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Plant Posters

P-2000

Enhancing Halotolerance in the Cyanobacterium *Fremyella diplosiphon* Through Biotechnological Approaches. B. TABATABAI[1], S. AnithaChristy[2], B. Montgomery[3], and V. Sitther[1]. [1]Morgan State University, Department of Biology, Morgan State University, 1700 E. Cold Spring Lane, Baltimore, MD 21251; [2]Department of Pathology, Methodist Hospital Research Institute, 6565 Fannin St., Houston, TX 77030; and [3]Department of Biochemistry and Molecular Biology, Michigan State University, 612 Wilson Road, East Lansing, MI 48824. Email: betab1@morgan.edu

Fremyella diplosiphon (SF33) is a cyanobacterial species that has great potential due to its ability to grow in a range of light intensities. Preliminary studies on its growth in various concentrations of sodium chloride (NaCl) indicated that the organism could tolerate only up to 15 g/L NaCl. The present study was aimed at increasing salt tolerance in *F. diplosiphon* to enable its growth in brackish waters. In one set of experiments, *F. diplosiphon* cells in the logarithmic phase were subjected to heat shock at 42°C to induce mutations that enhance halotolerance. Results showed that *F. diplosiphon* could tolerate only up to 20 g/L NaCl, when exposed to two successive 15 minute heating cycles, a slight increase in halotolerance. In order to further enhance halotolerance, genetic transformation was carried out. Candidate halotolerance-linked genes, *NhaP, NapA, GMST,* and *DMT* from the naturally halotolerant cyanobacterium, *Aphanothece halophytica*, were identified in *F. diplosiphon*. BLAST searches of these genes revealed that the nucleotide sequences of the potential homologs were present in the *F. diplosiphon* genome. Using *F. diplosiphon* genomic DNA as a template, amplified fragments at the expected gene sizes were obtained and sequenced. Using primers containing *Hind*III *and BamH*I sites, the products were digested and ligated into the pGEM-7Zf (+) expression vector. The resultant expression plasmids are being transformed into the wild type using heat shock/ electroporation methods. Future studies will be aimed towards molecular screening and physiological evaluation of the transformants. Results of the present study indicate that halotolerance in *F. diplosiphon* can be augmented through biotechnological approaches, thereby enabling the use of naturally available brackish waters for its large-scale cultivation. *Disclosure: Author received research grant from Morgan State University.*

P-2001

Targeting Methylglyoxal Detoxification Pathway: An Efficient Approach to Improve Multiple Abiotic Stress Tolerance in Leguminous Crop Plant. P. SINGH, D. Kumar, M. Yusuf, and N. Sarin. Jawharlal Nehru University, C/O Prof. Neera Bhalla Sarin , School of LifeSciences, New Delhi 110067, INDIA. Email: preetimku@gmail.com

Methylglyoxal (MG) is a cytotoxic compound which increases upon exposure of plants to various abiotic stresses. At higher concentrations MG is harmful to the system as it reacts with the major macromolecules like RNA, DNA and proteins leading to the cell death. MG is mainly catabolized by two major enzymatic pathways. The first is the ubiquitous pathway, the glyoxalase pathway. An alternate pathway involves aldose reductase that converts MG into acetol in a NADPH dependent two step reaction. It is this pathway that we have exploited in our study. The enzyme aldose reductase plays an important role in the osmo-protection mechanism and detoxification of reactive aldehyde compounds.A detailed functional validation of *Xerophyta viscosa* (a "resurrection plant") aldose reductase (*ALDRXV4)* was first carried out in model plant tobacco and subsequently used for the transformation of a highly recalcitrant pulse crop, *Vigna mungo* (black gram).Compared with wild type plants, transgenic showed improved photosynthetic efficiency, less electrolyte leakage and higher relative water content under drought and salinity stress. The increased synthesis of aldose reductase in the transgenic plants correlated with an elevated level of sorbitol and reduced MG accumulation under stress conditions, consistent with its suggested role in osmoprotection and detoxification. The transgenic lines showed normal growth, morphology and seed production as compared to the WT plants

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without any yield penalty under stress conditions. The overall results demonstrate the profound effect of *ALDRXV4* in bestowing multiple abiotic stress tolerance at cellular and whole plant level via ROS detoxification. To the best of our knowledge this is the only report of engineering multiple abiotic stress tolerance in blackgram. These finding suggests the potential application of aldose reductase in abiotic stress tolerance for engineering agriculturally important crop plants.

P-2002

Evaluation of a *Vitis*-derived Reporter Gene System for Precision Breeding of Cold-hardy Grapevine Cultivars. R. KANDEL[1], M. Dutt[2], J. Grosser[3], D. Gray[4], Z. Li[2], V. Sitther[5], D. Bergey[1], and S. Dhekney[1]. [1]University of Wyoming, 1348 Omarr Ave, Sheridan, WY 82801; [2]UF/IFAS, 700 Experiment Station Road, Lake Alfred, FL 33850; [3]University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850; [4]Mid-Florida Research & Education Center, University of Florida/ IFAS, University of Florida, 2725 S. Binion Rd., Apopka, FL 32703; and [5]Morgan State University, 1700 E. Cold Spring Lane, Baltimore, MD 21251. Email: rkandel@uwyo.edu

Cisgenic and intragenic reporter gene systems are valuable tools for precision breeding of perennial fruit species including grapevine. To evaluate the development of a grapevine-derived reporter gene system, expression of the *Vitis vinifera* MybA1 gene, which is involved in the production of anthocyanin pigment, was compared with existing reporter genes including the green fluorescent protein (gfp) and β glucuronidase (gus) genes. Each reporter gene sequence was individually placed along with a nptII gene under the control of a CaMV35S promoter. Embryogenic cultures of 'Bronx Seedless' and 'Thompson Seedless' were initiated from in vitro-derived leaf explants. Somatic embryos at the mid-cotyledonary stage were co-cultivated with *Agrobacterium* harboring either the MybA1, gfp or gus genes. Transient and stable gene expression levels were recorded in co-cultivated embryogenic cultures. The number of independent embryo and plant lines obtained after transformation with individual reporter genes along with gene expression patterns was also recorded. Transient anthocyanin expression was evidenced by bright red spots on hypocotyl and cotyledonary regions of somatic embryos. Gene expression levels of cultures expressing VvMyBA1, gfp or gus reporter genes varied with cultivar. Transient and stable gene expression levels varied from 65-95% and 41-61%, respectively, whereas conversion of stable embryo lines ranged from 6 – 9%. Growth and development of stable anthocyanin embryo and plant lines was slower compared to GFP and GUS expressing lines, possibly due to the hyper-accumulation of anthocyanin in cell vacuoles. We are currently studying the possibility of using embryo specific promoters for anthocyanin expression solely in cell cultures. Grapevine-derived embryo specific promoters would enable selection of transformed tissues at the cell culture stage while allowing normal plant development following embryo germination.

P-2003

The Effect of Silver Ions and Silver Nanoparticles on Plant Development. J. LIANG, Z. Li, Q. Hu, and H. Luo. Department of Genetics and Biochemistry, Clemson University, 110 Biosystems Research Complex, Clemson, SC 29634. Email: jiangol@clemson.edu

Silver ion is known to be one of the most toxic heavy metal ions to plants. With the wide application of nanomaterials including silver nanoparticles, it is crucial to understand the potential risk for consequent bioavailability of silver ions. Although silver ion-plant interaction has been previously reported in *Arabidopsis*, the mechanism of its impact on plants remains unknown. We have investigated the effect of silver ions on plant development and the underlying molecular mechanisms in *Arabidopsis thaliana*. The results showed that in the presence of silver ions, lower concentration treatment has no obvious impact on seed germination, but can significantly promote plant growth, whereas higher concentration of silver ions treatment strongly inhibits both seed germination and plant growth. The plant growth improvement is associated with enhanced lateral root development, which may have facilitated nutrient uptake, while the inhibition of plant development may be caused by decreased nutrient absorption due to reduced root hair growth. Further study revealed that the silver ions can be transformed into silver nanoparticles by reaction with ferrous ions in the 1/2 MS culture medium, which is then uptaken by roots. The effect of silver nanoparticles on plant development was also studied, and similar results to silver ions were obtained. The molecular mechanisms underlying silver ion- and silver nanoparticle-mediated plant development change are currently been investigated by studying expression profiles of stress- and plant development-related genes. Data obtained from this research will allow better understanding of plant-nanoparticle interaction and provide information to develop molecular strategies mediating impact of nanomaterials on environment.

P-2004

Heterologous Expression of the Rice MicroRNA395h in *Nicotiana tabacum* Impairs the Sulfate Homeostasis. N. YUAN[1], Z. Li[1], D. Li[2], and H. Luo[1]. [1]Clemson University, 105 Collings Road, 104 Room, Clemson University, Clemson, SC 29631 and [2]Department of Genetics and Biochemistry,

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As a rudimental and essential element, sulfur participates in many important mechanisms and pathways of plant development. Inorganic sulfate (SO_4^{2-}), the most common source of sulfur in soil, is absorbed into root tissue and distributed into aerial part through vasculature system, where it is reduced into sulfite and finally sulfide within the subcellular organs such as chloroplasts and mitochondria and used for cysteine biosynthesis. MicroRNAs (miRNAs) are involved in regulating many metabolisms by repressing their target gene expression. MiR395 family in *Arabidopsis thaliana* has been reported to be an important regulator involved in sulfate transport and assimilation, and a high-affinity sulphate transporter (SULTR2:1) and three ATP sulfurylases (APS1,3, and 4) were verified to be the target genes of miR395. We have studied the function of miR395 in rice, and hypothesized that rice miR395 may have similar functions to that in *Arabidopsis*. Our results indicated that transcripts level of rice miR395 genes increased in sulfate deficiency mediums, and two predicted target genes of miR395 were down regulated under the same conditions. Overexpression of the rice miR395h (*OsamiR395h*) in tobacco (*Nicotiana tabacum*) impaired its sulfate homeostasis, but sulfate distribution was not impacted among leaves of different ages. One potential target gene of miR395 was identified in tobacco, which is predicated to be a sulfate transporter gene. These results indicate that the rice miR395 responds to sulfate deficiency by inducing degradation of two target genes in rice, and the function of miR395 is conserved in plant species. Our results suggest that miR395 expression could be manipulated to enhance the uptake and assimilation of sulfur, benefiting plant development.

P-2005

Regulatory Mechanisms of Sesquiterpenoid Biosynthesis Revealed by Gene Expression and Metabolite Profiling in *Valeriana officinalis*. V. RICIGLIANO and D. Howarth. St. John's University, 8000 Utopia Parkway, Queens, NY 11758. Email: vincent.ricigliano@gmail.com

Plant sesquiterpenoids are secondary metabolites implicated in a variety of ecological interactions such as herbivore defense and pollinator attraction. Tremendous structural diversity contributes to a broad spectrum of biological activity with pharmaceutical, herbal medicine, and nutraceutical applications. Sesquiterpenoids occurring in medicinal plants have been demonstrated to possess important pharmacological activity including anti-cancer, anti-inflammatory, anti-microbial, and anti-malarial properties. However, most medicinal compounds accumulate at low concentrations *in planta* and their chemical synthesis may be difficult. Expounding the taxonomically restricted sesquiterpenoid metabolism of medicinal plants will accelerate metabolic engineering for the production of phytotherapeutics and drug discovery. Root extracts of Valerian (*Valeriana officinalis*) are the most widely recognized herbal sedative and anxiolytic. Biological activity is attributed primarily to *Valeriana*-specific sesquiterpenoid metabolites. Here, we report the development of an *Agrobacterium rhizogenes*-mediated transformation system its application to probe the gene-metabolite relationships of sesquiterpenoid biosynthesis in *V. officinalis*. Wild-type plant roots and hairy roots induced by transformation with *A. rhizogenes* possessed similar biosynthetic capacities. Overexpression and chemical elicitation strategies were employed in hairy roots and the effects of altered gene expression were evaluated with respect to their sesquiterpenoid content. The availability of cytosolic farnesyl pyrophosphate is a potential rate-limiting step in the production of sesquiterpene hydrocarbons. Downstream jasmonate-dependent regulation contributes significantly to the accumulation of oxygenated sesquiterpenoids. Our findings contribute to understanding the biosynthetic mechanisms underlying sesquiterpenoid metabolism in *V. officinalis* and perhaps other pharmacologically relevant plant species.

