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Plant Symposia

P-1

Genetic Transformation: Bridging from Functional Genomics to Specialty Crop Improvement. GUO-QING SONG. Plant Biotechnology Resource and Outreach Center, Department of Horticulture, Michigan State University, East Lansing, MI 48824. Email: songg@msu.edu

Michigan is the US leader in the production of numerous specialty crops including bedding plants, blueberries, dry beans, cherries and pickling cucumbers. The use of biotechnology to improve specialty crops has fallen far behind the agronomic crops due mainly to technical barriers as well as the lack of investment. The Plant Biotechnology Resource and Outreach Center (PBROC) of Michigan State University (MSU) is dedicated to the safe deployment of plant biotechnological advancements to Michigan and the world. The overall research goal of the PBROC is to provide biotechnology tools for the unique needs of Michigan agriculture to ensure healthier, more productive specialty crops. Over the past decade, the PBROC has made many solid advancements including: 1) greatly improving the transformation efficiency of blueberry, celery, and cherry, 2) developing efficient transformation systems for biofuel crops (rutabaga, canola, and switchgrass), 3) assembling a library of useful vectors, producing the first transgenic blueberry and celery carrying herbicide resistance, 4) initiating a RNA interference (RNAi)-mediated cherry virus resistance project, 5) producing transgenic blueberry with cold tolerance using an intragenic transformation strategy, and 6) producing mannitol-added transgenic tomato using celery mannose-6-phophate reductase gene (*M6PR*). As a proof of concept, these advancements have demonstrated the potential application of genetic transformation for improvement of specialty crops. Moving forward in the genomic era, reliable biotechnology tools will play an important bridge role in using functional genomics to improve specialty crops.

P-2

Progress in the Development of Intragenic Grapevine. D. J. GRAY[1], Z. T. Li[1], and S. A. Dhekney[2]. [1]Mid-Florida Research & Education Center, University of Florida/IFAS, 2725 Binion Road, Apopka, FL 32703 and [2]Department of Plant Sciences, University of Wyoming, Sheridan Research & Extension Center, 663 Wyarno Road, Sheridan, WY 82801. Email: djg@ufl.edu

Intragenic technology differs from transgenic technology in that it uses only genetic elements from the host plant to improve/modify target-specific traits. Implementation of "intragenics" requires availability of functional endogenous genes, promoters and terminators. In grapevine, the sequenced genome provides a wealth of information with which endogenous genetic elements can be readily identified and utilized. To date, we have tested approximately 40 endogenous promoters. The *Vitis vinifera* MybA1 (VvMybA1) transcription factor gene, which promotes anthocyanin production, is being developed as a visual marker for selection. The *V. vinifera* thaumatin-like protein gene (VvTL-1) has been shown to confer broad spectrum fungal disease resistance in greenhouse and field trials. Putative genes to induce seedlessness, as well as additional disease resistance genes are being evaluated. Efforts are ongoing to combine all of the endogenous elements necessary to create true intragenic plants with improved agronomic performance.

P-3

Clonal Propagation of GMO Virus Resistant Papaya Hybrids and, Someday, Maybe Non-GMO Ones. MAUREEN M. M. FITCH. Hawaii Agriculture Research Center, P.O. Box 100, Kunia, HI 96759. Email: mfitch@harc-hspa.com

In the 20 years that transgenic Rainbow, SunUp, and Laie Gold papayas have been sold, most of the plants have been produced from hybrid or inbred (SunUp) seeds. About 85 % of Hawaii's papayas are transgenic with *Papaya ringspot virus* (PRSV) resistance. Private companies produce the F1 hybrid seeds from transgenic X non-transgenic parent crosses or self the inbred cultivar. Micropropagated and rooted cuttings of high quality Rainbow and Laie Gold hermaphrodites have been generated on small scale at the Hawaii Agriculture Research Center for research. We produced several thousand hermaphrodite Rainbow plants that have borne fruit one to three months earlier, lower on the trunk, and resulted in significantly increased yields compared to traditionally multiple-planted seedlings that were thinned to a single hermaphrodite at flowering time (Fitch et al., 2005a, b). Sufficient quantities of plants have not been

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generated to supply growers as a result of low rooting ability in micropropagation and high labor costs associated with both methods. Taiwan and China reportedly have developed low-cost in vitro and acclimatization methods that provide plants to part or most of the industries. While we will try to acquire and adapt the techniques employed by these groups, in the meantime, we are attempting to improve in vitro rooting, the most important bottleneck. The low commercialization rate of transgenic virus resistant papayas, only by the US and China to date, suggests a need for alternative solutions. Introgression of PRSV resistance from wild relatives by R. Drew of Australia and colleagues (O'Brien Siar et al., 2011) may yield useful backcrosses. Experiments are ongoing in Hawaii with hybrids from Dr. Drew. Micropropagation of selections may be the preferred method of propagation if resistance levels between seedlings vary.

P-4

Manipulating Fruit Genomes – Discovery to Application. A. DHINGRA[1,2], K. Nicholson[1], N. Tarlyn[1], D. Jiwan[1], S. Schaeffer[2], M. Swanson[3], and K. Evans[4]. [1]Department of Horticulture, PO Box 646414, Washington State University, Pullman, WA 99164; [2]Molecular Plant Sciences Graduate Program, Washington State University, Pullman, WA 99164; [3]School of the Environment, PO Box 646410, Washington State University, Pullman, WA 99164; and [4]Tree Fruit Research and Extension Center, 1100 N. Western Ave., Wenatchee, WA 98801. Email: adhingra@wsu.edu

As more sequenced genomes from perennial fruit-bearing plant species become available, there is an urgent need to establish gene(s)-trait relationships to develop desirable fruit varieties incorporating multiple traits in an efficient and timely manner. Recently sequenced fruit genomes of apple, peach, grape (available publicly), pear and sweet cherry (unpublished) have opened up avenues for the development of physiogenomics-mediated in-field management strategies and the utilization of gene-based knowledge in varietal improvement. However, establishment of gene(s)-trait relationships and development of new varieties in perennial crops are time-consuming owing to long juvenility periods. Genetic populations for some perennial fruit-bearing species are inadequate to carry out forward genetic analysis to establish gene-trait relationships. We are addressing these issues by developing platforms for reverse genetics approaches in *Vitis* sps., for example, to enable reverse genetics studies; using phylogenetically related surrogate systems to test mechanistic gene action such as disease resistance in Rosaceae; and transgenic induction of rapid flowering in advanced apple breeding selections to obtain pollen in a single year to speed up the process of varietal improvement. Availability of these tools, in combination with rapidly accumulating physiological and genetic information in fruit-bearing perennial crops, will enhance the efficiency and speed of managing perennial crops in the field and the development of novel and desirable varieties.

