cause position effects (like silencing of transgenes) and mutation of genes at the integration site. Therefore, it would be an advantage if integration could be targeted to a specific locus. Such gene-targeting (GT) would also be of great advantage for the modification or inactivation of genes in the plant genome. GT can be achieved by homologous recombination. This process is efficient in yeast but a very rare event in higher eukaryotes, like animals and plants. Our laboratory has found that T-DNA integration in yeast is mediated by the non-homologous end-joining (NHEJ) enzymes [1]. Inactivation of NHEJ indeed resulted in much higher GT frequencies in yeast and fungi [2, 3]. In plants, NHEJ-mutants still showed considerable non-homologous integration. Therefore, we are exploring alternative routes to further increase GT in plants such as by using specific zinc finger nucleases and site specific recombination enzymes. 1. Van Attikum H, Bundock P and Hooykaas PJJ (2001) Non-homologous end-joining proteins are required for *Agrobacterium* T-DNA integration. *EMBO J* 20, 6550–6558. 2. Van Attikum H and Hooykaas PJJ (2003) Genetic requirements for targeted integration of *Agrobacterium* T-DNA in *Saccharomyces cerevisiae*. *Nucleic Acids Res* 31, 826–832. 3. Kooistra R, Hooykaas PJJ and Steensma HY (2004) Efficient gene targeting in *Kluyveromyces lactis*. *Yeast* 21, 781–792.

#### P-8

Towards Zinc Finger Nucleases-mediated Gene Targeting in Plants. Andriy Tovkach, Vardit Zeevi, and TZVI TZFIRA. Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI 48109. Email: ttzfira@umich.edu

Double-strand breaks (DSBs) in plant genomes are typically repaired by the plant nonhomologous end-joining machinery, which usually leads to local deletions and mutagenesis at the repair site. Interestingly, artificial induction of DSBs by various restriction enzymes results not only deletions, but also in insertions of foreign DNA molecules into the repair site. This phenomenon could potentially be used for mutating specific sites in the plant genome and targeting foreign DNA molecules into them with zinc finger nucleases (ZFNs). ZFNs are a new type of artificial restriction enzymes that are custom-designed to recognize and cleave specific DNA sequences, producing DSBs. However, technical difficulties in the design, assembly, and analysis of ZFNs have hindered the use of ZFNs for plant gene targeting. We have recently designed a set of constructs and cloning, biochemical, and *in planta* analysis procedures for newly designed ZFNs. Cloning begins with *de novo* assembly of the DNA-binding regions of new ZFNs from overlapping oligos containing modified helices responsible for DNA triplet recognition, and their insertion between a nuclear localization signal and the *FokI* endonuclease domain. Following the transfer of fully assembled ZFNs into *Escherichia coli* expression vectors, bacterial lysates were found to be most suitable for *in vitro* digestion analysis of palindromic target sequences. An *in planta* activity test was also developed to confirm the nucleic activity of ZFNs in plant cells. The assay is based on the reconstruction of GUS expression following bombardment of a reporter and ZFN-expressing plasmids into mesophyll cells, as well as integration of the tested reporter gene in transgenic calli. Our new procedures, plasmids, and assays bring us one step closer to efficient implementation of ZFN-based technology for gene targeting in plant species.

#### P-9

Zinc Finger Nuclease-Mediated Gene Targeting in Plants. JOSEPH F. PETOLINO<sup>1</sup>, Yannick Doyon<sup>2</sup>, Lisa Baker<sup>1</sup>, Russell DeKelver<sup>2</sup>, Andrew Worden<sup>1</sup>, Fyodor Urnov<sup>2</sup>, and Charles Cai<sup>1</sup>. <sup>1</sup>Dow AgroSciences, 9330 Zionsville Rd., Indianapolis, IN 46268 and <sup>2</sup>Sangamo BioSciences, 501 Canal Blvd., Richmond, CA 94804. Email: jfpetolino@dow.com

Gene targeting via homology-driven repair occurs at a very low frequency in plant cells compared to random integration, making targeted gene modifications impractical. Most recently, substantial increases in the frequency of gene targeting have been observed following the induction of double-stranded breaks in host cell DNA followed by the apparent stimulation of cellular repair mechanisms. Strategies to achieve targeted DNA double strand breaks have been developed by fusing zinc finger DNA binding proteins with sequence-independent nuclease domains derived from

type IIS restriction endonucleases. Using this strategy, site-specific modifications of native genes have been demonstrated. Implications for novel trait development and crop improvement will be discussed.

#### P-10

Plant Genome Manipulation Using *Cre/lox* Technology. VIBHA SRIVASTAVA. University of Arkansas, Department of Crop, Soil & Environmental Sciences, 115 Plant Science Bldg., Fayetteville, AR 72701. Email: vibhas@uark.edu

More than 15 years ago, site-specific recombination system, *Cre/lox*, was utilized for excising marker genes from transgenic tobacco plants. A few years later, it was successfully used for precisely integrating transgenes into a previously introduced genomic site. These experiments provided two important applications for transgenic plants: marker excision and site-specific gene integration. Since then, other recombination systems have been developed that are functional in a variety of plant species. However, only the *Cre/lox* system has so far been effectively applied for site-specific gene integration in plants. We studied the utility of *Cre/lox*-mediated site-specific gene integration by introducing a transgene construct consisting of  $\beta$ -glucuronidase gene (*gusA*) into rice cells, where it integrated into a preselected genomic site via *Cre/lox* recombination. All of the transgenic lines displayed high GUS activity; in addition, the expression variability between transgenic lines was greatly reduced. Each of the *Cre/lox* applications offers solutions to the major problems in biotechnology: presence of undesirable marker genes and expression variability in transgenic plants. However, these applications have not been widely implemented so far. Perhaps streamlining *Cre/lox* technology and developing appropriate resources will encourage its widespread use among biotechnologists. An important step towards streamlining the recombination technology is to combine the two applications into a single technology and provide founder lines for plant transformation. Therefore, we are developing the “marker-free site-specific gene integration” technology by utilizing both *Cre/lox* and *FLP/FRT* recombination systems. The next step would be to develop founder lines for important crop species such as maize and rice.