P-2006

Identification and Characterization of Genes Involved in the Fruit Color Development of European Plums (*Prunus domestica* L.). D. SELVARAJ[1], S. Sherif[1], K. Tiwari[1], G. Paliyath[1], and J. Subramanian[2]. [1]University of Guelph, Department of Plant Agriculture E. C. Bovey Building, Room 3107, 50 Stone Road East, Guelph, ON N1G 2W1, CANADA and [2]University of Guelph, Department of Plant Agriculture, University of Guelph, Plant Agriculture, Vineland Station, ON L0R 2E0, CANADA. Email: dselvara@uoguelph.ca

European plums (*Prunus domestica* L.) are normally purple black as the fruit ripens. The pigments that impart characteristic purple color to the plums are primarily anthocyanins. However, there are some genotypes that exhibit lack of this dark color and remain green or yellow even after ripening. It is hypothesized that in such genotypes one or more genes responsible for biosynthesis of anthocyanins are not functional possibly due to mutation or genetic regulation. In this study, six varieties of European plums that vary in color from dark purple to green are investigated for expression of five critical anthocyanin biosynthetic pathway genes and overexpressing them in tobacco plants to reveal the control points for flux into anthocyanin biosynthesis. The five genes chosen are phenylalanine ammonia lyase (PAL), chalcone synthetase (CHS), dihydroflavonol 4-reductase (DFR), anthocyanin synthetase (ANS), UDP-glucose:flavonoid 3-O-glucosyltransferase

(UFGT). In addition we also estimated, total polyphenols and individual flavonoid compounds in six genotypes of European plums and transgenic tobacco plants overexpressing CHS, DFR, ANS, UFGT. PAL was not overexpressed, based on its relative gene expression levels and it is not attributed to cause major metabolic flux towards anthocyanin synthesis. By comparing the regulation of these critical genes with polyphenol compound analysis, the loss of normal purple color resulting in green or yellow genotypes can be narrowed down.

Disclosure: Author received research grant from University of Guelph.

P-2007

A Self-regulating *MYB10* Transgene from Apple Confers Ectopic Anthocyanin Accumulation to Flowers but Not Leaves in Petunia. M. BOASE, C. Brendolise, D. Lewis, L. Wang, H. Ngo, K. Schwinn, K. Davies, R. Espley, and N. Albert. The New Zealand Institute for Plant & Food Research Limited, Palmerston North 4410, NEW ZEALAND. Email: murray.boase@plantandfood.co.nz

Anthocyanin biosynthesis is regulated by a complex of R2R3-MYB, bHLH and WDR proteins. This regulatory complex activates transcription of anthocyanin biosynthetic genes by binding to their promoters, thereby leading to production of anthocyanins. Previously we showed that an allelic rearrangement in the promoter of the apple anthocyanin-regulating transcription factor, *MYB10*, conferred autoregulation to the gene, and this allele occurred in all red foliage apple varieties (Espley et. al. 2009:*The Plant Cell* 21:168-183). We also reported on how a network of transcriptional activators and repressors operate in a hierarchy to control anthocyanin floral and vegetative pigmentation in petunia (Albert et al 2014:*The Plant Cell,* In press). In this study, we investigated whether a construct comprised of a promoter containing the *MYB10* autoregulatory element driving *MYB10* could give ectopic anthocyanin accumulation to the white-flowered Mitchell petunia line. The floral and vegetative phenotypes obtained from *Agrobacterium*-mediated stable transformation experiments will be shown and discussed.

P-2008

Metabolic Redesign of Vitamin E Biosynthesis in *Brassica juncea* for Human Health and Stress Alleviation in Plants. D. KUMAR[1], M. Yusuf[1], P. Singh[1], M. Sardar[2], and N. Sarin[1]. [1]Jawharlal Nehru University, C/O Prof. Neera Bhalla Sarin, School of Life Sciences, New Delhi 110067, INDIA and [2]Department of Bioscience, Jamia Millia Islamia, New Delhi, New Delhi, INDIA. Email: deepakinjnu@gmail.com

Oxidative stress is one of the major manifestations of unfavourable environmental conditions faced by plants as also of several different diseases, including cancers, in humans. Alpha (α)-tocopherol, the biologically most active form of vitamin E, is a major antioxidant that bulwarks the cells. It constitutes a small fraction of the total tocopherol pool in most oilseed crops. We generated transgenic (TR) *Brassica juncea* plants with ~6 fold higher α-tocopherol levels by overexpressing γ-tocopherol methyl transferase. To better understand the roles of different tocopherol forms in plants we compared the performance of TR plants under conditions of abiotic stresses induced by salinity, heavy metal and drought. This resulted in an increase in total tocopherol levels in both the WT and TR plants. Seed germination and leaf disc assay showed that TR *B. juncea* had enhanced tolerance to these stress and that induced by high temperature and methyl viologen. Damage caused by the induced stress was lower in TR plants compared to WT plants as assessed by their higher relative water content, lower MDA and electrolyte leakage. Lesser superoxide and H_2O_2 accumulation was observed in TR seedlings exposed to these stress. The levels of antioxidant enzymes and molecules were higher in TR plants when compared to WT plants under similar stress. Analysis of chlorophyll a fluorescence kinetics showed that there were differential effects of the stress on different sites of the photosynthetic machinery. These effects were found to be alleviated in TR plants. We further checked the efficacy of feeding α-tocopherol enriched seeds in securing the antioxidant defense in mice. We found significant increase in the content of various phase I and phase II enzymes with a corresponding decrease in peroxidative damage. Also, the transgenic seeds were found to have chemopreventive effects against DMBA-induced skin papillomagenesis in mice model. Our results highlight the potential of increased α-tocopherol in transgenic *B. juncea* in health of humans and agricultural crop plants.

P-2009

Novel In-vitro Biosynthesis of Lignin. V. SHARMA. Meadow Park Summa MS, 5508 NW 131st Ave., Portland, OR 97229. Email: sindpower@hotmail.com

Lignin is an essential biopolymer found naturally in trees, and is a crucial ingredient in products such as paper and structural components including hardwood. High demand of these products results in much deforestation. This paper details the in vitro synthesis of lignin precursors and an artificial biosynthetic pathway that results in the B, O, and S bonds found primarily in lignin. Both methods, while used together in this

paper, can also be employed independently. Precursors are directly created from an altered central dogma where transfer RNA is replaced with a synthetic equivalent engineered to produce lignin precursors, skipping several stages of metabolism/respiration (e.g. direct production of phenylpyruvate). Then, in an apparatus, enzymes and cofactors are manually inserted to replicate the shikimate and phenylpropanoid metabolic pathway, resulting in varying structures of lignin. Serving as proof of concept, this novel approach proves that polymers and substances derived from plant matter in high demand can be synthesized in vitro.

P-2010

Developmental Regulation of the *VvmybA1* Transcriptional Factor Gene of Grapevine (*Vitis vinifera* L.) to Produce Reporter Gene Expression Free Transgenic Citrus. L. SORIANO[1,2], M. Dutt[2], B. Januzzi Mendes[1], and J. Grosser[3]. [1]Universidade de Sao Paulo, Sao Paulo, Brazil; [2]UF/IFAS, 700 Experiment Station Road, Lake Alfred, FL 33850; and [3]University of Florida, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850. Email: lsoriano@ufl.edu; lsoriano@cena.usp.br

Agrobacterium-mediated transformation of embryogenic cell suspension cultures of 'Hamlin' sweet orange (*Citrus sinensis* (L.) Osbeck), 'W. Murcott' mandarin (*C. reticulata* Blanco), and 'Page' tangelo (*C. x tangelo* Ingram & Moore) were transformed with the *VvmybA1* transcriptional factor gene of grapevine (*Vitis vinifera* L.) that induces anthocyanin biosynthesis driven by one of three embryo specific promoters (An *Arabidopsis thaliana* At2S2 promoter, the *Daucus carota* DC3 promoter and our proprietary *Citrus sinensis* 6105 promoter). Regenerated transgenic somatic embryos from all three cultivars expressed anthocyanin with each of the three gene constructs. Anthocyanin expression was not visualized in any germinating embryos and we were able to recover transgenic plants without any visual anthocyanin production. Gene expression analysis confirmed the presence of the *VvmybA1* but failed to detect any product in well-developed plants. We conclude that tightly regulated embryo specific expression can be used to target reporter gene expression in the somatic embryos as an aid in transgenic plant selection and then switched off.

P-2011

Continued Improvement of *Agrobacterium*-mediated Sugar Cane Transformation. S. DONG, E. Bedani, J. Ke, P. Mai, G. Baldacin, P. deLucca, K. Mayo, G. O´Hata-Held, M. Sainz, E. Dunder, and J. Geijskes. Syngenta, 3054 E Cornwallis Rd, RTP, NC 27519. Email: shujie.dong@syngenta.com

Sugar cane (*Saccharum* spp.) account for nearly 70% of sugar produced worldwide and is among the most promising cellulosic biofuel crops. Sugar cane variety improvement is difficult due to complex genetic characteristics and resulting lengthy breeding process. Genetic engineering offers a great alternative for trait specific sugar cane variety improvement. One key enabling technology to ensure the successful application of biotechnology is genetic engineering. Considerable progress has been made on different transformation approaches during the last decade. However, challenges have been reported with low transformation efficiency and low proportion of high quality transgenic events. Another concern for sugar cane genetic engineering is the potential for somaclonal variation during the tissue culture process. We developed a high throughput *Agrobacterium*-mediated sugar cane transformation platform at Syngenta to support large scale transgenic plant production. Various factors throughout the transformation process have been tested. For example, the use of young explant material demonstrated great potential to produce transgenic plants more efficiently for some varieties and with a reduced risk of somaclonal variation. In addition to the desiccation cocultivation we had reported previously, which plays a critical role in T-DNA delivery and stable transgenic events generation, other factors can significantly improve transformation efficiency. Evaluation of *Agrobacterium* strains, modified binary vectors and improved expression of selection genes have shown we can further improve transformation efficiency and production of high quality events efficiency (3-4 fold increase) with an optimized combination. The high throughput transformation protocol has been validated in multiple varieties including US, Australian and Brazilian elite varieties. Syngenta is now applying this technology on a large scale in sugar cane research program and commercial development pipeline.

P-2012

Transient Expression of a Viral Insecticide in Plants. S. GANAPATHY[1], M. Parajulee[2], H. Zhang[1], and S. Bilimoria[1]. [1]Texas Tech University, Department of Biology, Main and Flint, Lubbock, TX 79409; and [2]Texas A&M University AgriLife Research and Extension Center, Lubbock, TX. Email: saranya.ganapathy@ttu.edu

Insect pests are a serious threat to agricultural productivity. Use of chemical pesticides, the predominant control method thus far, has resulted in environmental damage, pest resurgence, and negative effects on non-target species. Genetically modified (GM) crops offer a promising alternative, and *Bacillus thuringiensis* toxin genes have played a major role in this respect. However, to overcome insect tolerance issues and to broaden the target range, it is critical to identify

alternative insecticidal toxins working through novel mechanisms. Our research group is working on viral-insecticidal toxins toward the generation of insect-resistant transgenic plants. Chilo iridescent virus (ClV, family *Iridoviridae)* is the only virus known to induce mortality and metamorphic deformity in the cotton boll weevil. We have identified a kinase gene from CIV that has insecticidal activity. The CIV toxin, expressed in yeast systems, induces 50% mortality in cotton aphids and 100% mortality in green peach aphids. Attempts to transform and express this gene in plants yielded viral kinase-specific RNA but no protein. Expression of foreign genes in plants is complicated by codon usage, mRNA instability, translational efficiency, and proteolytic degradation. Therefore, the viral toxin gene was codon optimized to favor translation and stability in planta. The results of transient expression studies of this modified gene in tobacco and aphidicidal activity of the gene product will be presented.