P-5

Public Sector Delivery of Transgenically Enhanced Cassava to African Farmers. NIGEL TAYLOR, Mark Halsey, Eliana Gaitan-Solis, Paul Anderson, and Claude Fauquet. Donald Danforth Plant Science Center, 975 N. Warson Rd. St Louis, MO 63130. Email: ntaylor@danforthcenter.org

The tropical root crop cassava (*Manihot esculenta*) is an essential component of food and economic security throughout much of tropical Africa. Two large projects based at the Donald Danforth Plant Science Center are committed to production and delivery of transgenically enhanced cassava to small-holder farmers in East and West Africa. The Virus Resistant Cassava for Africa (VIRCA) project is utilizing RNAi technologies to produce cassava with resistance to cassava brown steak disease (CBSD) and cassava mosaic disease (CMD) for farmers in Uganda and Kenya, while BioCassava Plus is developing cassava with nutritionally enhanced storage roots for deployment in Nigeria. Both projects encompass all steps of a product delivery process including scientific proof of concept, large scale transgenic plant production and molecular screening, multiple confined field trials for event selection, and for trait and yield determinations, intellectual property rights, environmental and food safety regulatory assessments and dissemination of the de-regulated product. Very little experience exists within the public sector for handling complex, multi-institutional projects of this type, most especially when most field and regulatory work must be performed within the African partner countries, where systems for handing transgenic plants are still under development. Focusing mostly on the VICRA project, progress will be reported on the technologies, field trials, logistics, capacity building and associated challenges that are being met, and still face these projects, if they are to result in delivery of improved planting materials for the intended small-holder farmers.

P-6

Biofortified Sorghum for Africa. Z.-Y. ZHAO, K. Glassman, P. Che, and M. Albertsen. Pioneer Hi-Bred International, Inc., Du Pond Ag Biotech, 7300 NW 62nd Ave. Johnston, IA50131. Email: zuo-yu.zhao@pioneer.com

The African Biofortified Sorghum (ABS) project, a joint project with multiple organizations and coordinated by Africa Harvest Biotech Foundation International (AHBFI), was funded by the Bill and Melinda Gates Foundation from 2005 to 2010 to develop nutritionally improved sorghums

for the arid and semi-arid tropical areas of Africa. Additional technology development funding for four years was provided by the Howard G. Buffet Foundation in 2011. Sorghum is used as the primary staple food for 300 million people in Africa. People that rely upon sorghum as their staple diet exhibit significant deficiencies in micronutrients, such as iron and zinc and often exhibit severe vitamin A deficiency. In addition, sorghum protein digestibility is reduced 20-40% by cooking. Therefore a diet based mostly on sorghum is not adequate to meet the nutritional growth or maintenance requirements for children and adults. Without micronutrient and provitamin A supplementation, young children are doomed to a life of poor vision, anemia, immune deficiencies, and numerous other maladies. The goal of the ABS project is to develop transgenic sorghum varieties that will overcome most of the described nutritional deficiencies by substantially improving the bioavailability of iron and zinc, by enhancing levels of provitamin A and by improving grain protein digestibility. The development of improved sorghum lines relies upon transgenes and biotechnologies that have shown efficacy in other transgenic crops. As a proof of concept, a number of nutrition-related genes have been transformed into sorghum plants via *Agrobacterium* transformation. Molecular and biochemical analysis of grain produced on these sorghum transgenics has demonstrated that significant improvements in levels of provitamin A, in improved bioavailability of iron and zinc, and in improved grain protein digestibility can be achieved.

P-7

Public-private Partnerships for Delivering Stress-tolerant Maize Hybrids to African Farmers: CIMMYT's Experience. G. N. ATLIN. International Maize and Wheat Improvement Center (CIMMYT) and Dept of Plant Breeding and Genetics, Cornell University, 310 Bradfield Hall, Ithaca NY 14853. Email: g.atlin@cgiar.org

Maize productivity in Africa is limited by pests, diseases, drought, low soil fertility, and poor access to inputs. CIMMYT works with multinational seed companies to access technology, unavailable in-house, that can help us deliver improved germplasm to African smallholders. Our two largest public-private partnerships (PPPs) are "Water-Efficient Maize for Africa" (WEMA), a collaboration with Monsanto, and "Improved Maize for African Soils" (IMAS), with Pioneer Hi-Bred. Both collaborations are complex partnerships involving national research programs, regional seed companies, and the donation of transgenic technology by the private-sector partner to the project under humanitarian license. The private partners provide technical support for transgene testing, deregulation, deployment, and stewardship, as well as for molecular genetic analysis and, in the case of WEMA, conventional breeding. CIMMYT and its regional partners contribute germplasm, breeding capacity, native trait genetic analysis, and coordination. Donor support (from the Gates Foundation and USAID) for these projects makes possible the deployment of transgenic abiotic stress tolerance technologies in regions where they could not be profitably commercialized. The PPPs have become an important means for improving public sector capacity in transgenic technology, native trait analysis, phenotyping, and breeding pipeline management. Private sector partners benefit from the development of new potential markets in the medium term, and from a deeper understanding of CIMMYT's elite stress-tolerant germplasm.

P-8

Increasing *Agrobacterium*-mediated Genetic Transformation by Manipulating the Plant Genome. STANTON B. GELVIN. Department of Biological Sciences, Purdue University, West Lafayette, IN 47907. Email: gelvin@bilbo.bio.purdue.edu

Agrobacterium-mediated plant genetic transformation is a core technology for both plant molecular sciences and for agricultural biotechnology. Although *Agrobacterium*-mediated transformation has been used to generate transgenic plants for more than 25 years, many agronomically important species, or particular genotypes of these species, remain highly recalcitrant to transformation. Scientists have increased transformation frequency by manipulating tissue culture conditions and by developing highly virulent *Agrobacterium* strains. As an additional approach, for the past decade we have investigated plant genes and proteins important for plant susceptibility to transformation. By characterizing *Arabidopsis* mutants that are either resistant or hyper-susceptible to *Agrobacterium* transformation (rat or hat mutants), we have identified a large number of host genes involved in the transformation process. In some cases, over-expressing these "transformation genes" can increase transformation frequency, even in heterologous plant species. I shall describe the processes by which we identified these genes and characterized their role in transformation. In particular, I shall describe our recent studies on a plant myb transcription factor that is a negative global regulator of *Agrobacterium*-mediated transformation. Manipulation of this myb transcription factor gene may enhance transformation susceptibility of important crop species.

P-9

Site-specific Integration and Trait Stacking in Corn; Implications for Transformation. C. J. SCELONGE[1], D. J. Peterson[1], D. L. Bidney[1], and C. Falco[2]. [1]Dupont Agricultural Biotechnology, 7300 NW 62nd Avenue, Johnston, IA, 50131 and [2]Dupont Agricultural Biotechnology, Dupont Experimental Station, Route

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Site-specific integration (SSI) in plants has the potential to change both basic transgenic research and product development. Routine use of the yeast FLP/FRT system for recombinase-mediated SSI in corn is creating the need for greater knowledge in areas such as frequency of perfect integration, and transgene expression at a given insertion site or target locus, in order to better realize the full potential of this technology. Agrobacterium-mediated plant transformation used for gene testing and biotech product development generates random T-DNA insertions in the genome of the plant with a good deal of associated variation in performance. Significant numbers of Agrobacterium insertions need to be characterized due to the variation in gene expression arising from the genomic insertion site. A more ideal state would be to eliminate the random variation of multiple insertion sites and focus on fewer selected insertion sites and controlled variation by construct design. SSI facilitates the reuse of an insertion site by providing the capability to replace the original genes in a site with new genes. Preferred insertion sites for this work are made from SSI capable constructs and then selected using standard empirical procedures for having the best efficacy and agronomic properties. Introduction of new genes at sites pre-selected for the best performance provides an opportunity to obtain more informative data on introduced genes with less confounding effects due to the insertion site. In this manner, the need to generate large numbers of independent insertion sites in construct testing could be reduced. Cre-loxP mediated gene excision can be combined with SSI to allow complete replacement of genes at a preferred insertion site by excision of the original genes followed by SSI to introduce new genes. SSI alone at the preferred site permits the addition of genes for molecular gene stacking. Implementing SSI technology will fundamentally change the transformation process deployed for gene testing and product development.