#### P-11

Searching for New Antiviral Agents from Brazilian Biodiversity. CLAUDIA MARIA OLIVEIRA SIMOES, Celia Barardi, Flávio Reginatto, Janine Bettega, Adriane Freitas, Thais Sincero, Cibele Gaido, Luciane Savi, Carla Andrighetti-Fröhner, Alexandre Cordeiro Silva, Marcia Carriel-Gomes, Vanessa Müller, Jadel Kratz, Débora Luck-

emeyer, and Thais Isabel Silva. Laboratório de Virologia Aplicada, CIF, CCS, Universidade Federal de Santa Catarina, Florianópolis, SC, BRAZIL. Email: claudias@reitoria.ufsc.br

The current antiviral drug armamentarium comprises nearly 40 drugs that have been approved for clinical use. Most of these compounds date back over the last 7 years, and at least half of them are used for the treatment of HIV infection. Additionally, the appearance of viral strains resistant to the available antiviral drugs is a significant problem. Taking these situations into account, the research and development of more effective new antiviral agents, with low cytotoxicity and minor side effects, is a necessary and highly desirable task. Our research group [Laboratory of Applied Virology from Universidade Federal de Santa Catarina (UFSC), Florianópolis, SC, Brazil] has been searching for new antiherpes agents, especially from natural sources, and we describe here our contributions to this area. Considering that the Brazilian biodiversity is one of the richest in the world, and that just a small part of it has been investigated for biological activities, these studies must be encouraged. As part of our screening program by using HSV-1 (strains KOS and 29-R, sensitive and resistant to acyclovir, respectively) and HSV-2 (strain 333), we evaluated several taxa of native medicinal plants (*Achyrocline satureioides*, *Araucaria angustifolia*, *Cuphea carthagenensis*, *Ilex paraguariensis*, *Lafoensia pacari*, *Passiflora spp.*, *Rubus imperialis*, *Slonea guianensis*, *Cecropia spp.*, *Alamanda spp.*, *Glycine max*, *Lippia alba*, *Tillandsia usneoides*, *Wilbrandia ebracteata*, *Bromelia antiacantha*, *Dodonea viscosa*, among others), marine sponges from the Brazilian coastline, violacein from *Chromobacterium violaceum*, peptides isolated from animals (spider, tunicate, frog, shrimp, and mussel), hemolymph from two species of oysters, as well as several phenolic compounds isolated from tea (catechin and derivatives) and a series of alkyl gallates synthesized from gallic acid (present in various medicinal plants). Some of these samples showed important antiherpes activity and their mechanisms of action have been elucidated. It is important to state that all these works have been conducted by graduate and undergraduate students from UFSC.

#### P-12

Improving Cat's Claw Alkaloid Production by Stimulating In Vitro Plant Cultures. A. RAMOS-VALDIVIA<sup>1</sup> and C. Cerdá-García Rojas<sup>2</sup>. <sup>1</sup>Departamento de Biotecnología y Bioingeniería and <sup>2</sup>Departamento de Química, Centro de Investigación y de Estudios Avanzados del IPN (CINVESTAV-IPN), Av. Instituto Politécnico Nacional 2508.Col. Pedro Zacatenco, 07360 México, D.F., MEXICO. Email: aramos@cinvestav.mx

Alkaloid accumulation in plants shows pattern variation in different tissues as a consequence of their constitutive defense mechanisms. Furthermore, biosynthetic pathways for these compounds often become activated by herbivore or pathogens. *Uncaria tomentosa* (cat's claw), a native plant from the Amazon rainforest, produces pentacyclic monoterpene indole alkaloids (PMOA), which possesses immunomodulatory, cytotoxic, antileukemic, and anti-AIDS properties. In order to understand the accumulation pattern of PMOA, we investigated the alkaloid content in micropropagated plantlets, roots, and cell suspension cultures. The aerial vegetative organs showed the highest PMOA content (8–14 mg/g), while the root lines produced ca. 2 mg/g of PMOA. In the aerial vegetative organs from plantlets,  $^{14}\text{C}$ -tryptophan (100  $\mu\text{M}$ ) was rapidly transformed in radioactive tryptamine and the highly oxidized PMOA, while in root lines, radioactivity was incorporated into PMOA and into a glucoindole alkaloid, which was not previously detected in the wild plant. The structure of this compound was verified by mass spectrometry and 1D and 2D nuclear magnetic resonance spectroscopy as  $3\alpha$ -dihydrocadambine. In plantlets and root cultures, jasmonate addition induced a threefold increment in total PMOA accumulation, as well as changes in the total content and pattern of PMOA. Green cell suspension lines produced 10 to 20 times less PMOA than differentiated cultures, although PMOA production can be stimulated through oxidative stress induction when cells are cultivated in a bioreactor. These findings and the putative role of the glucoindole alkaloid in the PMOA biosynthesis will be discussed. The complete biosynthetic pathway of PMOA in *U. tomentosa* still remains to be investigated, although we proposed that they are formed from monoterpene indole alkaloids.