P-2013

Molecular Characterization of Transgenic 'W. Murcott' (Nadorcott) Mandarin Produced Using a Protoplast-GFP Transformation System. A. OMAR and J. Grosser. University of Florida, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850. Email: omar71@ufl.edu

Genetic transformation offers an attractive alternative to the conventional genetic improvement of *Citrus* sp. Genetic transformation of several *Citrus* genotypes has been achieved by co-cultivating different explants (mainly juvenile) with *Agrobacterium tumefaciens*. However, mandarin is considered to be the most difficult *Citrus* sp. for transformation using the *Agarobacterium*-mediated system. 'W. Murcott' mandarin (a hybrid of 'Murcott' and an unknown pollen-parent) is a commercially important cultivar grown in many regions around the world. Protoplast-GFP transformation opens a new avenue to produce transgenic plants especially from seedless or other polyembryonic cultivars not amenable for the *Agraobacterium*-mediated system. We undertook the study to develop efficient direct DNA uptake using PEG-mediated transformation of mandarin protoplasts and regeneration of transgenic plants, using our protoplast-GFP transformation system. Plasmid DNA (pAO3), encoding the non-destructive selectable marker *GFP* (Green Fluorescent Protein) gene and the cDNA of the *Xa21 Xanthomonas* resistance gene from rice, was transformed into 'W. Murcott' embryogenic protoplasts. Eleven stable transgenic lines have established in the greenhouse for more than two years. PCR analysis revealed the presence of the *Xa21* and the *GFP* genes in the transgenic Plants. The transgenic plants have shown stable GFP expression all the time. ELSIA and molecular characterization including Southern blot analysis and Western blot analysis are showing integration and expression of the transgene in most of the generated transgenic plants. Transgenic lines have been propagated for greenhouse and field disease resistance assays.

P-2014

Site-specific Integration of Transgenes into the Sugarcane Genome for Elimination of Transgene Position Effects. Y. ZHAO[1], J. Kim[1], J. Jung[1], C. Fan[2], W. Yu[2], S. Dong[2], V. Srivastava[3], and F. Altpeter[1]. [1]University of Florida, 1692 McCarty Drive, McCarty Hall D, RM 3062, Gainesville, FL 32611; [2]Syngenta Biotechnology Inc., Research Triangle Park, NC; and [3]University of Arkansas, University of Arkansas, Fayetteville, AR 72701. Email: yangzhao779@ufl.edu

Transgenic sugarcane plants with improved agronomic and value-added traits have already been reported. Commercialization of transgenic sugarcane is expected in the near future, which will benefit the U.S. and global sugar and biofuel industries. Future transgenic strategies will aim at incorporating several traits or entire pathways using transformation vectors harboring multiple transgene expression cassettes. Transgenes integrate randomly into the genome causing line to line variability in the level and stability of transgene expression. Therefore, it is highly desirable to develop strategies for site directed integration of transgenes into the sugarcane genome. Our goal is to achieve transgene integration into a pre-characterized genomic site which supports stable and high transgene expression. Our objective is to develop a site-specific recombination platform for sugarcane and apply this technology for gene stacking. The series of experiments include: 1) Construct and introduce a vector that incorporates a site-specific recombination target downstream of a constitutive promoter into sugarcane. 2) Characterize these target lines to select single copy events for re-transformation by site-specific recombination. 3) Deliver promoter less selectable marker gene with site specific recombination site and stacked reporter gene expression cassette into selected target lines. 4) Evaluate transgene performance and integration. We will present data describing the generation and characterization of sugarcane target lines for retransformation by site specific recombination and promoter trapping.

P-2015

Functional Characterization of a Soybean SUMO E3 Ligase GmSIZ1-1. Z. LI, Q. Hu, J. Zhao, K. Rebholz, H. Knap, and H. Luo. Clemson University, 105 Collings Street, Biosystems Research Complex, Clemson, SC 29634-0318. Email: zhiganl@clemson.edu

SUMO E3 ligasesmediating SUMO attachment to other proteins in Sumoylation process, modulates protein stability, subcellular localization and activity, thus it regulates most cellular functions including response to environmental stress in plants. Here, we report the isolation and molecular characterization of *Glycine max* SIZ1-1 (*GmSIZ1-1*), one of the soybean SIZ1 homologs. The soybean SIZ1-1 protein is localized to the nucleus and showed sumoylation activities in model plant *Arabidopsis*. The expression of *GmSIZ1-1* in *Arabidopsis siz1-2* mutant complemented the mutant phenotype. Overexpression of GmSIZ1-1 in wild-type *Arabidopsis* impacts plant response to abscisic acid treatment, and enhances plant performance under adverse environmental conditions including extreme temperature, drought and salinity stresses. The results suggest that GmSIZ1-1 is a functional SUMO E3 ligase that is involved in protein sumoylation pathway in plant cells, demonstrating the great potential of using *GmSIZ1* in genetic manipulation of sumoylation process in transgenic crop species for improved resistance to broad abiotic stresses.

P-2016

Determination of Apple Mosaic Virus (*ApMV*) in Some Turkish Hazelnut Cultivars (*Corylus avellana* L.) via Reverse Transcription-Polymerase Chain Reaction (RT-PCR). K. EFE, M. Gül Şeker, B. Köksal, M. Divyapıcıgil, Y. Özden Çiftçi, and E. Kaya. Gebze Institute of Technology, Istanbul Str., Kocaeli 41400, TURKEY. Email: kemal_efe@hotmail.com

Hazelnut (*Corylus avellana* L.), which is an important agricultural products of Turkey, is known as the living source of almost 395 thousand farmers. Our country meets 70-75 % of the world's production in 540 thousand hectar. However, hazelnut can be infected by several viruses that can affect its production. The most economically damaging *Ilarvirus* affecting hazelnut on a worldwide scale is related to apple mosaic virus (*ApMV*). *ApMV* is present world-wide and preferentially exist on woody hosts such as apple, apricot, cherry, almond, plum, and peach. The investigation of hazelnut infestation with *ApMV* is conducted mostly in the countries where the plant is cultivated commercially: USA, Spain, Italy, and Turkey. Thus, it is important to detect *ApMV* infection in hazelnut. Although virus infection can be screened symptomatically and DAS-ELISA, the most suitable method for detection of even the low titration of the virus is the usage of reverse-transcription polymerase chain reaction (RT-PCR). Therefore, we aimed to develop an efficient procedure for detection of *ApMV* in some symptomatically infected Turkish hazelnut cultivars ("Fosa", "Mincane", "Palaz" and "Tombul") via one-tube RT-PCR technique in this study. Our results indicated that RT-PCR can be used efficiently for the detection of *ApMV* in hazelnut.

P-2017

A Protocol for Direct Somatic Embryogenesis in Medicinal Tree *Murraya koenigii* (L.) Spreng. R. JOSHI. Government College, M.D.S University, Ajmer, C-1043, Near St. Stephen's School, Panchsheel Nagar, Ajmer 305004, INDIA. Email: drrameshjoshi10@gmail.com

A reproducible protocol for direct somatic embryogenesis was established in *Murraya koenigii*. The conventional method of propagation of this tree is limited to seeds only, which retain their viability for only a short period. No information was gathered for the genetic improvement of *M. koenigii*. Hence, a biotechnological approach might have an advantage edging over traditional breeding as well as the genetic improvement of *M. koenigii* within a short period. The hypocotyle, cotyledons and root and nodal segments were excised from 60 days old *in vitro* grown seedlings. globular somatic embryos were induced on MS medium supplemented with 2.0mg/lit 6-benzyladenine (BA), 0.5mg/lit. α-naphthaleneacetic acid (NAA) and 1.0mg/lit thidiazuron (TDZ). The best result obtained for the average frequency of somatic embryos was from hypocotyle explants, which was significantly higher than cotyledons and root explant.The highest frequency of somatic embryos was recorded from hypocotyle segments in 6 weeks. The age and type of explant and concentration of TDZ played an important role in the development of somatic embryos. The heart and torpid stages of all the somatic embryos were also recorded. Most of the somatic embryos (above 95%), irrespective of their origin, germinated after 4 weeks in 1/2 MS basal media. Well-rooted plantlets were successfully acclimatized.

P-2018

Callus Induction from *In Vitro* Grown Sugarcane Plants . G. SANAHUJA, Y. Zhao, and F. Altpeter. University of Florida, IFAS, Agronomy Department, Plant Molecular and Cellular Biology Program, 3062, McCarty Hall, Gainesville, FL 32611. Email: georgina.sanahuja@gmail.com

Sugarcane (*Saccharum officinarum* L.) is a prime crop for sugar and biofuel production and is widely cultivated in tropical and subtropical regions. Due to its global importance,

research efforts in sugarcane crop improvement through biotechnology have recently intensified. Application of tissue culture techniques and genetic engineering are providing opportunities for producing sugarcane with multiple value added traits. Protocols for somatic embryogenesis of sugarcane are available. These involve the induction of callus from various explants of field or greenhouse grown donor plants including immature inflorescences, young leaves and axillary meristems. Repeated rounds of transformation of sugarcane for transgene stacking requires vegetative propagation of the primary transgenics. *In vitro* propagation of sugarcane through direct organogenesis from leaf whorl cross seections is routinely used for accelerating the vegetative propagation. The objective of this study was to explore the potential of axillary and apical meristems of such *in vitro* grown plantlets to induce embryogenic callus. This not only reduces the risk of contamination posed by soil grown plants but also accelerates the process. Different meristems of *in vitro* grown plants (axillary and apical), different growth regulators and differences in explant preparation (longitudinal and cross sections) and orientation on the media were compared in a factorial and randomized design. The frequency of callus induction and the associated callus quality from these different treatments will be reported.

P-2019

A Modified Protocol for Isolation of Genomic DNA from *Commiphora wightii* (Arnott.) Bhandari. - An Endangered Medicinal Plant. P. VYAS[1] and R. Joshi[2]. [1]Government College, M.D.S University, Ajmer, 1-DA-21, Near Subhash Udhyan Jodhpur 342003, INDIA and [2]Government College, M.D.S University, Ajmer, C-1043, Near St. Stephen's School, Panchsheel Nagar, Ajmer 305004, INDIA. Email: lumossolem1@gmail.com

For molecular studies, a paramount requirement is the isolation of DNA of sufficient molecular wt. & purity suitable for restriction analysis, cloning and selective sequence amplification. A simple and improved DNA extraction method from polysaccharide rich leaves of *Commiphora wightii* (guggul) has been developed. Fresh green leaves weighing 0.5 g were de-veined and grinded to a fine powder in mortar pestle using liquid N_2. A 60 ml homogenization buffer stock was prepared by adding 9ml 150 mM Tris-Cl, 3 ml 25mM EDTA, 18ml 1.5 M NaCl (all at pH 8.0) to 30 ml of DDW, and warmed at 65ºC. 2.1g CTAB and 1.8g PVP was added to the pre-warmed solution, 180 µl Beta Mercaptoethanol was added prior to the process of homogenization. The fine leaf powder was then suspended in 3 ml of pre-warmed CTAB solution. This 3 ml suspension was transferred to a sterile centrifuge tube & 20 µl of RNase was added it. The solution was incubated for 45 minutes at 65ºC with gentle inversions. The tube was then cooled to room temperature & 3 ml of Chloroform: IAA ratio (24:1) was added to it. The tube was inverted gently 20-25 times to form an emulsion. The emulsion was centrifuged at 10,000 rpm for 10 min. at RT. The upper aqueous layer was pipette out, transferred into sterile centrifuge tubes without disturbing the interphase. 3 ml of 3M NaCl was added to the aqueous phase and once again subjected to centrifugation at 10,000rpm at RT. 0.6 volumes (1.8 ml) Isopropyl alcohol was added to the aqueous phase, mixed well and incubated for 30 min. at RT. The solution was centrifuged at 10,000 rpm for 15 min. at RT. The supernatant obtained was gently poured off. The pellets obtained were washed thoroughly with 750 µl of 70% ethanol & spun at 10,000 rpm for 5 minutes. The supernatant was discarded and the white pellet obtained was air dried (~45 min), & then re-suspended in 30 µl of TE (10 mM Tris HCl+ 0.1 mM EDTA; pH 8.0) at 4ºC overnight. This method allowed recovery of good quality DNA, suitable for complete digestion by restriction endonucleases and amplifiable in PCR as compared to other methods.