P-10

Endophytes for Enhanced Plant Growth, Pollutant Degradation and Biofuels. S. L. DOTY, Zareen Khan, Jun Won Kang, Jenny Knoth, David Roman, Hannah Morrison, and Amy Baum. School of Environmental and Forest Sciences, College of the Environment, University of Washington, Seattle, WA. Email: sldoty@uw.edu

We isolated from poplar and willow trees a variety of microbial endosymbionts with properties including plant growth promotion, pollutant degradation, nitrogen fixation, and biofuel production. With the range of beneficial traits provided, these natural endophytes provide an alternative to plant or microbial genetic engineering. The microbes that produced phytohormones and fixed atmospheric nitrogen enhanced the growth not only of poplar but also of agronomically-important crops including tomatoes, pepper, rice, corn, and turf grasses. Endophytes were also isolated that degrade the environmental pollutants, TCE, PAHs, and explosives. When in partnership with plants, there is a greater potential for pollutant remediation that can be achieved by either alone. Some of the endophytes that were isolated were identified as yeasts. These strains ferment both pentoses and hexoses and are resistant to common inhibiting phytochemicals, making them valuable for the biofuel industry. By utilizing nitrogen-fixing endophytes instead of chemical fertilizers in growing biomass, biofuel production can be more environmentally-sustainable and economically-viable. By including pollutant-degrading endophytes as well as nitrogen-fixing endophytes, high biomass plants such as poplar and willow could be grown on poor sites, providing biofuel and environmental services.

P-11

Overview of Next Generation Transformation Technology and Vector Design to Improve Plastid Transformation Efficiency. D. J. OLDENBURG, R. A. Kumar, and A. J. Bendich. Department of Biology, University of Washington, Box 351800, Seattle, WA 98195. Email: delene@uw.edu

Recent advances in plant transformation include the use of minichromosomes, mechanisms for targeted transgene delivery, and removal of selectable markers from the transgenic plant. Most approaches to improving plant transformation focus on transgene introduction and expression in the nuclear genome. There are, however, certain advantages in transformation of the plastid genome, including multiple transgenes on operons, high gene copy number, no gene silencing, and biological containment by maternal inheritance. Four issues will be considered in the development of procedures for plastid transformation in maize, for which plastid transformation has not yet been reported. First, knowledge of cpDNA replication, maintenance, and degradation indicates that the integrity of resident cpDNA molecules is lower in green leaves than in the base of the stalk and dark-grown leaves, making the latter the tissues of choice for plastid transformation. Second, the efficiency of plastid transformation with liverwort and tobacco is higher using a linear vector rather than a circular vector. Third, identification of the sequences at the ends cpDNA molecules from maize allows the design of vectors with recombinogenic ends that should improve transformation

efficiency. Lastly, information on nucleus-integrated plastid DNA (NUPT) sequences can be used to design PCR primers and introduce unique restriction sites into the plastid vectors, so as to identify true transgene integration into the plastid genome and establish homoplasmy without interference from NUPTs.

P-12

Development of Site-specific Recombinase Technology for Precise Crop Plant Genome Modification. JAMES THOMSON. Crop Improvement and Utilization Unit, Western Regional Research Center, USDA, Albany, CA. Email: James.Thomson@ARS.USDA.GOV

Plant biotechnology has a role in addressing global needs for food, fiber and fuel, by developing new crop varieties with increased pest resistance, biofortification, and abiotic stress tolerance. Publicly acceptable forms of biotechnology offer an avenue for meeting these demands. Recombinase-mediated genetic engineering provides a favorable direction for enhancing the precision of biotechnological approaches. Concerns over the presence of antibiotic resistance genes in the food supply and their escape into the environment can be relieved through the use of recombinase technology to excise unwanted DNA from the genome of genetically engineered (GE) crops prior to marketing or release. Previous studies have documented how use of site-specific recombination can produce transgenics with stable gene expression over multiple generations and also resolve multicopy transgene inserts, initially silenced for expression, to a single functional genomic copy. Research in this lab addresses the need of novel publicly available recombinases. Our team has developed a series of novel recombinases for use as molecular tools. We have identified and characterized the recombinase Bxb1, CinH, ParA and phiC31 for practical use towards genomic engineering of crop plants. We describe here the unique features of each recombinase, practical application and potential combinatorial approaches for recombinase utilization.

P-13

An Integrated Dual Recombination System for Use in Producing Clean Transgenic Plants. H. LUO, Z. Li, Q. Hu, M. A. Machado, R. L. McLaurin, and M. Zhou. Department of Genetics and Biochemistry, Clemson University, 100 Jordan Hall, Clemson, SC 29634. Email: hluo@clemson.edu

The environmentally responsible utilization of transgenic techniques in crop species requires the development of transgene containment strategies. We have developed an integrated dual site-specific recombination system for eliminating unwanted DNAs in transgenic plants. The functionality of the system has been tested using FLP/*FRT*, Cre/*lox* and reporter genes in plants by producing two independent transgenic lines. In the first line, the *Cre* recombinase gene linked to a hygromycin resistance gene, *hyg* and an inducible promoter is flanked by FLP recombinase target site, *FRTs* and serves to separate a constitutive promoter from a reporter gene *gusA*. In the second line, the Cre target site *loxP*-flanked herbicide-resistant gene, *bar* linked to the *FRT*-flanked *FLP* gene serves to separate a constitutive promoter from a green-florescent gene, *gfp*. When the two transgenic lines are cross-pollinated, the Cre in the hybrids catalyzes the excision of the *loxP*-flanked *bar*, which brings into proximity the upstream constitutive promoter and the downstream *FRT*-flanked *FLP*, resulting in FLP expression and consequently self-excision of *FLP* gene. This in turn brings together the constitutive promoter and the downstream *gfp*, resulting in GFP expression. FLP also catalyzes excision of the *FRT*-flanked *Cre* and its linked *hyg* as well as the inducible promoter, bringing together the constitutive promoter and *gusA*, leading to GUS expression. The reporter genes serve to evaluate the efficacy of the system and will be replaced, in commercial scenarios, with gene of interest and a sterility-inducing construction. The inducible promoter serves as a safeguard in case of incomplete DNA recombination.

P-14

Plant Genome Editing: Knockouts, Gene Stacking and Targeting. L. A. LYZNIK, V. Djukanovic, and S. Jones. Pioneer Hi-Bred International, A DuPont Business, Research Center, Johnston, IA 50131. Email: alex.lyznik@pioneer.com

Advances in genetic engineering have been changing the methods of transgenic plant production. Site-specific (SSR) and homologous recombination (HR) are key elements in making the genome editing process a consistent option for controlled genetic modification of any genome. While site-specific recombination has effectively been used for gene stacking or re-configurations of the inserted foreign DNA fragments, the HR technology based on the introduction of double-strand breaks (DSB) provides precision, flexibility, and universality in introducing any genetic modifications of our choice. The efficiency and specificity of re-designed endonucleases are two crucial factors that are taken into account when weighting the usefulness of this technology for applied genome editing activities. Whether zinc-finger nucleases, homing endonucleases, or TAL-effector nucleases (TALENs) are concerned, they provide a sufficient number of double-strand breaks for dependable targeted mutagenesis in virtually any tested organism and chromosomal locus. However, when HR-based DSB repair mechanisms are employed instead of the non-homologous

end joining reactions (NHEJ), the process may be feasible, but not reliable. The practical implications related to the development of the DSB-based gene editing technology will be illustrated on maize plants using the I-*Cre*I-based, redesigned homing endonucleases as the double-strand break-making reagents. Regardless of the experimental system used, the challenge is to either make more double-strand breaks or to divert the existing double-strand breaks into the HR-based repair pathways. With the advancements of protein engineering and genetic transformation technologies, the truly controlled genetic engineering of plant species becomes a reality in many laboratories around the world.