### P-13

Production and Neuroprotective Properties of Natural Resveratrol Analogs from Hairy Root Cultures of Peanut. FABRICIO MEDINA-BOLIVAR<sup>1,2,3</sup>, Malathi Srivatsan<sup>2</sup>, Ganapathy Sivakumar<sup>1</sup>, Mahadevappa Badanavalu<sup>1</sup>, Jose Condori<sup>1</sup>, and Maureen Dolan<sup>1,3</sup>. <sup>1</sup>Arkansas Biosciences Institute and <sup>2</sup>Department of Biological Sciences, Arkansas State University, P.O. 639, State University, AR 72467 and <sup>3</sup>Nature West, Jonesboro, AR 72401. Email: fmedinabolivar@astate.edu

Resveratrol and its natural analogs are polyphenolic inducible compounds known as stilbenoids. These specialized metabolites are produced in a selected group of taxonomically unrelated plant species such as grape and peanut. Among the stilbenoids, resveratrol has been the most studied. Several health benefits impacting cardiovascular disease, various cancers, and aging have been associated with resveratrol. To study the production of

stilbenoids and identify novel biological activities associated with these compounds, we developed hairy root cultures of peanut. Sodium acetate and methyl jasmonate were effective in inducing and secreting these compounds in the medium of the hairy root cultures, thereby facilitating their purification. Chemical profiling of the ethyl acetate fraction of the medium by high-performance liquid chromatography–mass spectrometry confirmed the presence of resveratrol and several other stilbenoids such as piceatannol, arachidin-1, and arachidin-3. Scale-up of the peanut hairy root cultures in 5-l airlift balloon-type bioreactors was effective in providing significant quantities of stilbenoids for bioassays. In order to identify compounds with potential applications in neurodegenerative disorders such as Parkinson's disease, we focused on the neuroprotective activity of stilbenoids. Rat pheochromocytoma (PC12) cells were differentiated into neurons and exposed to hydrogen peroxide to induce oxidative stress. However, when the neurons were pretreated with a peanut hairy root extract enriched on stilbenoids, the neurons were protected from this oxidative damage. Our results indicate that the hairy root cultures provide a valuable tool for the production and identification of bioactive compounds with potential applications in human health.

### P-14

The Use of the Worm *Caenorhabditis elegans* as a Model to Investigate Functional Ingredients. D. RAMON<sup>1</sup>, P. Martorell<sup>1</sup>, J.V. Forment<sup>1</sup>, L. Moulay<sup>2</sup>, L. Cienfuegos<sup>2</sup>, B. Muguersa<sup>2</sup>, M. Pasamar<sup>2</sup>, S. Laghi<sup>2</sup>, O. Vilanova<sup>2</sup>, Y. Castilla<sup>2</sup>, R. De Llanos<sup>1</sup>, and F. Montón<sup>1</sup>. <sup>1</sup>Biópolis S.L. Polígono de la Coma s/n; 46980-Paterna; Valencia, SPAIN and <sup>2</sup>Department of Research and Development, Natraceutical Group. Autovía A-3, Salida 343, Camí de Torrent s/n; 46930 Valencia, SPAIN. Email: daniel.ramon@biopolis.es

By the year 2050, it is estimated that the elderly population (aged 65 or older) will double that of children (aged 0–14) for the first time in history. The increase in the elderly population has already taken a toll on health care systems. In this context, nutritional intervention using food products specially designed for elderly people will be one of the tools to improve the life quality of this class of people. It is well documented that the oxidative stress is a typical consequence of cell aging. Cocoa is a rich source of polyphenols, mainly the flavonoids (procyanidins and flavan-3-ols). However, it is difficult to assess the beneficial antioxidant effects of these components in aging by using short-term dietary supplementation studies due to the high cost and duration. Both *Saccharomyces cerevisiae* and *Caenorhabditis elegans* are organism models widely used for biological research due to their short life, the availability of full genome sequence data and the existence of a good

collection of mutants in both organisms. Therefore, the nematode *C. elegans* is the appropriate organism to identify and characterize compounds that delay aging and extend the life-span cycle. The aim of the present work was to analyze the in vivo antioxidant effect of a high-rich cocoa polyphenol extract ingredient (CocoanOX 12%, CCX, Natraceutical Group), to define its molecular target, and to study its effect on the life span of *C. elegans*. In order to assess the protective effect of CCX on the oxidative stress, a preliminary experiment was carried out using different doses (from 0.5 to 8 mg/mL) of this ingredient in the yeast *S. cerevisiae*. The results showed that a marked antioxidant effect was observed when a dose of 4 mg/mL was applied. Using *S. cerevisiae* DNA arrays, we have determined that CCX mainly induces the expression of genes encoding DNA-binding proteins. Most significantly, CCX increases four times the expression of the gene *HST3* encoding a sirtuin. To confirm this result, an experiment using a knock-out mutant strain of *S. cerevisiae* in a sirtuin gene was carried out. Results indicate a lack of effect of CCX on this mutant suggesting a role for sirtuin induction on the biological effect of CCX. Oxidative stress was also assayed using *C. elegans*, and again, this effect was observed. To determine whether CCX expanded life span, we further assessed its effect in a wild-type *C. elegans* strain. For this assay, NG agar plates containing a CCX concentration of 4 mg/mL was used. The results revealed that an increase from 15.2 days in the control media without CCX to 17.8 days in the presence of this compound was observed. In addition, several *C. elegans* mutant strains were used to determine the involved biochemical and molecular pathways that are affected by CCX. *Caenorhabditis elegans* mutant strains used in this experiment were VC199 (*Sir-2.1*), GR1307 (*daf-16*), and AM1 (*osr-1*). The results showed that the increase in life span was dependent of *Sir-2.1* and independent of *osr-1* proteins. A minor effect was detected in the case of the *daf-16* mutant.

In conclusion, CocoanOX 12% was found to exhibit antioxidant stress effects and prolonged life span in wild-type *C. elegans*. This effect is mediated by at least the sirtuin circuit. These results suggest that the polyphenolic compounds present in CCX have a preventive effect on oxidative stress and, therefore, strong benefits during aging.