P-2020

Development of Fluorescence Protein Marked Horseweed (*Conyza canadensis*). R. YE[1], Y. Peng[1], J. Wang[2], R. Millwood[1], and C. Stewart[1]. [1]Department of Plant Sciences, University of Tennessee, 252 Ellington Plant Sciences, Building 2431 Joe Johnson Drive, Knoxville, TN 37996-4561 and [2]Illinois State Water Survey, Prairie Research Institute, University of Illinois at Urbana-Champaign, Champaign, 2204 Griffith Dr., Champaign, IL 61820. Email: yerongjian@utk.edu

The evolution of glyphosate resistance (GR) in weedy species places an environmentally benign herbicide in peril. Horseweed (*Conyza canadensis*) with evolved GR has become an especially problematic weed in crop production across the United States. The GR horseweed gene flow research can provide valuable information for field horseweed control and biosafety evaluation. Horseweed is a self-pollination plant, so, most of the published horseweed gene flow studies have been focused on seed spread. There is little knowledge about transfer of GR via pollen as a mechanism of horseweed gene flow. The objective of this study was to produce transgenic GR-horseweed with fluorescence

protein-tagging for gene flow research. The green fluorescence protein (mGFP5-ER) and red fluorescence protein (tdTomato-ER) were driven by the CaMV 35S promoter and a tomato pollen-specific LAT52 promoter, respectively. These genes were inserted into expression vector PMDC99, and the construct was introduced into horseweed TNR (glyphosate resistant horseweed from Tennessee) by *Agrobacterium*-mediated transformation. These transgenic events will be characterized using conventional molecular methods and also via epifluorescence microscopy. After that, the homozygous transgenic lines will be used in the pollen dispersion and outcrossing experiments. The horseweed transformation experiments resulted in multiple events of transgenic callus characterized by epifluorescence microscopy. Transgenic plant regeneration is under way.

P-2021

Molecular Characterization of Some Turkish Olive (*Olea europaea* L.) Cultivars Based on IRAP Markers. B. KÖKSAL[1], E. Kaya[1], K. Efe[1], B. Uzan[2], and Y. Özden Çiftçi[1]. [1]Gebze Institute of Technology, Istanbul Str., Kocaeli 41400, TURKEY and [2]Republic of Turkey General Directorate of Forestry, Poplar Research Institute, D-100 Karayolu, Hasat S., PK. 93, 41001, Izmit, Kocaeli. Email: busrakoksal91@gmail.com

Olive (*Olea europea* L.), which has nearly more than 1200 cultivars, is one of the oldest cultivated plants originated in the Mediterranean area. It has two forms, wild (*O. europaea* subsp. *europaea* var. *sylvestris*) and cultivated ones (*O. europaea* subsp. *europaea* var. *europaea*). The cultivated olive (*O. europaea L. var. europaea*) is propagated by cutting or grafting whereas wild olive (*O. europaea L. var. sylvestris*) reproduced from seeds. Olive trees have high levels of heterozygosity and thus genetic diversity among cultivars is so high that they are predominantly allogamus. This variability, coupled with the confusion in olive cultivar identification, make necessary the evaluation and characterization of olive genetic resources since both olive productivity and oil quality are traits inherited to a variety. Therefore, molecular markers, such as inter-retrotransposon amplified polymorphism (IRAP) markers, are environment-independent and efficient to both identify olive varieties and detect synonymous and homonymous. Thus, ten selected IRAP markers are used for determination of relationships among twenty individuals belonging to important Turkish olive cultivars ("Gemlik", "Hatay", "Mardin" and "Mugla"). Our results showed that retrotransposon-based IRAP markers can be used to differentiate olive cultivars.

P-2022

Expression of Thermostable Starch Synthase Genes in Wheat Improves Grain Fill at Elevated Temperatures. H. TRICK[1], B. Tian[1], S. Talukder[2], H. Lee[1], and A. Fritz[1]. [1]Kansas State University, Plant Pathology, 4024 Throckmorton, Manhattan, KS 66506 and [2]The Samuels Robert Noble Foundation, Forage Improvement Division, 2510 Sam Noble Parkway Ardmore, OK 73401. Email: hnt@ksu.edu

Heat stress is one of the major environmental constraints for wheat production worldwide. Wheat is a temperate cereal with an optimum growth temperature range of 15-22°C during the grain filling stage of development. For every 1°C rise above the optimum temperature, yield is reduced ~3-4%. This reduction in yield is primarily due to the loss of activity to one key heat-labile enzyme in the starch biosynthesis pathway: soluble starch synthase (SSS). If the conversion of sucrose to starch could be increased during higher temperatures, greater test weights would follow, bringing greater yields and improved grain quality to the producers. One method to increase starch synthesis during high temperature is to genetically engineer wheat with a replacement SSS gene that is more stable at high temperatures. Tropical cereals (e.g. rice), for example, are more resistant to high temperatures than temperate cereals. Expression of putative thermostable SSS in wheat may increase the productivity under heat stress. We have independently expressed the SSS from rice under regulatory control of both the maize ubiquitin and wheat high molecular weight glutenin (HMW-Dy10) promoters in transgenic wheat and carried events out to the T_3 generation. Under optimum growth conditions all agronomic traits evaluated were not significant. However with elevated temperatures during grain fill (34/28 °C (d/n)), transgenic lines have demonstrated between 20 and 34% increase in 1000 seed weight. In addition to the SSS gene from rice, we have identified addition starch synthase genes from other species with greater heat tolerances based on a thermostabililty prediction algorithum. We will report progress on the transformation and recovery of engineered events with these genes as well.

P-2023

Developing Intragenic Biotechnology for Sugarcane. H. DERMAWAN, J. Jung, and F. Altpeter. Agronomy Department, Plant Molecular and Cellular Biology Program and Genetics Institute, University of Florida - IFAS, Gainesville, FL. Email: hugohd17@ufl.edu

Sugarcane produces 80% of the world's sugar and is Florida's most valuable field crop. Genetic engineering of sugarcane will contribute to sugarcane improvement. Intragenic biotechnologies may facilitate regulatory approval and require a selectable marker from sugarcane or sexually compatible species to select intragenic events. Acetolactate synthase (ALS) is a key enzyme in the biosynthetic pathway of branched-chain amino acids. It is the target site of highly selective ALS-inhibiting herbicides including sulfonylureas and imidazolinones. In this study, a minimal expression cassette encoding a mutant acetolactate synthase gene from sorghum as well as a sorghum ubiquitin promoter and HSP 3' UTR were introduced into tissue cultures of sugarcane cultivar CP 88-1762. A selection protocol was developed by identifying a suitable herbicide and concentration for recovery of intragenic events. PCR, qRT-PCR and herbicide application were carried out to identify intragenic events.

P-2024

Development of Regeneration and Mutagenesis Systems for *Arundo donax*. Y. DAN and A. Kekkonen. Institute for Advanced Learning and Research, 150 Slayton Avenue, Danville, VA 24540. Email: yinghui.dan@ialr.org

Giant Reed(*Arundo donax L.*) has drawn increasing attention as a promising bioenergy crop due to its great biomass productivity and cropping under low input conditions for irrigation, fertilization and weed treatment. Since Arundo is a sterile plant, its traditional propagation is based on vegetative propagation via fragmentation of rhizomes or canes, making it very expensive for large-scale crop establishment. Furthermore, conventional breeding is restricted due to its sterility and narrow genetic background. Therefore, Arundo improvement will rely on mutagenesis, transgenic approaches and ecotype selection. We are developing regeneration systems via callus using leaf and root explants and mutagenesis systems to overcome these limitations. We have developed a regeneration system via callus phase using leaf explants. With this system 78% of explants produced organogenic calli 3 to 4 weeks after culture, and plant regeneration was obtained approx. 3 weeks after culture. We have developed a mutagenesis system using ethyl methanesulfonate (EMS) to enhance biomass and cold tolerance of giant reed. The explants were treated with different EMS concentrations in combination with various levels of plant growth hormones in culture media. Four to five weeks after EMS treatments, 95.3% to 100% of explants produced calli for all concentrations tested. Optimal frequency of 46.7% of putative mutant shoot and/or bud regeneration was obtained from the calli approx. 4 to 5 weeks after culture. Putative mutant plants were regenerated from the calli. Development of the regeneration and mutagenesis systems using leaf, root and meristem explants, and their application to corn and wheat regeneration systems will be discussed in detail in the poster.

P-2025

Marker-gene Excision by Cre/lox and Its Stable Inheritance in Site-specific Gene Integration Lines of Transgenic Rice Generated by FLP/*FRT* Recombination. S. NANDY and V. Srivastava. University of Arkansas, 115 Plant Sci. Bldg, Fayetteville, AR 72701. Email: snandy@uark.edu

Plant transformation technology is creating 'transgenic' crop plants to enhance agricultural productivity so as to meet the future needs of food, fiber and energy. Along the way the technology also brings in the marker genes that allow the selection of regenerated transgenic plants. However, these marker genes are generally superfluous once an intact transgenic plant has been established. To remove these marker-genes and to create a safe transgenic crop, marker-free transgenic technology is running in the fore-front. Strategies are being developed to generate marker-free transgenic plants that would help allay the concern. Here, a 'clean' transformation system, based on two independent recombination systems, FLP/*FRT* and Cre/*lox,* is reported. These recombination systems are versatile tools for precise genomic manipulations such as DNA excision and integration. By using FLP/*FRT*, site-specific integration lines were developed in rice, consisting of gene-of-interest and selection marker genes. The placement of 4 *lox* sites within the integration locus was an important part of the strategy, which primed the locus for excision of marker genes and other undesirable genes within the locus by heat-inducible Cre activity, generating a 'clean transgene locus'. Young plants of four integration lines were subjected to heat-treatment that resulted in production of the 'clean' locus. Each line transmitted the 'clean' locus to next generation, establishing 'clean' transgenic lines. The efficiency of the transmittance of 'clean' locus varied from 100% to 40%. This work demonstrates the integration of two applications of recombination systems, marker excision and precise transgene integration, for efficient production of 'clean' transgenic plants.

P-2026

Tobacco Hairy Roots as a Unique Platform to Study Plant Hydroxyproline- O-glycosylation Process and a Proposed Role of Hyp-glycans in Secretion. N. ZHANG, F. Medina-Bolivar, B. Savary, and J. Xu. Arkansas State University, P. O. Box 639, State University, AR 72467. Email: ningning.zhang@smail.astate.edu

Hydroxyproline (Hyp)-rich glycoproteins (HRGPs) are a superfamily of plant cell wall proteins that function in diverse aspects of plant growth and development. Structurally, HRGPs are modular glycoproteins with marked peptide periodicity. They are produced through Hyp-O-glycosylation involving post-translational hydroxylation of proline to Hyp and subsequent O-glycosylation, a modification unique to plants and green algae. Our earlier work showed that peptide sequence directs Hyp-O-glycosylation of HRGPs. Furthermore, Hyp-O-glycosylation facilitates secretion and improves stability of recombinant proteins expressed in plant cell cultures. However, the precise process of Hyp-O-glycosylation has yet been elucidated so far. Plant hairy root culture, in which fully differentiated root tissues propagate rapidly in liquid media, is regarded as a unique platform to study the Hyp-O-glycosylation process. Two major types of HRGP motifs, an extensin consisting of tandem repeats of "Ser-Hyp-Hyp-Hyp-Hyp" motif, and an arabinogalactan protein (AGP) consisting of tandem repeats of "Ser-Hyp" motif, were each engineered in tobacco hairy roots as fusion with a reporter protein EGFP. As in plant cell cultures, significantly enhanced secretion of the HRGP-tagged EGFP was determined in hairy root cultures. While fully glycosylated extensin motif was detected in both the culture media and roots, interestingly, two types of glycoforms of the AGP motif were observed; they were completely segregated with the partially glycosylated form retained inside the roots while the fully glycosylated form was recovered in the media. Treatment of the hairy roots with brefeldin A that blocks protein transport from ER to Golgi was further conducted to track the Hyp-O-glycosylation process in hairy roots.