P-15

Application of FLP-FRT System for Site-specific Gene Integration in Rice. V. SRIVASTAVA. Department of Crop, Soil & Environmental Sciences, University of Arkansas, Fayetteville, AR. Email: vibhas@uark.edu

Precise full-length integration of transgenes is desirable for ensuring optimum gene expression. Site-specific recombination systems are versatile tools for catalyzing DNA integration, excision or inversion. A number of site-specific recombination systems have been isolated and shown to excise specific DNA fragments from the transgene locus; however, only a few have been used for transgene integration in plant genomes. We have developed FLP-FRT system for efficient targeting of foreign gene into the engineered genomic site in rice. The transgene vector containing a pair of directly oriented FRT sites was introduced by particle bombardment into the cells containing an engineered target site. FLP activity generated by the co-bombarded FLP gene generated the backbone-free gene circle that integrated into the target site. The majority of the transgenic events contained the precise integration locus, expressed the transgene, and transmitted the stable site-specific integration locus to progeny.

P-16

In Vitro Conservation Tools in the Age of Extinction. K. W. DIXON and E. Bunn. Kings Park and Botanic Garden, West Perth, 6005, Western Australia, AUSTRALIA. Email kingsley.dixon@bgpa.wa.gov.au

We are now recording the highest rates of species extinction on record across global biomes. Accelerated levels of habitat and species loss in the 34 global biodiversity hotspots mean conservation and restoration efforts need to be redoubled in these regions if we are to halt extinction. Here we present a review of the current in vitro approaches being employed across hotspots and find that there are significant deficiencies in the application of in vitro conservation approaches that would significantly improve the conservation lot for many plant species. We provide an outline of a systems approach for a global effort to fast-track in vitro technology with examples from the biodiversity hotspot of southwest Australia.

P-17

Ex Situ Plant Conservation in Global Biodiversity Hotspots and Island Countries: Importance of In Vitro Methods. VISWAMBHARAN SARASAN. Conservation Biotechnology, Jodrell Laboratory, Royal Botanic Gardens Kew, Richmond, Surrey TW9 3AB, UK. Email: v.sarasan@kew.org

Global biodiversity hotspots hold over 50% of the world's plant species yet cover only 2.3% of Earth's land surface. Plant species endemic to these habitats are facing extinction due to climate change, overharvesting, habitat destruction, invasive alien species and several other factors. The last decade was the most productive for seed banking for species with bankable seeds. However seeds that are difficult to collect, recalcitrant seeds and taxa with no seed at all require other options for long-term conservation. Good quality propagules underpin the success of ex situ conservation programmes. In groups such as orchids, the largest and the most diverse of all plant families, this is more relevant than any other plant group. The tropics and subtropics have more biodiversity hotspots and have the most diverse and numerous orchids compared to other parts of the world. Building capacity and increasing the number of research-driven initiatives are critical to improve the success rate of reintroduction and restoration activities especially for orchids and plant groups that are in peril.

P-18

Optimizing Cryopreservation Protocols for Plant Conservation and Restoration: The Genotypic Effect. J. J. SADLER and M. E. Kane. Environmental Horticulture Department, PO Box 110675, University of Florida, Gainesville, FL 32611–0675. Email: jjsadler@ufl.edu

Cryopreservation has proven to be an effective *in vitro* conservation tool for many plant species. Utilizing liquid nitrogen (−196°C) virtually halts physiological functions for an indefinite time period. This has led to germplasm storage facilities that utilize cryopreservation to preserve multiple species. Yet, plant vegetative tissue (i.e., meristematic tissue) must be pretreated with various solutions and temperatures to withstand immersion into liquid nitrogen. Currently, most cryopreservation optimization protocols screen few genotypes

to determine the most effective pretreatment phases for a species. However, similar to differential genotypic responses to *in vitro* culture, the ability to withstand the cryopreservation pretreatment stages and ultimate immersion into liquid nitrogen must be considered between genotypes of a species. Then, survivability following regrowth can be compared to determine the optimal protocol across genotypes. If a genotype requires a very different set of pretreatments than others, the cryopreservation protocol could result in a lower survivability rate. Therefore, optimization protocols utilizing cryopreservation for long-term germplasm storage should include a screening of genotypes before a large-scale effort is conducted. To illustrate this we present the survival results of multiple cryopreserved genotypes of sea oats (*Uniola paniculata* L.) using a protocol previously optimized with seedlings.

P-19

Maize Protoplast-based Transient Assay System for Analysis of Gene Functionality and Controlling Sequences. PAUL D. MILLER. dow AgroSciences, Agronomic Traits Discovery, Transformation, 16160 SW Upper Boones Ferry Rd., Portland, OR, 97224. Email: Miller@dow.com

A system for the isolation and transfection of maize protoplasts is being utilized as a transient assay to obtain quick feedback for testing regulatory elements and genes of interest. Leaves or roots from dark-grown seedlings are digested overnight in cellulytic enzymes and the following morning protoplasts are prepared for transfection or other manipulations. Protoplast transfection is accomplished by PEG/$CaCl_2$ induction with a typical efficiency around 50%, based on microscopic observation of fluorescence. Protoplasts prepared and transfected by this method have been used in the transient assay of: marker genes (YFP, GFP, GUS); luciferases; amiRNAs; transcription factors; plastid transit peptides; promoter testing; gene of interest function. These assays help direct vector constructions that may be used in the transformation pipeline.

P-20

The Use of Protoplasts for Citrus Improvement. MANJUL DUTT, Fred G. Gmitter, Jr., and Jude W. Grosser. Citrus Research and Education Center, University of Florida/IFAS, 700 Experiment Station Road, Lake Alfred, FL 33850. Email: manjul@ufl.edu

The application of protoplast fusion technology in citrus improvement has resulted in the regeneration of somatic hybrid plants from more than 500 parental combinations, and many of these have been evaluated in field trials. In rare cases, a new somatic hybrid may have direct utility as an improved scion or rootstock cultivar; however, the most important application of somatic hybridization is the building of novel germplasm as a source of elite breeding parents for various types of conventional crosses. Somatic hybridization is beginning to have a major impact on our scion improvement program by the generation of superior allotetraploid breeding parents for use in interploid crosses to generate seedless triploids. Seedlessness is a primary breeding objective for new fresh fruit citrus varieties, and hundreds of triploid hybrids have been produced using somatic hybrids as the tetraploid parent. Successful somatic hybridization in citrus rootstock improvement has allowed for the creation of a rootstock breeding program at the tetraploid level that achieves maximum genetic diversity in zygotic progeny and has great potential for tree size control via polyploidy. Rootstock breeding at the tetraploid level also facilitates the packaging of the many required traits for an improved rootstock into individual hybrids, including wide adaptation to diverse soil and environmental conditions, ability to consistently produce high yields of quality fruit, and especially disease and insect resistance. Several protoplast derived tetraploid selections have also been genetically modified to express insecticidal genes as potent trap plants for control of Asian Citrus Psyllid, a vector spreading huanglongbing (HLB), a devastating bacterial disease caused by a gram negative, non-indigenous but now endemic pathogen. Protoplasts have also been directly transformed with antimicrobial genes using a PEG mediated method to generate transgenic plants that can potentially control HLB. Finally, continued feedback from field trials of both scion and rootstock somatic hybrids and their progeny is facilitating the identification of superior breeding parents and cross combinations, thereby accelerating the development of improved citrus varieties.