#### P-15

Humanization of N-glycosylation of *Nicotiana benthamiana* for Production of Biotherapeutics Using MagnICON. KOEN WETERINGS<sup>1</sup>, Annemie Boets<sup>1</sup>, Johan Botterman<sup>1</sup>, Herta Steinkellner<sup>2</sup>, and Gerben van Eldik<sup>1</sup>. <sup>1</sup>Bayer BioScience NV, Technologiepark 32, 9052 Ghent, BELGIUM and <sup>2</sup>Institute for Applied Genetics and Cell Biology, University

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Using our capabilities in plant biotechnology, process science, and product development from Bayer CropScience and Bayer Schering Pharma, respectively, Bayer is uniquely positioned to establish a platform and integrated process for the production of plant-made pharmaceuticals. We have developed the magnICON expression system allowing the high-level production of biotherapeutics in the leaves of the host plant *Nicotiana benthamiana*. For instance, this system allows us to generate protein levels of 300 mg mAb per kg fresh weight within 7–9 d. Because N-glycans of *N. benthamiana* carry the potentially immunogenic core  $\alpha$ 1,3-fucose and  $\beta$ 1,2-xylose, we have set out to humanize the N-glycosylation pathway by downregulating the mRNAs coding for  $\alpha$ 1,3-fucosyltransferase (FucT) and  $\beta$ 1,2-xylosyltransferase (XylT). In plants carrying the individual RNAi constructs for FucT or XylT, we observed some, albeit low, residual amounts of fucose and xylose on expressed mAbs. Surprisingly, however, no xylose and fucose was detected on expressed mAbs from plants that carried both FucT and XylT RNAi constructs. These plants have a normal phenotype under standard growth conditions, and the RNAi is stable for several generations. Together, the data shows that XylT and FucT in *benthamiana* may be down-regulated without any deleterious effect and that these plants can serve as production platform for biotherapeutics with more human-like N-glycosylation.

#### P-16

The Power of One: Glyco-optimized Therapeutic Antibodies in Lemna. JOHN R. GASDASKA<sup>1</sup>, Jason D. Sterling<sup>1</sup>, Jeffrey T. Regan<sup>1</sup>, Kevin C. Cox<sup>1</sup>, Steven Sherwood<sup>2</sup>, Lynn F. Dickey<sup>1</sup>. <sup>1</sup>Biolex Therapeutics, Pittsboro NC 27312 and <sup>2</sup>Aragen Bioscience, Morgan Hill CA 95037. Email: jgasdaska@biolex.com

Monoclonal antibodies are one of the fastest growing classes of protein therapeutics. For many antibodies, the structure and extent of the N-glycans on the Fc region of the H-chain plays a significant role in the therapeutic function. A glyco-optimized rituximab was expressed in the small aquatic plant *Lemna*. The optimized glycosylation was accomplished by co-expressing an interfering RNA construct targeting the endogenous alpha-1,3-fucosyltransferase and beta-1,2-xylosyltransferase genes (Cox et al., 2006). The resulting rituximab contained a single major G0 N-glycan without any detectable xylose or fucose. In cell-based functional assays, the glyco-optimized rituximab

showed similar CD20 binding kinetics as Rituxan<sup>®</sup> produced in mammalian cells but with significantly enhanced antibody-dependent cellular cytotoxicity and B-cell depletion in whole blood. In conjunction with decreased complement-dependent cytotoxicity (CDC), Lemna-derived rituximab offers the potential for an optimized anti-CD20 antibody therapeutic with improved efficacy and potency while simultaneously decreasing the side effects that are associated with CDC activity (Clark and Ledbetter 2005).

#### P-17

Sustainable Glyco-engineering and Production of Optimized Biopharmaceuticals in Bryophytes. G. GORR. Greenovation Biotech GmbH, 79111 Freiburg, GERMANY. Email: ggorr@greenovation.com

Genetically engineered plants are promising systems for the production of therapeutic proteins. Among the different plant-based systems, bryophytes show unique properties. The mainly used moss *Physcomitrella patens* is cultivated as haploid, photoautotrophically active, and fully differentiated gametophytic tissue performed as suspension cultures in bioreactors. Cultivation of the tissue is performed in a clonal way, and it has been shown that transgenic strains are highly stable showing no variations on the molecular level—even after several years of subcultivation. Cultivation conditions are simple, robust, highly flexible, and allow doubling times of once per day. No animal-derived products are used in the whole process. *Physcomitrella* is a well-characterized organism, e.g., the genome is sequenced. In contrast to other plants, no codon optimization is needed. Homologous recombination is used for sustainable gene knockouts, opening the possibility of genetic engineering of the glycosylation pathway. Removal of plant-specific *N*-glycan structures in *Physcomitrella* was achieved by targeted knockout of corresponding genes and included quantitative elimination of core fucosylation, resulting in improved performance of antibody-dependent cellular cytotoxicity-related antibodies. Here, the features of the moss-based expression technology in combination with improved product performance by glyco-engineering – through knockout and/or heterologous integration of glycosyltransferases encoding genes – will be presented and discussed.

#### P-18

Regulating Transgenic Plants for Academic Research. C. NEAL STEWART, JR. Department of Plant Sciences, University of Tennessee, Knoxville, TN 37996. Email: nealstewart@utk.edu

The laboratory and greenhouse regulation of transgenic plants fall to institutional biosafety committees (IBCs), whereas United States Department of Agriculture (USDA)–Animal and Plant Health Inspection Service (APHIS)–Biotechnology Regulatory Services (BRS) regulates field releases and interstate movement and importation of regulated transgenic organisms. BRS notifications, requiring a trimmer application, are typically used for plants and traits that fit the notification eligibility criteria, and permits are required for organisms and traits that require more oversight, e.g., phytoremediation, plant-produced pharmaceuticals, and perennials. The ePermits system that APHIS has instituted requires user registration and validation of identity at local USDA offices (<http://offices.sc.egov.usda.gov/locator/app>). There are numerous boxes to be filled in the linear web-based system. Also, lab PIs (not graduate students or post docs) act as the “responsible person” for permits or notifications and must be the ones to apply. The first few applications to be filled out by an applicant are time-consuming compared with the old e-mail application system. Once pertinent information has been entered into the system, however, it can be copied and pasted into subsequent applications. Since the availability of the ePermits system, most applications are handled much more quickly than was typical just 2 years ago. BRS is cognizant of academic research needs and timelines and their staff are helpful, while at the same time maintaining biosafety standards. They have a web site that contains online notification guide and inspection and compliance information ([http://www.aphis.usda.gov/biotechnology/brs\\_main.shtml](http://www.aphis.usda.gov/biotechnology/brs_main.shtml)). They encourage applicants to contact BRS by telephone or e-mail about any issues relating to the permit process. It is a good practice to keep the local IBC informed of BRS permits and notifications, especially with regards to field releases, and my institution mandates this practice. Unlike many parts of the world, it is still facile to perform academic field research with transgenic plants in the USA in which complete life cycles, including flowering and seed set, may be permitted in the field. Cooperation between applicants and BRS, as well as collecting and communicating new data to BRS on real biosafety risks (the absence thereof), is necessary for the continuation of transgenic plant field research.