P-2027

Fostering Plant Science Research at MU Plant Transformation Core Facility. L. ZHOU, S. Valdes, H. Lee, W. Neng, X. Yin, H. Li, S. Lu, M. Folta, C. Wu, M. Mookkan, P. Do, K. Duan, Z. Zhang, and Z. Zhao. University of Missouri, 1-31 Agriculture Building, Columbia, MO 65211. Email: zhouliw@missouri.edu

Since its establishment in 2000, the University of Missouri (MU) Plant Transformation Core Facility has been providing state-of-the-art research support services in genetic engineering of several major crops species. Located inside the new MU central campus greenhouse, the Sears Plant Growth Facility, the Plant Transformation Core Facility is aiming at promoting gene discovery, crop improvement, and funding opportunities for the plant science research community. Our staff is strongly dedicated to providing various types of transformation support services and conducting research in transgenic technology development with a focus on maize (*Zea mays*), soybean (*Glycine max*), switchgrass (*Panicum virgatum)*, and sorghum (*Sorghum bicolor*). The facility service categories include both standard and customized transient as well as stable transformation for maize and soybean upon the user's request. The transformation systems for all the above crops utilize *Agrobacterium*-mediated approaches and somatic embryogenesis processes except for soybean. We employ the *Agrobacterium*-mediated cot-node transformation system coupled with organogenesis regime for soybean transformation. We are now establishing efficient transformation system and soon will be ready for wheat transformation services. We are also advancing sorghum transformation system and soon will be offering very competitive price. In addition, we are ready to take on new service projects to transform new plant species as user's requests. Current research activities are geared towards developing high-throughput transformation, improving the quality of transgene integration and sufficient gene regulation to meet the needs of crop improvement and functional genomics. Our specific interest in soybean genetic engineering is to regulate several economically important genes conditioning soybean seed traits, abiotic stress, virus resistance, etc. Some of these studies are conducted as collaborations with on- and off-campus researchers. More details of these activities will be presented at the conference.

P-2028

Engineering Improved Photosynthesis into Sugarcane. R. KARAN[1], X. Zhang[1], A. Vilharinho[1], H. Wu[1], J. Jung[1], Y. Zhao[1], X. Chen[1], A. Grennan[2], D. Ort[2], and S. Long[2]. [1]University of Florida, Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, Gainesville, FL and [2]Department of Plant Biology, Department of Crop Sciences, University of Illinois. Email: karan.ratna@gmail.com

Sugarcane is one of the most productive biofuel crops due to its superior photosynthetic efficiency. Sugarcane has a C4 type metabolism for fixation of carbon, allowing it to be very well adapted to biomass production in tropical and subtropical regions. Genetic improvement of photosynthetic efficiency can be achieved by targeting higher photosynthetic rates/unit of leaf area and by developing the most photosynthetically effective canopy. We introduced several different transgenes into sugarcane and will report on transgene expression and its correlation to altered phenotypes and the consequences for photosynthesis.

P-2029

Gladiolus Plants Transformed with D4E1, a Synthetic Antimicrobial Peptide, for *Fusarium oxysporum* Resistance. K. KAMO[1], L. Dilip[1], G. Bauchan[2], K. Rajasekaran[3], J. Cary[3], and J. Jaynes[4]. [1]USDA, 10300 Baltimore Ave., Floral & Nursery Plants Research Unit, Beltsville, MD 20705; [2]Electron & Confocal Microscopy Unit; [3]USDA Food and Feed Safety Research Unit; and [4]Tuskegee University, 1200 W Montgomery Road, Tuskegee, AL 36088. Email: kathryn.kamo@ars.usda.gov

The main pathogen of *Gladiolus* plants is *Fusarium oxysporum*, a soilborne fungus that causes rotting of its corms and kills the plant. Purified D4E1, a synthetic antimicrobial peptide, was found to effectively inhibit 100% of *F. oxysporum* f. sp. *gladioli* spores from germinating *in vitro* at a concentration of 12.5 μM. *Gladiolus* cv. Peter Pears plants were transformed with the gene for D4E1 using particle bombardment, and 14 independent transformants were obtained. The gene for D4E1 consists of 66 bp and was under the control of the CaMV 35S promoter. Transgenic plants were tested *in vitro* for resistance to *F. oxysporum*, and a few lines appeared to be more resistant than the control plants that lacked D4E1. Cell extracts of transgenic *Gladiolus* lines 6(1) and 7(1) inhibited germination of *F. oxysporum* spores by 34 and 38%, respectively, *in vitro*. *F. oxysporum* was transformed with the ECFP (cyan) gene allowing us to follow the growth of *F. oxysporum* during infection of D4E1 in transformed and non-transformed roots. Non-transformed shoot and root tissue appeared to be disintegrating 10 days after inoculation with *F. oxysporum*. Fluorescence observations using the confocal laser scanning microscope showed that *F. oxysporum* covered the surface of the root at this time, but mycelia had not penetrated the root. In contrast, transformed *Gladiolus* line 7(1) showed relatively few fungal mycelia around the root and on its surface 10 days after inoculation and no mycelial penetration of root tissue.

P-2030

Genetic Transformation of Basil. R. DAME. Western Kentucky University, 710 Madison Ave., Owensboro, KY 42301. Email: damevedder1@hotmail.com

The purpose of this study is to create transgenic *Ocimum sanctum*, Holy Basil, and other related basil species using the reporter gene, Green fluorescent protein (GFP) from the jellyfish *Aequorea victoria*. This is an important innovation that allows gene expression to be visually inspected by GFP fluorescence in the transgenic basil plants. If the plants exhibit bright green fluorescence then this is proof that a gene can be expressed. The GFP reporter gene can replace the more widely used technique that incorporates the GUS gene to signal expression, where some of the plants must be sacrificed for chemical analysis. Also, when GFP is used then no time is wasted caring for plants that were not transformed. I will be presenting a poster depicting the transformation pathway used to introduce the GFP gene into the Holy Basil and exhibit my results.

Disclosure: Author received research grant from Western Kentucky University.

P-2031

Development of Innate Potato Varieties by Marker-free Transformations. M. SINGH, T. Carroll, C. Richael, and T. Weeks. JR Simplot Co., 5369 W Irving St, Boise, ID 83706. Email: manmeet.singh@simplot.com

New technologies that eliminate the need for selective markers (antibiotic/herbicide) and foreign DNA could greatly improve public acceptance of genetically modified plants. We have developed a transformation method for the production of commercial potato (*Solanum tuberosum*)-varieties that are marker-free, backbone-free and only contain native potato promoters/genes. This method employs a vector containing the bacterial isopentenyltransferase (*ipt*) gene which drives regeneration and acts as a backbone integration marker. *Agrobacterium* strain carrying a marker-free/*ipt* commercial construct targeting bruise resistance, low sugars, and low acrylamide was used to infect potato explants of Russet Burbank, Ranger Russet, and Atlantic. Upon transfer to hormone-free medium, infected explants produced shoots with a gene of interest (GOI) frequency of 1% and

P-2032

Recombinase-mediated Cassette Exchange (RMCE) for Gene-stacking in *Saccharomyces cerevisiae*. S. HOTTON[1], M. Cook[2], and J. Thomson[2]. [1]University of California-Davis, 800 Buchanan Street, Albany, CA 94710 and [2]USDA-WRRC, 800 Buchanan Street, Albany, CA 94710. Email: skhotton@ucdavis.edu

Presented here is work done in the model *Saccharomyces cerevisiae* (budding yeast) to integrate multiple genes-of-interest (GOIs) at a targeted locus, using recombinase-

mediated cassette exchange (RMCE). RMCE is a method of genomic engineering that uses site-specific recombinases for precise integration of GOIs and removal of excess DNA, and it has broad application as a high-throughput cloning tool to produce predictable gene expression. A gene-stacking tool is presented here that alternates use of large serine recombinases, Bxb1 and *Phi*C31, for multiple GOI integration events, combined with the small serine recombinase CinH for excision. Yeast TAG lines were developed by disruption of the *URA3* locus with recombinase recognition sequences (RS) flanking a selectable marker. EXCH plasmids were then developed for gene-stacking. The first GOI is introduced with RS1 for precise integration at the TAG site, and removal of the recombinase and selectable marker sequences from the system. The EXCH vector also brings in a unique RS2 for the *next* integration event. After the first integration/excision cycle, only the GOI with RS remain. The second GOI is then introduced via the RMCE strategy bringing a unique RS1 for continued stacking. By alternating use of two EXCH plasmids with different RS sites and integrating recombinases, gene-stacking is achieved. Currently, I am using these tools to introduce multiple copies of a GOI into yeast for trait enhancement studies. Work presented here is done in yeast – due to its sequenced/annotated genome, short life-cycle, and genetic tractability, with investigations in plant systems also underway.

P-2033

Soybean Anther Culture: A Preliminary Data Report. M. GARDA and G. Phillips. Arkansas State University, 504 University Loop East, ABI Rm 343, Jonesboro, AR 72401 Email: gardamartina@gmail.com; martina.garda@smail.astate.edu

Food is a constant world demand, and soybeans are among the most complete food crops which have fed people since its domestication (Guo et al.2010; Hartman et al. 2011). Unfortunately, soybeans are difficult to work with in the biotechnology lab (Pratap et al. 2010; Somers et al. 2003; Thomas et al. 2003). In an effort to overcome this challenge, we report some preliminary data to produce double haploids (DH) in just one generation (Barnabas et al. 1999; Jain et al. 1996; Seymour et al. 2012). The overwhelming majority of anther culture responses consisted of filament-derived callus formation, regardless of the extent of filament tissue attached to the anthers. Very few anthers in culture developed calli that appeared to emerge from the inside of the anthers. If we are successful, soybeans will become more malleable by shortening the *in vitro* growth time and obtaining superior yields, allowing future development of new and better varieties in a shorter and less expensive approach (Ferrie & Caswell 2011; Seymour et al. 2012).

P-2034

Cell Suspension Culture of *Magnolia dealbata* Zucc in Bioreactor. J. CANTOR DEL ANGEL[1], M. Mata[2], A. Del Villar Martínez[1], G. Sepúlveda Jiménez[1], and M. Rodríguez Monroy[1]. [1]CEPROBI-IPN, Carretera Yautepec-Jojutla, Km. 6, calle CEPROBI No. 8, Col. San Isidro, Yautepec, Morelos, México, Carretera Yautepec-Jojutla, Km. 6, calle CEPROBI No. 8, Col. San Isidro, Yautepec, Morelos, Yautepec 92630, MEXICO and [2]INECOL, Carretera antigua a Coatepec 351, El Haya, Xalapa, Veracruz 91070, MéXICO. Email: hi_can@hotmail.com

Magnolia dealbta Zucc is an endemic plant from Mexico and could be used to treat cancer disease and cardiovascular affections. Honokiol and magnolol are two phenilpropanoid responsible of the pharmaceutical activity. *M. dealbata* suspension culture growing in Erlenmeyer flask producing phenilpropanoid has been reported. However, it is necessary to delineate the operation conditions in bioreactor to obtain the successfulcellgrowth. Thus, the aim of this work was to know the effect of supply oxygen over the cell viability of *M. dealbata*. Cell suspension cultures were grew in Erlenmeyer flask (500 mL) containing 100 mL of Murashige & Skoog media and closed with different caps. *M. dealbata* cultures in Erlenmeyer flasks closed with aluminum, cotton and silicone caps generated oxygen transfer rate (OTR) values of 0.07, 0.58 and 1.04 kg O_2 m^{-3} d^{-1}, respectively. Culture closed with an aluminum cap had a viability of 70%, while the cell viability of cultures closed with cotton and silicone caps was 20 %. Considering that *M. dealbata* cells were affected negatively by the high OTR, conditions to grow *M. dealbata* in stirred tank bioreactor (3 L) was defined to prevent a possible stress by oxygen. It was used a Rushton impeller, 400 rpm, and 0.1 vvm of aeration rate. Growth kinetic parameters of cells suspension cultures developing in Erlenmeyer flask and bioreactor were similar. Maximum biomass reaching in bioreactor was 16.79 ± 1.05 g DW L^{-1} and the generated in Erlenmeyer flask was 18.95 ± 1.40 g DW L^{-1}. Specific growth rate of culture was $\mu = 0.15$ day^{-1}. Cell viability was over 70 %. pH values of culture broth were between 5.1 to 5.3. The production of honokiol and magnolol is subject of study to confirm the successful growth of *M. dealbata* in bioreactor.