P-21

In Vitro Chemical Mutagenesis for Improvement of Turf Quality of Bahiagrass. F. ALTPETER, B. Kannan, and P. Lomba. Agronomy Department, Genetics Institute, Plant Molecular and Cellular Biology Program, University of Florida - IFAS, Gainesville FL-32611. Email: altpeter@ufl.edu

Bahiagrass accounts for 24 % of the total sod produced in Florida. This low-input turfgrass is used for large residential lawns and as utility turf along highways. The popularity of bahiagrass is attributed to its tolerance of marginal soil fertility, excellent persistence supported by drought tolerance, heat tolerance, insect- and disease resistance and nematode suppression. However, the turf quality of bahiagrass is limited by its open growth habit, light green color and tall

seedheads. Improvement of the bahiagrass (*Paspalum notatum* Flugge) cultivar 'Argentine' by conventional breeding is very difficult due to its apomictic reproduction mode. Our objective was to explore the potential of chemical and tissue culture derived mutagenesis for genetic improvement of apomictic bahiagrass for generation of uniform mutagenized seed progeny with improved turf quality. Scarified and surface sterilized bahiagrass seeds were treated with the mutagen sodium azide at various concentrations and exposure times. Callus was induced from these seeds and regenerated via somatic embryogenesis to obtain uniformly mutagenized plants. 2,000 of the 20,000 regenerated seedlings were selected based on their vigor or morphological characteristics and transferred to soil. 46 independently mutagenized lines with reduced stem length, higher tiller density or reduced or delayed seedhead formation were established under field conditions in 1.2 m x 1.2 m plots in a randomized block design with 4 replications for further evaluation of density, leaf texture, tiller length, color, growth pattern, biomass, seedhead and seed production, as well as seedling vigor. A superior mutagenized bahiagrass line with improved turf characteristics and production of viable seeds was identified. Data describing the performance of this elite line in comparison to non-mutagenized bahiagrass under non-irrigated conditions will also be presented.

P-22

Oil Modification via Transcriptional Activation of Canola KASII Using an Engineered Zinc Finger Transcription Factor. Manju Gupta[1], Russell C. DeKelver[2], Asha Palta[1,3], Carla Clifford[1], Sunita Gopalan[2], Jeffrey C. Miller[2], Stephen Novak[1], Daniel Desloover[1], Daniel Gachotte[1], James Connell[1], Josh Flook[1], Thomas Patterson[1], Kelly Robbins[1], Edward J. Rebar[2], Philip D. Gregory[2], Fyodor D. Urnov[2], and JOSEPH F. PETOLINO[1]. [1]Dow AgroSciences, 9330 Zionsville Rd., Indianapolis, IN 46268; [2]Sangamo BioSciences, 501 Canal Blvd., Richmond, CA 94804; and [3]Current address: Targeted Growth Inc., 6767 East 276th St., Atlanta, IN 46031. Email: jfpetolino@dow.com

Targeted gene regulation via designed transcription factors has great potential for precise phenotypic modification and acceleration of novel crop trait development. Canola seed oil composition is dictated largely by the expression of genes encoding enzymes in the fatty acid biosynthetic pathway. In the present study, zinc finger proteins (ZFPs) were designed to bind DNA sequences common to two canola β-Ketoacyl-ACP Synthase II (KASII) genes downstream of their transcription start site. Transcriptional activators (ZFP-TFs) were constructed by fusing these ZFP DNA binding domains to the VP16 transcriptional activation domain. Following transformation using *Agrobacterium*, transgenic events expressing ZFP-TFs were generated and shown to have elevated KASII transcript levels in the leaves of transgenic T_0 plants when compared to 'selectable marker only' controls as well as of T_1 progeny plants when compared to null segregants. In addition, leaves of ZFP-TF-expressing T_1 plants contained statistically significant decreases in palmitic acid (consistent with increased KASII activity) and increased total C18. Similarly, T_2 seed displayed statistically significant decreases in palmitic acid, increased total C18 and reduced total staturated fatty acid contents. These results demonstrate that designed ZFP-TFs can be used to regulate the expression of endogenous genes to elicit specific phenotypic modifications of agronomically relevant traits in a crop species.

P-23

RNA Interference and Plant Biotechnology. JOHN MCMILLAN and Peifeng Ren. BASF Plant Science, 26 Davis Dr. Research Triangle Park, NC 27709. Email: john.mcmillan@basf.com

The use of RNAi technology to silence expression of specific gene targets has been a boon to plant research both in academia and in industry. Today this technology is starting to be applied to many agriculturally important plant species. RNA interference can be induced in plants by the expression of long dsRNAs or by the expression of a microRNA (miRNA) gene. Of these two approaches, the more frequently used is long dsRNA. Typically, in plants, an engineered long dsRNA is created by combining several hundred bases of the sense and antisense sequence of the RNA to be targeted, often with a spacer sequence inserted between the inverted repeats. When transcribed, a long stem loop structure is formed that is recognized and processed by members of the dicer family of endonucleases. This processing typically yields an abundance and variety of small interfering RNAs (siRNAs). In contrast, with miRNA technology, a single, predominate small RNA species is produced. miRNAs are embedded within longer non-coding RNAs and are also processed by a member of the dicer family, yielding the mature miRNAs. In this talk, we will compare these two technologies (long dsRNAs and miRNAs), and discuss the advantages and disadvantages of each approach. In addition, we will describe novel ways in which miRNA technology can be used for fine-tuning of gene expression and potential applications in plant biotechnology.

P-24

Next Generation Gene Silencing Vectors in Soybean. THOMAS B. JACOBS[1], Peter R. LaFayette[1], Lila O. Vodkin[2], and Wayne A. Parrott[1]. [1]University of Georgia, Center for Applied Genetic Technologies, Athens, GA and

[2]University of Illinois, Department of Crop Science, Urbana, IL. Email: tbj03001@uga.edu

Most gene-silencing attempts involve the use of hairpin constructs, in which a portion of a target gene is cloned as an inverted repeat separated by an intron or a spacer sequence. An alternative approach is to utilize the *trans-acting* siRNA (tasiRNA) pathway in plants, whereby a short 22-nt 'tag' is fused to a target gene to induce the production of siRNA. Montgomery et al. (2008) demonstrated in arabidopsis that a target of miR173 fused to a *phytoene desaturase* (PDS) gene silenced native expression of PDS. Chen et al. (2010) and Cuperus et al. (2010) further showed that miRNAs that induce the tasiRNA pathway are generally 22-nt-long. Preliminary results indicate the 22-nt miR173 fused to a portion of the *GUSPlus* (GP) gene suppresses GP expression in soybean homozygous for GP. Although the sequencing of the small RNA populations in these constructs has yet to be completed, sequencing of soybean hairy root cultures identified several miRNAs that might be inducing the tasiRNA pathway in hairy roots, as phased siRNAs were found homologous to sequences beyond the predicted miRNA cut sites. Genes were targeted by fusing the recognition site for the putative tasiRNA-inducing miRNA, miR1514, to a portion of target cDNA. RT-PCR analysis confirms the silencing of the targeted genes, indicating that miR1514 is a bona fide tasiRNA-inducing miRNA. Together, these results suggest that simple vectors with a 22-nt silencing 'tag' fused to a target gene may be able to effectively silence gene expression.