#### P-19

Field Evaluation of Regulated Transgenic Plants in an Academic Environment. T. E. CLEMENTE. Center for Plant Science Innovation, University of Nebraska, Lincoln, NE 68588. Email: tclemente1@unl.edu

Evaluation of traits derived from biotechnology requires extensive field testing, beyond the characterization of the

target phenotype, to ensure the agronomic qualities of the crop expressing the novel trait have not been compromised. Field tests on regulated transgenic material must be carried out in accordance with the Federal guidelines governing the movement and release of the regulated items. The Federal guidelines are crafted to maximize confinement and, therefore, limit the possibility of escape of the regulated material outside of the designated locations. The University of Nebraska has developed the capabilities in the area of field testing of regulated crop plants by building infrastructure to ensure identity preservation, containment, and chain of custody tracking of regulated seed. These resources include a Field Coordinator who is responsible for training of personnel and oversight of all field-testing of regulated items, isolated storage facility, separate planting and harvesting equipment and dedicated acreage. This infrastructure permits the researcher to evaluate transgenic traits from the laboratory to the field under strict identity preservation. Importantly, this infrastructure provides an ideal environment for the hands-on training of plant breeding students in overall management and evaluation of regulated transgenic traits.

#### P-20

Controlled Field Release of Pharmaceutical Corn in Iowa: Lessons and Strategies. K. WANG. Biopharmaceutical Initiative, Plant Science Institute, and Department of Agronomy, Iowa State University, Ames, IA 50011-1010. Email: kanwang@iastate.edu

Plant-made pharmaceuticals (PMPs) offer great promise as efficacious and cost-effective products for the treatment of human and animal disease. However, the benefits of this technology must be balanced against potential health and environmental risks that may be associated with its use. Because PMPs presently have no provision for regulatory tolerances, their inadvertent occurrence in foods or feeds remains an important economic consideration, even when the health and environmental risks are low. The Plant Science Institute Biopharmaceutical Initiative at Iowa State University has been conducting a case study on open field release of an antigen-producing transgenic maize line. The transgenic corn expresses a well-characterized nontoxic antigen of *Escherichia coli* heat-labile enterotoxin subunit B (LT-B) that has potential use as an orally administered antidiarrheal agent or as a pharmaceutical adjuvant. Here I will present open field release experiments conducted in the past 5 years. In addition, I will discuss our risk assessment studies of low-level exposure of this transgenic corn from human health and ecological perspectives.

#### P-21

Imaging Live Plants and Plant Cells. SIDNEY L. SHAW. Department of Biology, Indiana University, Bloomington, IN 47405. Email: SiShaw@Indiana.edu

Modern microscope systems provide tremendous opportunities for investigating biological processes in living plants. Both genetically encoded probes and chemical labels have found routine use in screens and for diagnostic testing of protein function. Imaging live plants presents challenges to the microscopist due their inherent interactions with light and their sensitivity to environmental stimuli in the imaging experiment. In this workshop, a variety of microscopic techniques will be discussed along with their application to living plant materials. Conventional and spinning disk confocal microscopy will be highlighted and new technologies, including multiphoton and multispectral imaging, will be discussed with reference to their advantages and disadvantages for living plant specimens.

#### P-22

Plant Microscopy: Perils and Promises. ELISON B. BLANCAFLOR. Plant Biology Division, The Samuel Roberts Noble Foundation Inc., Ardmore, OK, 73401. Email: eblancaflor@noble.org

The structural components of a cell form the basis of its function. Cellular components are ordered into regulatory networks that allow for highly efficient transfer of information to specify how the cell grows and responds to environmental stimuli. Microscopy has contributed significantly to our understanding of plant cell function and continues to evolve from a group of descriptive techniques into a variety of quantitative tools that have allowed investigations into the molecular organization of cells, tissues, and organs. For instance, new fluorescent protein reagents and advanced instrumentation have allowed imaging of the dynamics of living plant cells with unparalleled spatial and temporal resolution. Despite the growing popularity of live cell imaging, plant microscopy continues to rely on traditional fixation protocols to verify observations in living samples. This section of the workshops will provide a survey of prospects and perils in handling plant samples for light microscopic analysis in both fixed and live cell preparations. We will draw upon our work on root biology to highlight common problems in plant microscopy and provide suggestions as to how such problems can be addressed. Methods on immunolabeling, sectioning, minimizing cross-talk during colocalization, choice of fluorescent tags for protein localization, and different types of equipment for light microscopy will be

discussed. Although most of the examples presented will be on plant roots, the lessons learned from working with this tissue should be applicable to other plant tissues.