P-2035

Endpoint cDNA Expression Analysis of Genes Involved in Host Defense to Disease in Developing Sweetpotato Storage Roots. G. BERNARD, M. Egnin, S. Samuels, D. Mortley, W. Witola, C. Bonsi, O. Idehen, and C. Lee. Tuskegee University, Tuskegee, AL 36830. Email: gcbernard4673@mytu.tuskegee.edu

Background Sustainable sweetpotato production is constrained by the presence of disease resulting in decreased crop yields and overall quality as well as reduced profits for producers. Differential genetic expression patterns of a variety of transcripts involved in the host response to disease have been used to distinguish resistant and susceptible plant genotypes. Objectives In this study, a molecular fingerprinting analysis was conducted using sweetpotato cultivars in efforts to investigate significant differences in expression patterns of transcripts involved in overall disease defense. Our goal is to develop an efficient preliminary molecular screening analysis for existing and newly developed sweetpotato cultivars. Materials and Methods 40 day-old sweetpotatos with different levels of disease tolerance grown under heavily infested field conditions were collected for this study. Developing storage roots were lyophilized and ground to powder for subsequent total RNA extraction and cDNA synthesis. Candidate disease resistant primers were developed from previously reported expressed sequence tags and used to PCR amplify transcripts in the selected cultivars. Results An increased expression of resistance gene *CC-NBS-LRR* transcripts was demonstrated in disease susceptible cultivar Georgia Jet in comparison to resistant sweetpotato cultivar Jewel and newly developed DMO1 and TU-02. Conclusion Although the genes that are involved in disease resistance are numerous, dynamic and encompass multiple converging pathways that confer the resistant or susceptible phenotype, the differential expression patterns shown in this study may indicate genotype specificity to disease tolerance and resistance to major pests of sweetpotato. Our analysis may promote efficiency in marker assisted breeding programs and the development and analysis of new resistant cultivars in efforts to promote sustainable agricultural production of sweetpotato. Research supported by USDA-Evans-Allens and NSF-CREATE-IGERT.

P-2036

Potential Utility of a Parthenogenesis Gene for Apomixis and Haploid Induction. P. OZIAS-AKINS, J. Conner, M. Muruganantham, K. Chae, and H. Huo. University of Georgia, 2360 Rainwater Road, Tifton, GA 31793. Email: pozias@uga.edu

A candidate gene for parthenogenesis, *ASGR-BABY BOOM-like* (*ASGR-BBML*), was cloned from *Pennisetum squamulatum*, a natural apomict closely related to the forage and grain crop, pearl millet. The gene is tightly linked with apomixis in populations segregating for mode of reproduction, and a rare recombinant without the gene also is defective in parthenogenesis. *ASGR-BBML* is predicted to encode an AP2-domain transcription factor, and similar genes have been shown to induce ectopic embryos when overexpressed in transgenic *Brassica* and *Arabidopsis*. The gametophytic form of apomictic reproduction, or clonal reproduction through seeds, requires the formation of female gametes from chromosomally unreduced cells. Furthermore, these unreduced female gametes develop into embryos without fertilization by a male gamete. We have shown that the *ASGR-BBML* promoter drives expression of GUS in the egg or egg apparatus of embryo sacs. Expression of the native gene in sexual tetraploid pearl millet led to the recovery of dihaploid progeny from transgenic tetraploid pearl millet, i.e., meiotically derived eggs became parthenogenetic. Expression of *ASGR-BBML* to confer parthenogenesis potentially could be utilized as a breeding tool for synthesizing apomixis or inducing haploids in crops.

P-2037

High Density SNP Mapping and Genome Synteny to Identify Candidate Genes Underlying QTL for Pre-harvest Sprouting. M. JORDAN, A. Cabral, C. McCartney, and G. Humphreys. Agriculture and Agri-Food Canada, 101 Route 100, Morden, MB R6M 1Y5, CANADA. Email: mark.jordan@agr.gc.ca

Previous work by our group identified significant QTL for pre-harvest sprouting (PHS) traits on chromosomes 4A and 7D in the spring wheat population RL4452/AC Domain (Rasul G, Humphreys, DG, Brule-Babel A, McCartney CA, Knox RE, DePauw RM, Somers DJ. *Euphytica* 168:363-378) utilizing a simple sequence repeat based genetic map. Recently, a large number of SNP markers has become available in wheat and through community efforts many SNPs are now available via public databases http://www.cerealsdb.uk.net/, http://129.130.90.211/snp/) and a 90,000 gene-associated SNP genotyping assay is available based on the Illumina Infinium platform. Genotyping the 193 line RL4452/AC Domain population with this assay identified 12,351 polymorphic markers that were added to 419 existing SSR, DArT and EST markers. QTL analysis using phenotypic data from Rasul et al. (2009) revealed two QTL for sprouting index not identified in the previous study on chromosomes 3B and 7B and narrowed down the flanking region for the 4A and 7D QTL. The 7D QTL was for falling number and coincided with QTL for maturity and heading date. The genomic region on 4A was associated with QTL for germination index, falling number and sprouting index. Utilizing synteny with *Brachypodium distachyon* the gene encoding gibberellin 20-oxidase was identified as a candidate for the dormancy trait in the population RL4452/AC Domain. Analysis of the 5'-upstream region of the gene revealed an insertion in the non-dormant parent RL4452. The insertion marker was mapped into the region corresponding to the quantitative trait locus (QTL) for germination index. For 7D more markers are needed to further narrow down the region. Markers based on synteny to the

newly sequenced *Aegilops tauschii* genome have been developed and are currently being evaluated.

P-2038

Wheat Gene Expression Differences Induced by Six Races of *Puccinia triticina*. K. NEUGEBAUER[1], M. Bruce[2], and J. Fellers[2]. [1]Kansas State University, 4024 Throckmorton, Manhattan, KS 66502 and [2]USDA-ARS-HWWGRU, Manhattan, KS. Email: kerrin@ksu.edu

Puccinia triticina, the casual agent of wheat leaf rust, is a devastating disease that can cause up to 20% yield loss. During fungal infection the host plant recognizes proteins, secreted effectors, and other molecules, which trigger a host defense response. Changes in the pathogen effectors and strong varietal selection pressure are responsible for the rapid development of new rust races. Six races of leaf rust were evaluated on a single variety of wheat in a time course study to determine if different rust races utilize different pathways in wheat. RNA was sequenced and sixty-three wheat genes were identified that showed varying expression in response to different races. Of the sixty-three, fifty wheat genes have been characterized for timing of expression and whether expression is necessary for rust infection. Race specific expression was found. RNAi is now being used to silence select wheat genes to further understand their role in leaf rust infection.

P-2039

Optimizing Gene Insertion and Plant Regeneration Parameters for Precision Breeding of Cold-hardy Grapevine Cultivars. R. KANDEL[1], B. Hallwachs[1], T. Pham[1], M. Dutt[2], D. Gray[3], Z. Li[2], V. Sitther[4], D. Bergey[1], and S. Dhekney[1]. [1]University of Wyoming, 1348 Omarr Ave, Sheridan, WY 82801; [2]UF/IFAS, 700 Experiment Station Road, Lake Alfred, FL 33850; [3]Mid-Florida Research & Education Center, University of Florida/IFAS, 2725 S. Binion Rd., Apopka, FL 32703; and [4]Morgan State University, 1700 E. Cold Spring Lane, Baltimore, MD 21251. Email: sdhekney@uwyo.edu; rkandel@uwyo.edu

Grapevine improvement via precision breeding involves using genetic sequences found solely in the grape genome and is a logical refinement of conventional breeding, made possible by advances in cell culture, gene insertion and computational technology. *Vitis* species and cultivars vary in their response to gene insertion and plant regeneration rates, and individual parameters must be optimized for specific cultivars. The effects of 3 *Agrobacterium* strains EHA105, GV3101 and LBA4404, photoperiod during co-cultivation and bacterial culture density on gene insertion, embryo production and plant regeneration was studied in grapevine cultivars 'Bronx Seedless' and 'Thompson Seedless'. Embryogenic cultures and a green fluorescent protein gene were used to optimize experimental procedures. Transient gene expression ranged from 53 – 96% for various cultivars. Among the various *Agrobacterium* strains, the highest stable gene expression levels were observed in 'Bronx Seedless' embryogenic cultures co-cultivated with LBA 4404 (60.2%) followed by 'Thompson Seedless' co-cultivated with EHA 105 (52.2%). 'Bronx Seedless' and 'Thompson Seedless' embryogenic cultures co-cultivated at a 16 h light/8 h dark photoperiod or solely in the dark resulted in a similar rate of stable embryo production. Embryogenic cultures co-cultivated with *Agrobacterium* at an optical density of 1.5 exhibited higher stable gene expression (52 – 57%) and embryo production compared to cultures co-cultivated at OD values of 0.5 and 1.0. Uniform plant regeneration was obtained following germination of stable embryo lines. We are currently studying the response of additional grapevine cultivars including 'Interlaken' 'Himrod' and 'St. Croix'. Optimization of gene insertion and plant regeneration protocols for cold-hardy grapevine cultivars will enable transfer of functional traits including drought and salinity tolerance utilizing precision breeding technology.

P-2040

Over-expression of *AsHSP17*, a Creeping Bentgrass (*Agrostis stolonifera*) Small Heat Shock Protein, Increases Abiotic Stress Sensitivity in Transgenic *Arabidopsis thaliana*. X. SUN, C. Sun, Z. Li, and H. Luo. Clemson University, Department of Genetics and Biochemistry, 110 Biosystems Research Complex, Clemson, SC 29634. Email: xinbos@clemson.edu

Heat shock proteins (HSPs) are universal stress proteins that accumulate in response to heat and other abiotic stressors. Small heat shock proteins (sHSPs) are the most ubiquitous HSP subgroup with molecular weights ranging from 12 to 42 kDa. We have cloned a new sHSP gene, *AsHSP17* from creeping bentgrass (*Agrostis stolonifera*) and studied the role it plays in plant response to environmental stress. *AsHSP17* encodes a protein of 17 kDa. Its expression was strongly induced in both leaf and root tissues by heat treatment. *AsHSP17* was also induced slightly in root by salt and abscisic acid (ABA) treatment, but not by water withholding. No expression was found in leaf tissue under salt, drought and ABA treatment. These results indicate that *AsHSP17* accumulates in response to heat, salt and ABA but not in response to drought. Transgenic *Arabidopsis* plants overexpressing *AsHSP17* exhibited higher sensitivity to heat stress than wild-type controls. Overexpression of *AsHSP17* also led to decreased seed germination in transgenic plants under salt and ABA treatment. The results obtained so far suggest that

AsHSP17 may be a negative regulator involved in plant response to adverse environmental stresses. This information would allow development of molecular strategies manipulating sHSP expression in transgenic plants, achieving improved plant resistance to various abiotic stresses in crop species.