P-25

Overexpression of miR156 for Switchgrass Improvement. C. Fu[1], R. Sunkar[3], C. Zhou[1], H. Shen[2,5], J. Zhang[2,5], J. Matts[3], J. Wolf[l], D. Mann[4,5], N. Stewart Jr.[4,5], Y. Tang[2,5], and Z.-Y. WANG[1,5]. [1]Forage Improvement Division, [2]Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK; [3]Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, OK; [4]Department of Plant Sciences, University of Tennessee, Knoxville, TN; and [5]BioEnergy Science Center. Email: zywang@noble.org

Switchgrass (*Panicum virgatum* L.) has been developed into a dedicated herbaceous bioenergy crop. Biomass yield is a major target trait for genetic improvement of switchgrass. microRNAs have emerged as a prominent class of gene regulatory factors that has the potential to improve complex traits such as biomass yield. A miR156b precursor was overexpressed in switchgrass. The effects of miR156 overexpression on SQUAMOSA PROMOTER BINDING PROTEIN LIKE (SPL) genes were revealed by microarray and quantitative RT-PCR analyses. Morphological alterations, biomass yield, saccharification efficiency and forage digestibility of the transgenic plants were characterized. miR156 controls apical dominance and floral transition in switchgrass by suppressing its target *SPL* genes. Relatively low levels of miR156 overexpression were sufficient to increase biomass yield while producing phenotypically normal plants. Moderate levels of miR156 led to improved biomass but the plants were non-flowering. However, high miR156 levels resulted in severely stunted growth. The degree of morphological alterations of the transgenic switchgrass depends on miR156 level. The improvement in biomass yield was mainly because of the increase in tiller number. Targeted overexpression of miR156 also improved solubilized sugar yield and forage digestibility, and offered an effective approach for transgene containment.

P-26

An Inducible Three-component Gene Expression System and Its Application for Inducible Flavonoid Overproduction in Transgenic *Arabidopsis thaliana.* H. KOIWA[1], Y. Feng[1], C. M. Cao[1], M. Vikram[1], S. Park[2], H. J. Kim[3], J. C. Hong[3], and L. Cisneros-Zevallos[1]. [1]Vegetable & Fruit Improvement Center, Department of Horticultural Science, Texas A&M University, College Station, TX 77843; [2]Dept of Horticulture, Forestry and Recreation Sources, Kansas State University, Manhattan, KS, 66506; and [3]Division of Applied Life Science (BK21 program) and Plant Molecular Biology and Biotechnology Research Center, Graduate School of Gyeongsang National University, Jinju 660–701, KOREA. Email; koiwa@neo.tamu.edu

Major goals in plant genetic engineering include increasing level of gene expression/protein production, and enabling induction of gene expression at the right timing. Inducible gene expression is a powerful tool to study and engineer genes whose overexpression could be detrimental for the host organisms. Our group has been using Arabidopsis thaliana to address these issues to facilitate functional studies of plant proeins, and potential application of technology to metabolic engineering. We have recently streamlined a lab-scale overproduction strategy as well as an inducible gene expression system. Our osmotically inducible system uses three components of plant origin, *RD29a* promoter, CBF3 transcription factor and *cpl1-2* mutation. The efficacy of this system was tested using *PAP1* transgene, a model transcription factor that regulates anthocyanin pathway in Arabidopsis. While transgenic plants with only one or two of three components did not reproducibly accumulate anthocyanin pigments above the control level, transgenic *cpl1* plants containing homozygous *RD29a-PAP1* and *RD29a-CBF3* transgenes produced 30-fold higher level of total anthocyanins than control plants upon cold treatment. Growth retardation and

phytochemical production of transgenic plants were minimum under normal condition, albeit some ectopic anthocyanin production was observed in transgenic *cpl1* lines. The flavonoid profile in cold-induced transgenic plants was determined by LC/MS/MS, which resembled that of previously reported *pap1-D* plants but enriched for kaempferol derivatives indicating PAP1 and environmental signals synergize to produce new blend of phytochemicals. These results establish the functionality of the inducible three-component gene expression system in plant metabolic engineering.

P-27

Evolution of the Tetracycline Repressor into a Sulfonylurea Herbicide Responsive Gene Switch for Crop Plants. K. E. MCBRIDE[1], N. K. Kakani[1], J. Fang[1], W. J. Gordon-Kamm[2], K. S. Lowe[2], B. L. Lenderts[2], B. McGonigle[3], Q. J. Liu[3], S. C. Falco[3], and L. L. Looger[4]. [1]Pioneer Hi-Bred International, Inc: 4010 Point Eden Way, Hayward CA 94545; [2]Pioneer Hi-Bred International: 7300 NW 62nd Avenue, Johnston, IA 50131–1004; [3]DuPont Experimental Station, Route 141, Henry Clay, Wilmington, DE 19880–0353; and [4]Janelia Farms Research Campus, Howard Hughes Medical Institute, 19700 Helix Drive, Ashburn, VA 20147. Email: Kevin.mcbride@pioneer.com

Incompatibility between agricultural chemistry and known genetic switch mechanisms has thwarted the deployment of chemically regulated gene expression in crop plants. The widely used tetracycline repressor (TetR) based switch is impractical due to the antibiotic nature and light sensitivity of the inducer. Through iterative cycles of directed evolution, we engineered mutants of TetR that respond to registered sulfonylurea (SU) herbicides in place of tetracycline. The evolved repressors exhibit low nanomolar affinity for ethametsulfuron-methyl (Es, Muster©), and bind to the Tet operator sequence only in the absence of Es. The crystal structure of one repressor variant in complex with Es reveals the nature of the high affinity interaction. These repressors (EsR's) conditionally repress expression of transgenes under the control of a TetO-linked 35 S promoter in tobacco, soy and corn. Spray experiments in tobacco with the switch linked to an SU resistance gene reveal widespread induction of a fluorescent marker continuing thru seed development. The sulfonylurea switch will enable alternative seed production and trait technologies as well as provide a powerful new tool for discovery research.

P-28

Genetic and Epigenetic Beauty, Ugliness and Complexity of Flower-specific Promoters and Enhancers. ZONGRANG LIU. USDA-ARS Appalachian Fruit Research Station, 2217 Wiltshire Road, Kearneysville, WV 25430. Email: Zongrang.Liu@ARS.USDA.GOV

Precisely engineering agronomical traits in crops requires highly regulated promoters in an inducible or tissue-specific manner. Many of these promoters have been characterized and become available. However, some of these highly regulated promoters, like other genetic elements, are highly interactive when inserted or placed in a new genomic context in the same or different hosts. Many of these promoters, especially those specific for flower meristem or organs, display a strong "personality" and are able to influence the activity of the adjacent promoters, while others lose their "confidence" and are completely silenced in the new genomic context in transgenic plants. The detailed genetic and epigenetic analyses and the mechanisms underlying the promoter's strange behaviors will be presented and discussed.