### P-23

Interchromosomal Transfer of Epigenetic Information. VICKI L. CHANDLER, BIO5 Institute, University of Arizona, Tucson, AZ 85721. Email [chandler@Ag.arizona.edu](mailto:chandler@Ag.arizona.edu)

Paramutation at the *b1* locus in maize is mediated by unique tandem repeats that are necessary and sufficient to communicate *in trans* to establish and maintain meiotically heritable chromatin states. The *mop1* gene (*mediator of paramutation1*) is required for paramutation, *Mutator* transposon silencing, and *mop1* mutants exhibit pleiotropic developmental phenotypes. Map-based cloning of *mop1* has revealed that it encodes an RNA-dependent RNA polymerase gene (RDRP), most similar to *RDR2*, the RDRP in Arabidopsis that is associated with production of siRNA (short interfering RNA) molecules targeting heterochromatin. Nuclear run-on assays reveal that the tandem repeats required for *b1* paramutation are transcribed from both strands and the presence of tandem repeat siRNAs depends on *mop1*. However, the tandem repeats are transcribed and siRNAs are produced in all genotypes, even those that do not undergo paramutation. These data suggest siRNAs are involved but are not sufficient. We hypothesize that the *mop1* RDRP is required to maintain a threshold level of repeat RNA, which functions *in cis* to regulate transcription of *b1* and *in trans* to establish and maintain the heritable chromatin states associated with paramutation. This work was supported in part by an National Institutes of Health Pioneer Award (NIH 1 DP1 OD000575-01) and by a grant from the National Science Foundation (NSF MCB 0235329).

### P-24

The iPlant Collaborative: A Cyberinfrastructure-centered Community for a New Plant Biology. R. A. JORGENSEN, L. Stein, S. Ram, G. Andrews, V. Chandler, et al. University of Arizona, BIO5 Institute, Plant Sciences, Management Information Systems and Computer Sciences, Cold Spring Harbor Laboratory, Arizona State University, Tucson, AZ 85721-0036; UNC-Wilmington; and Purdue University. Email: [raj@ag.arizona.edu](mailto:raj@ag.arizona.edu)

The iPlant Collaborative is a distributed, cyberinfrastructure-centered, international community of plant and computing researchers enabling new conceptual advances through computational thinking and addressing an evolving array of the most compelling grand challenges in the

plant sciences and associated, cutting-edge research challenges in the computing sciences. Initially providing services through a small, committed, centralized core (at Arizona and the Cold Spring Harbor Labs), the Collaborative will gradually become distributed throughout the community. The community, comprised of both plant and computing researchers, working together, will determine priorities and allocation of resources via the community's Board of Directors. The Board of Directors will prioritize several grand challenges through a process involving community-proposed, grand-challenge workshops and self-forming, grand-challenge teams. The community's Board of Directors (Chair, Robert Last, Michigan State University) will make all major project resource allocation decisions. Project personnel will facilitate community discussion and decision-making, and then design and build the cyberinfrastructure that best meets the community's needs.

### P-25

The Oryza Map Alignment Project: Genomes in Flux. ROD A. WING. Arizona Genomics Institute, Department of Plant Sciences and BIO Institute, Thomas W. Keating Bioresearch Bldg., 1657 E. Helen Street, University of Arizona, Tucson, AZ. Email: [rwing@ag.arizona.edu](mailto:rwing@ag.arizona.edu)

With the completion of a finished genome sequence, we must now functionally characterize the rice genome by a variety of methods including comparative genomic analysis between cereal species and within the genus *Oryza*. *Oryza* contains 2 cultivated and 21 wild species that represent 10 distinct genome types. The wild species, in particular, contain an essentially untapped reservoir of agriculturally important genes that must be harnessed if we are to maintain a safe and secure food supply for the twenty-first century. The Oryza Map Alignment Project (OMAP) was established 4 years ago to generate a comprehensive set of genomics resources to investigate genome evolution and enhance positional cloning efforts in the genus *Oryza*. To date, we have generated: (a) 14 high-quality BAC libraries that encompass the 10 genome types of *Oryza*, (b) ~1,000 Mb of BAC end sequence from these libraries, and (c) SNaPshot fingerprint databases for all 14 libraries. All of these resources are publicly available through the AGI BAC/EST Resource Center, GenBank, or at <http://www.omap.org/>. The fingerprints and end sequences (BES) have been combined to develop 14 phase I physical maps. Nine of these physical maps, *Oryza nivara* [AA], *Oryza rufipogon* [AA], *Oryza glaberrima* [AA], *Oryza barthii* [AA], *Oryza punctata* [BB], *Oryza officinalis* [CC], *Oryza australiensis* [EE], *Oryza brachyantha* [FF], and *Oryza minuta* [BBCC], have been heavily manually edited (HME)

and aligned to the reference rice genome sequence. These alignments have revealed a large array of genome rearrangements relative to the International Rice Genome Sequencing Project reference sequence and have allowed us to begin to draw a more complete picture of *Oryza* genome evolution. In this talk, I will present the current status of OMAP and discuss recent analysis of the HME maps, a global analysis of structural variation across the AA genome species, an analysis of the effect of differential and lineage specific long terminal repeat retrotransposon bursts on genome size variation, and comparative sequence analysis of select loci across the *Oryza*.

#### P-26

Unraveling the Catalytic Specificity of Terpene Biosynthetic Enzymes and Engineering the Biosynthesis of Novel Terpenes in Yeast and Plants. J. CHAPPELL, S. Wu, S. Takahashi and B. Greenhagen. Plant Biology Program, University of Kentucky, Lexington, KY 40546. Email: chappell@uky.edu

Many plants respond to pathogen attack by the synthesis and secretion of antimicrobial compounds. For example, solanaceous plants produce antimicrobial terpenes that inhibit germination and growth of several fungal species. The production of these chemicals has been interpreted as an important defense response. We have also hypothesized that an understanding of the mechanisms responsible for the biosynthesis of the antimicrobial terpenes should provide a means for engineering the generation of novel and more efficacious compounds. Towards that goal, we have elucidated a two-step biosynthetic pathway for capsidiol, an antimicrobial sesquiterpene di-alcohol. The pathway consists of a synthase that catalyzes the cyclization of farnesyl diphosphate to a bicyclic hydrocarbon structure, followed by the action of a P450 hydroxylase that introduces hydroxyl functions with stereo- and region specificity. Using several different approaches, we have mapped functional features of the respective enzymes and have used this information to evolve novel catalytic activities for the generation of unique chemical entities—biologically active chemicals and chemicals of industrial interest.