P-2041

Cell-free *In Vitro* Translation of Colchicine Pathway *N*-acetyltransferase. G. SIVAKUMAR, J. Condori, and G. Medrano. Arkansas State University, Arkansas Biosciences Institute and College of Agriculture and Technology, Arkansas State University, Jonesboro, AR 72401. Email: sivakumar@libero.it

Gloriosa superba seed is the pharmaceutical source of natural colchicine, which is approved by US FDA for gout treatment. The pharmaceutical demand for *natural* colchicine is growing. However, colchicine biosynthetic genes have not been identified. Previously we have identified one key candidate gene, *N*-acetyltransferase (NAT) in the colchicine pathway from our cDNA library. This gene is likely involved in the final step by catalyzing the conversion of deacetylcolchicine to colchicine. We successfully cloned the ORF of NAT into pF3K WG (BYDV) Flexi® vector and expressed in TnT® SP6 high-yield wheat germ protein expression system. Transformants were further confirmed with restriction digestion and Sanger sequencing. Analysis of the proteins in SDS-PAGE and western blot showed that the recombinant HisNAT protein was highly expressed in the soluble protein fraction. The functional confirmation of NAT could provide a necessary foundation for knowledge-based modification of the colchicine pathway using genetic engineering so that antigout drug substrate production can be maximized.

P-2042

Characterization and Overexpression of 5-*Enol*pyruvylshikimate 3-Phosphate Synthase in *Capsicum annuum*. W. RAJAPAKSE, S. Bagga, J. Ortega, and C. Gopalan. New Mexico State University, Dept. of Plant & Environmental Sciences, 945 College Dr., Skeen Hall, Room 127N, Las Cruces, NM 88003. Email: menik@nmsu.edu

Being a key enzyme in the shikimate pathway, 5-*Enol*pyruvylshikimate-3-phosphate synthase (EPSPS) plays a critical role in the biosynthesis of aromatic amino acids and numerous secondary metabolites in plants and microorganisms. EPSPS is the penultimate enzyme in the shikimate pathway and catalyzes the reversible production of 5-*Enol*pyruvylshikimate-3-phosphate and phosphate from shikimate 3-phosphate and phospho*enol*pyruvate. EPSPS has been well investigated in the field of plant biotechnology being the target of the world's most used herbicide, glyphosate. However, little is known about the regulation of the gene/s encoding EPSPS and the biochemical and molecular repercussions of overexpressing this gene in plants. Our research is focused on the characterization of the *EPSPS* gene and its regulation in *Capsicum annuum* (chile). Preliminary data has shown that there is a single *EPSPS* gene in chile and we have analyzed the tissue-specific expression of *the* gene at the RNA and protein level. We have isolated a full-length (chile) *EPSPS* cDNA from the leaves of chile, have engineered it behind the constitutive CaMV 35S promoter and introduced it into chile. Several independent transformants have been identified and the T1 progeny has been produced. The transformants are now being analyzed for the amino acid profile to check for any changes in the levels of the aromatic amino acids. Analysis will also be done to check for any changes in the secondary metabolites.

P-2043

Micropropagation of *Zanthoxylum armatum* DC – an Endangered Medicinal Plant of the Himalayan Region, and Assessment of Genetic Stability of *In Vitro* Raised Plants. S. PUROHIT[1], A. Jugran[1], S. Nandi[1], I. Bhatt[1], L. Palni[1], and A. Bhatt[2]. [1]G. B. Pant Institute of Himalayan Environment and Development, Kosi-Katarmal, Almora 263643, INDIA and [2]Department of Crop Improvement College of Forestry and Hill Agriculture, Ranichauri, Uttarakhand. Email: sumit.biotech2@gmail.com

Zanthoxylum armatum DC (family - Rutaceae; Common name - Winged prickly ash; Hindi name –Timur) is one of the important medicinal plant, has gained tremendous importance in recent times in Indian system of medicine as carminative & stomachic agent. The essential oil of the species is known for antibacterial, antifungal & antihelminthic activities. The increasing demand for this species by the pharma industries and in the traditional system of medicine has resulted in reckless harvesting & overexploitation. Hence, the situation demands sustainable utilization and conservation of this species. Thus present study aims to i). develop micropropagation protocol & ii). evaluate genetic fidelity of in *vitro r*aised plants using molecular markers. Nodal explants of *Z. armatum* were cultured on Murashige and Skoog's medium supplemented with various concentrations of plant growth regulators, namely 6 benzylaminopurine (BAP; 5–15 μM), indole 3 acetic acid (IAA; 0-1 μM) & gibberellic acid (GA_3; 0.5 μM). Amongst different combinations used, medium supplemented with 12 μM BAP, 0.5 μM IAA and 0.5μM GA_3 resulted in max rate of shoot multiplication (4.78) & shoot number (3.46 shoots/explants). Max rooting (100%) was achieved on ½

MS medium containing 50μM IBA for 24 hr, followed by subsequent culture on the same medium without PGRs. The micropropagated plants were hardened and transferred to field after a period of acclimatization; 75% survival was observed and *in vitro* raised plants appeared phenotypically similar to the mother plant. Genetic fidelity assessment of the regenerates through random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers did not reveal any variation between micropropagated & mother plants. This is first report of an efficient protocol, with high rate of multiplication, along with genetic stability of the micropropagated plants. This method can be utilized for obtaining large number of planting material for commercial cultivation.

P-2044

In Vitro Propagation of *Murraya koenigii L. Spreng* (Curry Leaf Plant) Through Adventitious Shoot Proliferation from Different Explants. R. JOSHI[1], N. Khatik[2], and P. Vyas[3]. [1]Government College, M.D.S University, Ajmer, C-1043, Near St. Stephen's School, Panchsheel Nagar, Ajmer 305004, INDIA; [2]Government College, M.D.S. University Ajmer, H.U Nagar, Ajmer; and [3]Government College, M.D.S University, Ajmer, 1-DA-21, Near Subhash Udhyan, Pratap-Nagar, Jodhpur 342003, INDIA. Email: lumossolem1@gmail.com; drrameshjoshi10@gmail.com

Murraya koenigii (L.) Spreng, commonly known locally as "curry patta" or "mitha neem" in India, is a valuable medicinal plant known for its biochemical and aromatic properties. Adventitious regeneration, which is a pre-requisite in most genetic transformation studies using Agrobacterium and ballistics, needs to be developed as a protocol for micropropagation of *M. koenigii*. This paper presents a procedure for the rapid, high frequency regeneration of *M. koenigii* plantlets from internode explants, hypocotyle, root segments, leaves, and cotyledons via adventitious shoot formation. The concentration of plant growth regulators (PGRs) in liquid MS medium exhibited a discrete role in the efficacy of adventitious shoot induction. N_6-benzyle adenine (BA), kinetin, adenine sulphate and indole-3-acetic acid (IAA) in combination were the most effective PGRs for adventitious shoot induction. Murashige and Skoog (MS) liquid medium with 9.29 μM kinetin, 13.317 μM BA, 2.854 μM IAA and 70 mg/l adenine sulphate yielded the maximum number (18) of shoot buds from internode explants. The number of shoots was further increased (27.30) after sub-culturing them into semi-solid (containing 8 g/l agar-agar) MS medium fortified with similar concentrations and combinations of PGRs. Most in vitro shoots (2.5-3.0 cm long), rooted (90%) on semi-solid MS medium containing 19.68 μM indole- 3-butyric acid within 28-30 days. The rooted plantlets were transplanted into pots containing a mixture of soilrite (mixture of peat moss + vermiculite + perlite in a 1: 1: 1 ratio that was mixed with natural soil in the ratio of 1: 1) at 70-80% relative humidity and 28°C -30°C for hardening. 85% of *in vitro*-raised plantlets survived under field conditions.

P-2045

Reducing Hyperhydricity in Shoot Cultures of *Cycladenia humilis* var. *jonesii,* an Endangered Dryland Species. V. PENCE[1], L. Finke[1], and R. Niedz[2]. [1]Cincinnati Zoo and Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220 and [2]USDA - ARS, 2001 S. Rock Rd., U. S. Horticultural Research Laboratory, Fort Pierce, FL 34945-3030. Email: valerie.pence@cincinnatizoo.org

Cycladenia humilis var. *jonesii* (Apocynaceae) is a federally threatened species known from only five locations in Utah and Arizona. The seeds have low viability and thus, in vitro methods were explored as a way of propagating this species for ex situ conservation and restoration. A few seeds germinated in vitro and shoot cultures were initiated from the seedlings. Shoots maintained on DKW medium with 0.5 mg/L BAP with gellan gum showed an extremely hyperhydric morphology, characterized by swollen, watery stems and minimal leaf development. In an attempt to normalize the morphology, the cultures were grown on the same medium with agar and venting. After one subculture, more normal growth was achieved, which increased with continued subculture under these conditions. Normal growth was characterized by greater leaf development, lower catalase activity, higher dry weight, and higher protein levels per gram dry weight than more hyperhydric shoots. In order to examine these and other factors more closely, a 6-factor 2-level fractional factorial design was used to determine the effects of KNO_3, $CaCl_2$, NH4:K, BAP, gelling agent (agar vs. gellan gum), and venting (+/-) on shoots grown in Magenta boxes during one subculture. Responses that were measured included tissue dry weight, stem growth and branching, stem thickness and opacity, leaf development, catalase activity, and protein levels. Results indicated that changes in these factors were significantly correlated with venting and gelling agent, as determined previously, as well as with nitrogen and cytokinin, depending on the response measured. This information will be important for improving in vitro propagation methods for the conservation of *C. humilis*, as normalized shoots showed approximately 30% rooting when transferred to medium with 1 mg/L IBA, compared with no rooting in hyperhydric shoots. These results indicate that 2-level fractionated factorial designs are useful for efficiently screening for factors that potentially affect hyperhydric growth; identified factors can then be studied in further detail.

P-2046

In Vitro Micrografting of Mastic Tree, *Pistacia lentiscus* L. V. SÜZERER[1], A. Onay[2], F. Kılınç[2], Y. Özden Çiftçi[3], A. Altınkut Uncuoğlu[4], H. Akdemir[2], E. Tilkat[5], N. Çalar[2], and H. Akdemir[6]. [1]Bingöl University, Vocational School of Health Services, Department of Medical Services and Techniques, Program of Medical Laboratory Techniques, Bingöl Üniversitesi, Selahaddin-i Eyyubi Mahallesi, Sağlık Hizmetleri Meslek Yüksekokulu, No: 1, Bingöl 12000, TURKEY; [2]Dicle University, Dicle University, Faculty of Science, Department of Biology, Diyarbakır 21280, TURKEY; [3]Gebze Institute of Technology, Istanbul Str., Kocaeli 41400, TURKEY; [4]Marmara University, Faculty of Engineering, Department of Bioengineering, 34722, Göztepe, Istanbul, TURKEY; [5]Batman-Turkey; and [6]Gebze Institute of Technology, Faculty of Science, Moleküler Biyoloji Ve Genetik Bölümü, Çayırova, Kocaeli 41400, TURKEY. Email: beyso1985@gmail.com

A successful micrografting technique for the lentisk was developed by using *in vitro* germinated seedlings as rootstocks and axenic shoot cultures established from mature tree sources as microscions. Shoot tips derived from axenic germinated mature seeds of lentisk micrografted onto *in vitro* juvenile rootstocks of *P. vera* L., *P. khinjuk* Stocks, *P. atlantica* Desf., and *P. terebinthus* L., resulted in the restoration of shoot-bud proliferation with a high success rate when the rootstock was decapitated to remove all leaves and a vertical slit was made on the stump; the scion base, cut in a v-shape, was fitted to the slit. Methodological approaches such as rootstock and scion production, micrografting method, effects of scion type on the success of the micrografts and effects of media on the micrograft development were examined. The 14-day-old seedlings of *P. vera L.* and *P. khinjuk* Stocks were used as rootstock because the mean shoot diameters for *P. vera L.* and *P. khinjuk* Stocks were reached to 2.38 mm and 1.44 mm, respectively in 2 weeks after culturing. The growth of rootstocks from mature seeds of *P. vera* L. and *P. khinjuk* Stocks were developed faster than *P. terebinhus* L. and *P. atlantica* Desf. Shoot culture initiation from two lentisk genotypes were successfully achieved by culturing mature shoot tips from forced nodal buds, about 8-10 mm, on a modified MS medium containing 1 mgl^{-1} BA and 0.5 mgl^{-1} GA_3. Slow growth and lack of axillary shoot development on the micrografts were noticeable when the micrografts were cultured on proliferation and rooting medium. *In vitro* micrografted plantlets were successfully acclimatized and no problems were encountered with the establishment of micrografted plants *in vivo*. The described micrografting technique could be used for rejuvenation of shoot explants of mature elite lentisk genotypes and it also has potential use for the development of a trans-grafting method.