P-29

Leaf-, Root- and Pollen-specific Promoters from Rice; Useful New Tools for Improved Crop Biotechnology. R. THILMONY, M. Cook, and M. E. Guttman. USDA-ARS Western Regional Research Center, 800 Buchanan St., Albany CA 94710. Email: roger.thilmony@ars.usda.gov

Biotechnology is a useful and important tool for crop improvement. Transgene expression control elements (i.e. promoters) are crucial components for plant genetic engineering, and relatively few promoters that function well in crop plants are available. To increase that number, we are developing a suite of monocot promoters that confer distinct patterns of organ- or tissue-specific expression in transgenic plants. Using microarray gene expression profiling, we identified candidate genes with distinct patterns of expression. The corresponding upstream promoter sequences of selected candidates have been fused to a reporter gene and transformed into rice, *Brachypodium distachyon* and/or *Arabidopsis* plants. The rice *Root3* promoter, derived from a putative plastocyanin-like gene, activates expression only in the vascular cells of rice roots. The *Pollen Specific 2* (*PS2)*, *PS3* and *OsGEX2* promoters control expression limited to the pollen of transgenic plants. The *LP2* promoter, derived from a LRR receptor kinase-like rice gene, controls light-responsive green tissue-specific expression primarily in the leaves of transgenic rice and *Brachypodium* plants. These promoters are useful biotech tools that enable spatially-defined transgene expression in rice and potentially other crop plants. They can facilitate the development of genetically engineered crops that express the introduced traits in specific tissues, reducing potential unintended effects that biotechnology may have on plant physiology and the environment.

P-30

Death Be Not Proud: Modulation of Programmed Cell Death for Disease/Stress Tolerance in Plants. MARTY DICKMAN[1], Brett Williams[1], Mehdi Kabbage[1], James Dale[2], and Harjeet Khanna[2]. [1]Institute for Plant Genomics and Biotechnology, Texas A&M University, College Station, TX, 77843 and [2]Centre for Tropical Crops and Biocommodities, Queensland, AUSTRALIA. Email: mbdickman@tamu.edu

The genes that control mammalian programmed cell death are conserved across wide evolutionary distances. Although plant cells can undergo apoptotic-like cell death, plant homologs of mammalian regulators of apoptosis have in general, not been found. This is in part due to the lack of primary sequence conservation between animal and putative plant regulators of apoptosis. Thus, alternative approaches beyond sequence similarities are required to find functional plant homologs of apoptosis regulators. Here we present the results of using advanced bioinformatic tools and functional genomics approaches to uncover the Arabidopsis family of BAG proteins. The *Arabidopsis* genome contains seven homologs of the BAG family, including four with domain organization similar to animal BAGs. Over expression of AtBAG4 confers robust drought tolerance. Inactivation of AtBAG6 results in increased susceptibility to the necrotrophic fungus *Botrytis cinerea*. Localization studies shows distinct organellar places for this family ranging from the mitochondria, to the endoplasmic reticulum (ER), to the vacuole. Consistent with their mammalian counterparts plant BAG members are also multi-functional and regulate PCD processes including those induced by pathogen attack, abiotic stress and development. We have also generated transgenic plants that express animal and plant genes that negatively regulate apoptosis. These genes also have the potential to generate effective disease resistance/stress tolerance in economically important crop plants. For example, the vast majority of bananas grown today have not undergone improvement through conventional breeding. The major reason for this is that most cultivars are essentially sterile. An alternate strategy is to improve current cultivars through genetic modification. We have found that *A. tumefaciens* exposure induces a programmed cell death in banana cell suspensions. More than 90% of embryogenic banana cells died after exposure to *A. tumefaciens* and cell death was accompanied by a subset of features associated with apoptosis in mammalian cells, including DNA laddering, fragmentation, and formation of apoptotic-like bodies. Importantly, these cellular responses were inhibited in cells expressing antiapoptosis genes. In addition, these plants were drought tolerant and resistant to the two major fungal pathogens of banana.

P-31

Protein Phosphorylation and Degradation in Plant Responses to Herbivory. J. W. STRATMANN. University of South Carolina, Department of Biological Sciences, Columbia, SC 29208. Email: johstrat@biol.sc.edu

Biotic and abiotic stress signals induce specific stress responses. However, in plants, most of the underlying signaling pathways include the phosphorylation and activation of only two or three MAP Kinases (MAPKs). It is not known how this limited number of MAPKs mediates specific responses. The tomato MAPKs MPK1 and MPK2 respond to herbivory, infection by pathogens, mechanical wounding, the wound signaling hormone systemin, and many abiotic stresses. To investigate whether the MPKs are required for specific stress responses, we used virus-induced gene silencing to silence MPK1/2. In the MPK-silenced plants, wound-induced increases in the hormone jasmonic acid (JA) were reduced. JA is required for expression of wound- and herbivory-responsive genes. As a consequence, MPK-silenced plants were defenseless against an attack by *Manduca sexta* larvae (Lepidoptera). We also found that stress responsive MAPKs assemble in a multiprotein complex that may be involved in modulating signaling specificity. JA perception requires the function of the E3-ubiquitin ligase SCF^{COI1}. The activity of this E3 ligase is regulated by an eight-subunit protein complex, the COP9 signalosome (CSN). Silencing subunits of the CSN (CSN5, CSN4, CSN3) in tomato plants resulted in reduced JA synthesis and increased performance of *M. sexta* larvae and a necrotrophic pathogen. Our data indicate that additional E3-ubiquitin ligases regulate tomato defenses, and that posttranslational protein modifications are an integral part of plant responses to herbivory.

P-32

Enoyl-ACP Reductase Is an Essential Component of Fatty Acid Biosynthesis and a Linking Point of Stress Response and Oil Biosynthesis. HUI CHEN[1,2] and Oliver Yu[2]. [1]Conagen Inc, 1005 N. Warson Rd., St Louis, MO 63132 and [2]Donald Danforth Plant Science Center, 975 N. Warson Rd., St. Louis, MO 63132. Email: chenhui_wsu@hotmail.com

Enoyl-Acyl Carrier Protein (ACP) reductase (ENR, EC 1.3.1.9) catalyzes the reduction of 2,3-*trans*-enoyl-ACP to the corresponding saturated acyl-ACP in an NADH or NADPH dependent manner, which is the final step of the chain elongation cycle in the de novo fatty acid biosynthetic pathway. In *E. coli*, it has been demonstrated that ENR-catalyzed reaction is a rate-limiting step, and over-produced ENR is toxic to growth of *E. coli*, indicating that the activity

of ENR must be tightly regulated in vivo. Previous proteomics experiments identified *E. coli* ENR as a putative partner of thioredoxin (Trx), suggesting that this protein might be regulated by a redox signaling pathway. Recently, Arabidopsis ENR was found to be a disulfide-bonded protein in vivo, so *E. coli* and plant ENRs may share similar regulatory mechanisms. Here we reported that Arabidopsis ENR physically interacted with chloroplast Trx y1/y2 in a yeast-two-hybrid assay, and that $CuCl_2$ could partially oxidize recombinant ENR expressed in *E. coli* and decrease its activity. In contrast, a mutant form of ENR, C198A, was insensitive to $CuCl_2$ treatment. We generated transgenic Arabidopsis, over-expressing ENR or C198A, and screened the transformants based on ENR activity. While most transgenic lines with moderate increases in enzyme activity did not show any visible phenotype, the transformants with very high ENR activities started to develop sick and dwarf phenotypes at the seedling stage, suggesting that the level of ENR is critical for the healthy growth and development of plants. Oil contents of the seeds harvested from normal-looking transgenic plants were significantly higher than that of the WT control, whereas oil contents of the seeds from dwarf plants were significantly lower than that of the WT control. In addition, we also found that the normal-looking ENR over-expressors were more resistant to salt stress compared to the WT control. These findings suggest that ENR might be involved in the detoxification of reactive electrophile species (RES) under stress conditions.