#### P-27

The Role of Ethnomedical Knowledge in Defining Methods for Large-Scale In Vitro Cultivation: Study Cases of Two Mexican Medicinal Plants. M. L. VILLARREAL, L. Caspeta, A. Cardoso-Taketa, A. Ortíz, C. Castillom, and R. Quintero. Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, Cuernavaca 62209, Morelos, MEXICO. Email: luisav@cib.uaem.mx

Our efforts in the area of plant technology for producing active compounds from Mexican medicinal plants have been carried out using a multidisciplinary approach, which primarily involves obtaining ethnomedical information concerning the popular use of plants, in order to select appropriate candidates for study. The selected plants are subjected to pharmacological evaluation and bioguided chemical fractionation, which permits the isolation of bioactive metabolites. The molecular structure of these compounds is elucidated using conventional spectroscopic and spectrometric procedures and various biotechnological strategies have been established for the in vitro cultivation of cells and organs from the plants that will eventually produce valuable chemicals. Large-scale culture systems have been carried out in airlift bioreactors and employing a novel fitting that was specifically developed for growing hairy root cultures. In addition, we developed metabolomic analyses for the purpose of the characterization and identification of therapeutic principles among plant species that are growing in a variety of locations within the country. As examples of our integrated focus; we present the study cases of *Solanum chrysotrichum* and *Galphimia glauca*, two plant species used by indigenous people in this country for the treatment of skin infections and nervous system disorders. The focus and technological procedures used in these investigations constitute a research area that promotes the rational exploitation and conservation of autoctonous medicinal resources in Mexico.

#### P-28

Sublethal Levels of Electric Current Elicit the Biosynthesis of Plant Secondary Metabolites. E. KAIMOYO, M. A. Farag, L. W. Sumner, C. Wasmann, J. L. Cuello, and H. VanEtten, Division of Plant Pathology and Microbiology, Department of Plant Sciences, University of Arizona, Tucson AZ 85721. Email: kaimoyoe@email.arizona.edu

Many secondary metabolites that are normally undetectable or in low amounts in healthy plant tissue are synthesized in high amounts in response to microbial infection. Various abiotic and biotic agents have been shown to mimic microorganisms and act as elicitors of the synthesis of these plant compounds. In the present study, sublethal levels of electric current are shown to elicit the biosynthesis of secondary metabolites in transgenic and nontransgenic plant tissue. The production of the phytoalexin (+)-pisatin by pea was used as the main model system. Nontransgenic pea hairy roots, treated with a 30–100 mA of electric current, produced 13 times higher amounts of (+)-pisatin than the nonelicited controls did. Electrically elicited transgenic pea hairy root cultures blocked at various enzymatic steps in the (+)-pisatin biosynthetic pathway also accumulated intermediates preceding the blocked

enzymatic step. Secondary metabolites not usually produced by pea accumulated in some of the transgenic root cultures after electric elicitation due to the diversion of the intermediates into new pathways. The amount of pisatin in the medium bathing the roots of electro-elicited roots of hydroponically cultivated pea plants was ten times higher 24 h after elicitation than in the medium surrounding the roots of nonelicited control plants, showing not only that the electric current elicited (+)-pisatin biosynthesis but also that the (+)-pisatin was released from the roots. Seedlings, intact roots, or cell suspension cultures of fenugreek (*Trigonella foenum-graecum*), barrel medic, (*Medicago truncatula*), *Arabidopsis thaliana*, red clover (*Trifolium pratense*), and chickpea (*Cicer arietinum*) also produced increased levels of secondary metabolites in response to electro-elicitation. Based on our results, electric current would appear to be a general elicitor of plant secondary metabolites and to have potential for application in both basic and commercial research.

#### P-29

Engineering Ascorbate for Enhanced Growth, Nutritional Content, and Stress Tolerance in Crops. K. A. Lisko<sup>1</sup>, R. S. Harris<sup>1</sup>, J. Yactayo<sup>1</sup> and A. LORENCE<sup>1,2</sup>. <sup>1</sup>Arkansas Biosciences Institute and <sup>2</sup>Department of Chemistry and Physics, Arkansas State University, P.O. Box 639, State University, AR 72467. Email: [alorence@astate.edu](mailto:alorence@astate.edu)

Vitamin C (L-ascorbic acid, AsA) has great importance for human and plant health. In plants, AsA is the most abundant water-soluble antioxidant, acting as a scavenger of reactive oxygen species generated during normal metabolism and under various types of stress. Ascorbate is also a cofactor for numerous enzymes and a regulator of multiple fundamental cellular processes such as the cell cycle and cell expansion. Increased vitamin C has been engineered in plants by us and others via overexpression of genes from various branches of the biosynthetic network, by increasing the activity of the dehydroascorbate recycling enzyme and by suppressing ascorbate oxidase or malate dehydrogenase. We have found that *Arabidopsis* lines overexpressing genes that participate in the inositol route to ascorbate [e.g., *myo*-inositol oxygenase (MIOX), glucuronate reductase (GlcUAR), or L-gulonolactone oxidase (GLOase)], containing 2–3 times more AsA than wild-type plants, are tolerant to multiple stresses such as salt, cold, heat, and methyl viologen. Moreover, these lines exhibit tolerance to common environmental pollutants such as trichloroethylene and pyrene, a model polycyclic aromatic hydrocarbon. We have also observed a remarkable positive effect of elevated AsA on the growth of both aerial and underground tissues of the *Arabidopsis* MIOX, GlcUAR, and GLOase overexpressers. An initial screening of mustards

with naturally high levels of AsA growing in Arkansas was carried out, of which watercress (*Nasturtium officinale*), and yellow rocket (*Barbarea vulgaris*) are prime subjects for further studies. Engineering crops and trees to have elevated vitamin C may lead to increased biomass, nutritional value, stress tolerance, and enhanced phytoremediation capabilities.