P-2047

Assessment of Clonal Fidelity Between Selected Lentisk Genotypes (*Pistacia lentiscus* L.) by IRAP and Florescent-based AFLP. V. SÜZERER[1], O. Karakaş Metin[2], Ţ Koç[3], A. Onay[4], F. Kılınç[4], Y. Özden Çiftçi[3], A. Altınkut Uncuoğlu[5], Ö. Akdemir[4], N. Çalar[4], E. Tilkat[6], and H. Akdemir[3]. [1]Bingöl University, Vocational School of Health Services, Department of Medical Services and Techniques, Program of Medical Laboratory Techniques, Selahaddin-i Eyyubi Mahallesi, Sağlık Hizmetleri Meslek Yüksekokulu, No: 1, Bingöl 12000, TURKEY; [2]TÜBİTAK, Marmara Research Center, Genetic Engineering and Biotechnology Institute, Gebze 41470, TURKEY; [3]Gebze Institute of Technology Faculty of Science, Department of Molecular Biology and Genetics, Çayırova, Kocaeli 41400, TURKEY; [4]Dicle University, Faculty of Science, Department of Biology, Diyarbakır 21280, TURKEY; [5]Marmara University, Faculty of Engineering, Department of Bioengineering, 34722, Göztepe, Istanbul, TURKEY; and [6]Batman University, Faculty of Science, Department of Biology, 72060, Batman, Turkey. Email: beyso1985@gmail.com

Pistacia lentiscus L. is commonly known as the Mastic tree, the Evergreen Pistachio, the lentisk or the schinos. The lentisk is a bush that plays a particularly important role in the ecosystem of the Mediterranean region maquis from Morocco, Italy, Greece and Iberia to Mediterranean and western Turkey. Determination of somaclonal variants at early stage of regeneration is prominent for a micropropagation system. The aim of the study was to investigate the availability of Inter Retrotransposon Amplified Polymorphism (IRAP) and florescent-based Amplified Fragment Length Polymorphism (AFLP) markers to detect the level of somaclonal variation in regenerated plants of lentisk (*Pistacia lentiscus* L.) obtained by tissue cultures. In this context, the 5 IRAP primers (LTR-2, LTR-3, LTR-5, LTR-6 and LTR-10) were used for determination of somaclonal variation. The mean percentage of genetic similarity was calculated as 80% and the low average polymorphic information content (PIC) value of 0.331 indicated the presence of high genetic similarity among the clones (6, 9 and 12 times subcultured). The Florescent-based AFLP technique with seven primer combinations (*Eco*RI AGG*D4- *Mse*I CAA*D4, *Eco*RI AGG*D4- *Mse*I CTT, EcoRI AGG*D4- *Mse*I CAT, *Eco*RI AGG*D4- *Mse*I CAC, *Eco*RI

AGG*D4- *Mse*I CCA) was also utilized to study genetic variation in true-to-type and cultured plants to deduce the nature and boundaries of genetic variation. The first principal component explained 35% of the AFLP variation among the 8 groups (3, 6, 24 times subcultured female and male clones together with their parents), while the second principal component explained 21%. High levels of reproducibility coupled with the high multiplex ratio over the other molecular markers techniques are the reasons for selecting AFLP markers for evaluating genetic fidelity of tissue culture-raised plants in this study. This study shows that IRAP and AFLP technique can be applied to the quality-control system of tissue culture seedlings for *P. lentiscus* L.

P-2048

Engineering Sweetpotato [*Ipomoea Batatas (L.) Lam*] Expressing Synthetic Lytic Peptide for the Potential Inhibition of Human Immunodeficiency Virus Replication. S. SAMUELS, M. Egnin, T. Nashar, J. Jaynes, C. Prakash, and I. Ritte. Tuskegee University, 1200 W. Montgomery Rd., Tuskegee AL 36088. Email: ssamuels1822@yahoo.com

The development of plants to produce therapeutic compounds can be used to supply low-cost drugs and vaccines for major diseases such as HIV to the developing world. Treatments of infectious diseases in humans and animals have traditionally been targeted by chemically synthesized drugs, with the majority of the burden of cost falling on the individual in need of treatment. With the new revolution of producing therapeutic compounds, such as peptides in plant based systems, the cost of production is dramatically decreased. The action of most antimicrobial peptides induces membrane defects such as phase separation or membrane thinning, pore formation, and bilayer disruption. Antimicrobial peptides have also been found to target intracellular molecules, such as DNA/RNA or enzymes. Synthetic lytic peptides *jc41n* and *jc41nd,* capable of inhibiting the progression of HIV have been developed at Tuskegee University and expressed in sweetpotato. Seven transgenic plantlets were PCR positive using primers specific for the JC genes, and primers targeting the 35S promoter and NOS terminator. The presence of the JC protein from plant extracts are currently being detected by Western blot using antibodies derived from injection of peptide protein in mice. To test efficacy and toxicity, crude and purified sweetpotato extracts showed minimal peptide cytotoxicity in dosing trials using Jurket cells in 4, 8, 12, and 18 hr treatments; prior to downstream dosing tests in mice. Further analysis using Southern blot on genomic DNA from PCR positive transformants, parental non transformed control, and JC plasmids will confirm stable integration of the transgene and gene insertion number by qPCR. Following verification of efficacy and dosing regiments for extracted proteins, confined field trials will test agronomic evaluation and performance to demonstrate plant merit. Successful development and approval of sweetpotato expressing this novel therapeutic compound can be both a powerful tool in treatment of the HIV epidemic, as well as a road map for future treatment of viral mediated diseases.

P-2049

Elucidation the Biosynthesis of Stilbenoids in Peanut. T. YANG[1], L. Fang[2], C. Nopo-Olazabal[2], L. Nopo-Olazabal[2], and F. Medina-Bolivar[1]. [1]Arkansas Biosciences Institute and Department of Biological Sciences, Arkansas State University, Jonesboro, AR 72401 and [2]Arkansas Biosciences Institute, Jonesboro, AR 72401. Email: linglingfang.0131@gmail.com; tianhong.yang@smail.astate.edu

Peanut (*Arachis hypogaea*) produces a large number of stilbenoids upon abiotic and biotic stress. Interestingly, the majority of these polyphenols are prenylated. Among them, arachidin-1 and arachidin-3 are potentially beneficial to human health due to their anti-inflammatory, antioxidant and anticancer properties. Interestingly these compounds exhibit higher metabolic stability *in vitro* when compared to their non-prenylated analogs. Despite of the importance of these prenylated stilbenoids, the genes involved in the prenylation reactions of stilbenoids have not been identified. To address this issue, we established hairy root cultures of peanut and induced them to produce stilbenoids upon treatment with the elicitors, methyl jasmonate (MeJA) and cyclodextrin. The combined elicitor treatment produced much higher levels than treatment with MeJA alone. Furthermore, when the combined treatment was compared to cyclodextrin alone, a higher diversity of induced compounds was observed. Furthermore, high performance countercurrent chromatography was used to purify the prenylated stilbenoids, arachidin-1 and arachidin-3 from the medium of elicited hairy root cultures. Ongoing studies focus on integrated metabolomics and transcriptomics approaches to elucidate and characterize the mechanisms that affect the biosynthesis and accumulation of the prenylated stilbenoids in peanut with the ultimate goal to improve the health-related properties of this important crop.

P-2050

Metabolic Engineering and Biotransformation of Flavonoids in Hairy Root Cultures of *Scutellaria lateriflora*. T. YANG[1], N. Joshee[2], and F. Medina-Bolivar[1]. [1]Arkansas Biosciences Institute and Department of Biological Sciences, Arkansas State University Jonesboro, AR 72401 and [2]Fort Valley State University, 1005 State University Drive, Fort Valley State University, Fort Valley, GA 31030. Email: tianhong.yang@smail.astate.edu

American skullcap (*Scutellaria lateriflora*), a perennial herb native to North America, is rich in bioactive metabolites. In particular, the flavonoids wogonin, baicalein and baicalin have proven anticancer properties *in vitro.* To study the mechanisms that affect the biosynthesis of these bioactive compounds, hairy root cultures of *S. lateriflora*were developed via *A. rhizogenes*-mediated transformation. HPLC and tandem mass spectrometry analyses showed that the phenylpropanoid verbascoside and the flavonoids baicalein, wogonin and their respective glucuronides, baicalin and wogonoside, accumulated in the root tissue. In order to increase the levels of these flavonoids, transgenic hairy root lines of *S. lateriflora* harboring the flavonoid-specific transcription factor AtMYB12 under the control of the superP:TEV expression system were produced. Semi-quantitative RT-PCR analysis showed an increase in the expression of the genes encoding for the enzymes phenylalanine ammonia lyase and chalcone isomerase in the transgenic hairy root line when compared to a wild-type line. Subsequently, the accumulation of wogonin was also increased. Furthermore, after feeding the cultures with the flavonoids naringenin and chrysin, the hairy root cultures were capable of transforming these compounds into glucuronides. In summary, the AtMYB12 transcription factor may affect the biosynthesis of selected flavonoids by upregulating the expression of genes involved in their biosynthesis. Furthermore, glucuronidation of flavonoids and potentially other phenolics by hairy roots of *S. lateriflora* provides a valuable tool for producing novel potentially bioactive compounds in these tissue culture systems.

P-2051

Metatopolin and Phloroglucinol Improve Shoot Production and Plant Quality in Micropropagated *Stevia rebaudiana* by the Activation of Lignin Metabolism. A. PIQUERAS. CEBAS (CSIC), Depertment of Plant Breeding, CEBAS, (CSIC), Murcia 30100, SPAIN. Email: piqueras@cebas.csic.es

Stevia rebaudiana is the natural source of steviosides and is considered as the most promising biosweetener for pharmacological and medical purposes in the European Union. Currently there is a growing interest in the cultivation of stevia in southern Spain and the demand of plants for potential growers cannot be covered by conventional methods of production in nurseries. At the same time, the use of sexual reproduction to generate plant material in stevia has been shown to be severely limited by low viablilty of seeds. The object of this work was to improve the current micropropagation protocols by using metatopolin instead of benzyladeneine in stage II of the micropropagation process using nodal segments as initial explants. By doing so, a higher multiplication rate (8-10 shoots for initial explants) was achieved when metatopolin was used at the same concentrations of BA (1 mg/L), shoot length and leaf development was significantly improved. The addition of Phloroglucinol (50- 150 mg/l) to the culture medium increased the lignification of the produced shoots with an activation of both guayacol and ascorbate peroxidase activities and polyphenol oxidase. The shoots produced in the medium with phloroglucinol showed a reduced period of rooting and the period required for their acclimatization to the ex vitro conditions was of three weeks compared to five for the plants cultured in medium without phloroglucinol (control). The formation of new leaves ex vitro as well as their photosynthetic activity was greatly improved by phloroglucinol and the survival rate of these plants was 95% compared to 75% for controls. The evaluation of Metatopolin and phloroglucinol for the micropropagation of stevia in liquid medium is in progress.