P-33

Functional Characterization of AtCLB Protein, a Novel Repressor of Abiotic Stress Response. M. KHODAKOVSKAYA and K. de Silva. University of Arkansas at Little Rock, Dept. Applied Science, 2801 S. University Avenue, Little Rock, AR. Email: mvkhodakovsk@ualr.edu

Understanding the mechanisms by which environmental signals are perceived and transferred from the membrane to activate adaptive stress responses is of fundamental importance to Plant Biology. We have identified a new *Arabidopsis* calcium-dependent, lipid-binding protein with a C2-like domain (AtCLB) that is expressed in all tissues of *Arabidopsis* and is located in the nucleus of cells. AtCLB protein is able to bind membrane sphingolipid ceramide in a Ca2+−dependent manner. Our data strongly suggest that the AtCLB acts as a negative regulator in abiotic stress signaling and interacts with the ABA signaling pathway. Mutation in the *At3g61050* (AtCLB) gene leads to increased tolerance to osmotic stress and changed gravitropic response in *Atclb* mutants. Bioinformatics analysis has revealed that the C2-like domain of *Arabidopsis* calcium-dependent, lipid-binding protein is identical to the C2 domain sequences of important crops, including tomato and rice. Detailed characterization of the AtCLB protein and AtCLB signaling pathway is important for clarifying the downstream components of Ca^{2+}-dependent stress-signaling and can be useful for establishing new genetic engineering strategies focused on improving stress tolerance in crops.

P-34

Sustainability in Agriculture: Building a Foundation for Food Security. JERRY L. HATFIELD. Laboratory Director, National Laboratory for Agriculture and the Environment, 2110 University Blvd., Ames, IA 50011. Email: jerry.hatfield@ars.usda.gov

Agriculture is often considered only from the viewpoint of production and profit; however, there is an emerging realization that agricultural systems have to be viewed in the multifunctional context. Sustainable agriculture has often been viewed from a singular viewpoint, e.g., soil improvement, reduced inorganic fertilizers of pesticides; however, these efforts have somewhat limited our ability to fully comprehend the potential for sustainable agricultural systems. Sustainable agricultural systems will balance productivity, environmental goods and services, product quality, biodiversity, and resilience to climate change. An overlooked part of the sustainability equation is the role of the soil as a reservoir of water and nutrients for efficient plant production but also for environmental quality. Soil erosion and water and air quality are impacted by the state of the soil resource and a degraded soil has a reduced production efficiency and greater potential for an increased negative environmental impact. Restoration of degraded soils is one component of constructing a sustainable agricultural system and will lead to improved production efficiency and enhanced environmental quality. Development of new metrics which quantify sustainability from multiple viewpoints will increase our ability fully utilize the genetic potential of crops and meet the future food demands of the world's population.

P-35

Will "Sustainability" Standards Shun Biotech and Nanotech Innovation? T. REDICK. Global Environmental Ethics Counsel (GEEC), LLC. 65 Arundel Place, Clayton, MO 63105. Email: tpr@geeclaw.com

This presentation will survey an array of sustainability standards in agricultural biotechnology, medical innovation and other technology sectors, discussing the expectations emerging in the marketplace for greater efficiency in energy, water, waste, social responsibility and other factors. In

particular, growers and seed company representative successfully intervened to stop anti-biotech provisions in an American National Standards Institute (ANSI) draft standard on sustainable agriculture (Leo 4000), the SCS-002 standard on Ecolabeling-Life Cycle Analysis and the Roundtable on Responsible Soybeans. In addition, other standards that continue to discriminate against emerging technology will be scrutinized, including Global GAP and the Roundtable on Sustainable Biofuels, which both have EU influences that make them impose unreasonable "prevent migration" standard on biotech growers. The role that nanotechnology could play, if not subject to similar discriminatory standards, will also be discussed. Lastly, the potential for green marketing laws to stop such standards and marketing claims in the marketplace will also be briefed.

P-36

Partnerships Enhancing Sustainable Agriculture. JULIE BORLAUG. Texas A&M University, Norman Borlaug Institute for International Agriculture, Teague Building, Room 123, 2477 TAMU, College Station, TX 77843–2477. Email: JBorlaug@ag.tamu.edu

Today, we as an industry face a great challenge - how to feed the world in 2050. This will require us to not only produce and more efficiently transport and distribute more food than ever before in order to feed the growing global population but also modify the diets and eating habits of people around world.. The situation is further exacerbated b/c now, we must also double food production sustainably by 2050 on approximately the same area of arable land, or possibly less due to urbanization and industrialization, using fewer resources, in particular, fossil fuels, water and nitrogen at a time when we must also mitigate some enormous challenges associated with climate changes. Furthermore, there is the critical and urgent humanitarian need to alleviate poverty, hunger and malnutrition. As the granddaughter of Dr. Norman E. Borlaug, I know firsthand that my grandfather understood that the only way to meet this mounting challenge is to engage in innovative partnerships between farmers, communities, researchers, governments, NGOs and the private sector in order to ensure the continuation of breakthroughs in international agricultural science and technology. At the Borlaug Institute for International Agriculture at Texas A&M University, we believe that the legacy of my grandfather demands effective partnerships, and we have successfully engaged in strong cross discipline partnerships in order to improve food security and rural development around the globe. Our programs provide researchers, policymakers, government officials and university faculty from developing countries the ability to strengthen sustainable agricultural practices through scientific training and collaborative research opportunities. Our primary focus is the administration and support for international development programs and short and long-term training programs. A perfect example of how the Borlaug Institute is effectively living out our institutional mission and vision is through our current work in Rwanda on the USAID-funded Sustaining Partnerships to Enhance Rural Enterprise and Agribusiness Development, or SPREAD, project. The Borlaug Institute first became engaged in Rwanda over ten years through partnerships with the US government, US land-grant institutions, the Rwandan government, the National University of Rwanda and local Rwandan farmers. These partnerships have led to agricultural growth, improved food security, increased access to health services, and enhanced educational services (at the primary, secondary and tertiary levels) in Butare and surrounding rural areas. This unique and highly successful program exemplifies the Borlaug Institute's commitment to innovative partnerships by demonstrating how cross-sectoral partnerships promote food security, resource conservation, sustainable agriculture practices and international development. The vision of my grandfather is continued by dozens of organizations and countless scientists and farmers around the world, the vast majority of whom will never be widely known. They work to increase food production, increase nutrition, and conserve the natural resources that are so vital to agricultural systems. The legacy of my grandfather will ultimately be defined by new generations trained in agricultural science and their ability to collaborate across borders and across disciplines to continue to provide meaningful solutions to the persistent issues of hunger and poverty.