#### P-30

Genetic Improvement of Dedicated Energy Crops. S. R. THOMAS. Ceres, Thousand Oaks, CA 91320. Email: [sthomas@ceres-inc.com](mailto:sthomas@ceres-inc.com)

A wide range of technical approaches are being deployed to meet the many and diverse needs of the emerging bioenergy industry. These needs include those of the feedstock producer and the various biorefinery owners and operators and the need to create an economically viable value chain in this nascent industry. Increased biomass yield remains the primary goal of the plant breeder, but optimization of production costs is also important. Biomass yield is important because of its impact on transport distance and delivered feedstock cost at the biorefinery. Biomass chemical composition and conversion characteristics are other important characteristics because of the direct relationship to theoretical and actual conversion yields. Many laboratories are tackling these issues in a range of crops. The US Department of Energy and United States Department of Agriculture are creating numerous new initiatives. The science base that is required to achieve these goals sufficiently rapidly is extensive. It encompasses the exploitation of diverse germplasm collections, characterization of the collections to discover useful genes and combinations of genes, and breeding programs to create and select transgenic and nontransgenic plants and populations with better performance in the field in various environments, as well as in various possible downstream processes. Examples of progress in these topics will be presented from the activities of Ceres and other organizations.

#### P-31

Genetic Manipulation of Lignin Biosynthesis to Improve Biomass Characteristics for Agro-industrial Processes. FANG CHEN and Richard A. Dixon. Plant Biology Division, Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401. Email: [fchen@noble.org](mailto:fchen@noble.org)

Lignocellulosic bioenergy crops hold promise as sustainable sources of biomass for ethanol production. Recalcitrance to saccharification, including the requirement for acid pretreatment, is recognized as the major limitation to

efficient conversion of lignocellulose to ethanol. Independent down-regulation of ten individual enzymes in the monolignol pathway by expression of antisense transgenes has generated a series of otherwise isogenic alfalfa lines with varying amounts of lignin and widely differing lignin compositions. These plants show various visible growth phenotypes, from essentially normal ones to plants with delayed flowering, reduced height, increased lodging or a branching habit, and possessing significant differences in vascular cell size and number. The relationship between lignin and saccharification of plant cell walls for bioethanol production were investigated to determine the relationships between lignin content/composition and chemical/enzymatic saccharification for bioethanol processing. Our results showed that recalcitrance to saccharification of stem tissues is directly proportional to their lignin content. Lignin composition does not affect enzymatic saccharification of acid-pretreated tissues. Genetic modification of lignin biosynthesis in alfalfa can improve enzymatic hydrolysis efficiency by twofold and possibly eliminate the requirement for costly chemical pretreatment in biofuel production.

### P-32

Modifying the Corn Genome to Improve Its Biomass Biofuel Production. MARIAM STICKLEN, Chuansheng Mei, Callista Ransom, Sang-Hyuck Park, Robab Sabzikar, and Spring Qi. Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824. Email: stickle1@msu.edu

Major costs associated with production of cellulosic ethanol include (1) production of hydrolysis enzymes in microbial bioreactors and (2) pretreatment processes to break down plant lignocellulosic matter into intermediates, remove lignin, and allow the access of hydrolysis enzymes to cellulose. The speaker's team recently reported production of *Acidothermus cellulolyticus* endo-1,4-beta glucanase (E1) in maize. The maize-produced transgenic E1 was able to convert ammonia fiber explosion-pretreated corn stover into glucose. Furthermore, the team produced *Trichoderma reesei* cellobiohydrolase I (CBHI) in plants, and studied the ratio of E1/CBHI needed for the best conversion of cellulose into glucose. In these studies, commercial beta-glucosidase (Novozyme 188, Sigma) was added to convert cellubiose into monomer glucose. Most recently, the team produced South African rumen *Butyrivibrio fibrisolvens* H17c beta-glucosidase in maize plants too. Maize-produced beta-glucosidase enzyme also showed strong biological

activity and could convert commercial cellubiose into glucose. To improve the level of biological activity of hydrolysis enzymes in maize, the team tested production of E1 in different subcellular compartments. Subcellular targeting of hydrolysis enzymes is recommended to allow higher accumulation of enzymes in the presence of less proteases, better folding of proteins where there are molecular chaperons, and keeping the hydrolysis enzymes away from cell walls where they could otherwise cause in situ cell wall deconstruction. Lignin biosynthesis pathway enzyme down regulation can modify lignin configuration and/or reduce plant lignin contents. At this session, eminent lignin experts will present lignin pathway enzymes silencing in other plants. Our team studied down regulation of three of lignin biosynthesis pathway enzymes in maize via interfering RNA technology and found that down regulation of certain enzymes might reduce the level of needs for biomass pretreatment processes.

### P-33

Agrobacterium-mediated Transformation of Switchgrass. Yajun Xi<sup>1,2</sup>, Chunxiang Fu<sup>1</sup>, Yaxin Ge<sup>1</sup>, Xirong Xiao<sup>1</sup> and ZENG-YU WANG<sup>1</sup>. <sup>1</sup>Forage Improvement Division, The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401 and <sup>2</sup>College of Agriculture, Northwest A and F University, Yangling, Shanxi 712100, CHINA. Email: zywang@noble.org

Switchgrass (*Panicum virgatum* L.) has been chosen as a model bioenergy crop by the US Department of Energy based on its high biomass production, high nutrient use efficiency, wide geographic distribution, and environmental benefits. Switchgrass contains abundant sugars in the form of cellulose and hemicellulose, which can be converted to ethanol by hydrolysis and subsequent fermentation. We have developed a protocol that allows for the generation of transgenic switchgrass plants by *Agrobacterium tumefaciens*-mediated transformation. Embryogenic calluses induced from caryopses or inflorescences were used as explants for inoculation with *A. tumefaciens* strain EHA105. Soil-grown switchgrass plants were regenerated about 6 months after callus induction and *Agrobacterium*-mediated transformation. Lignification of grass cell walls has been identified as the key factor affecting enzymatic hydrolysis and utilization of plant structural polysaccharides for industrial and nutritional purposes. We have cloned major genes involved in lignin biosynthesis and regenerated transgenic switchgrass plants with interfering RNA constructs of the lignin genes.