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Plant Posters

P-2000

Low-cost Methods for Production of Commercial-size Fruit from Sugar-loaf Pineapple (*Ananas comosus*) Plantlets in Jamaica. S. A. MITCHELL, and M. H. Ahmad. The Biotechnology Centre, 2 St. John's Close, University of the West Indies, Mona Campus, Kingston 7, Jamaica, WEST INDIES. Email: sylvia.mitchell@uwimona.edu.jm, sylviamitchell.biotech@gmail.com

Production of fruit from micropropagated pineapple plantlets although commonplace in several tropical countries, has not yet been achieved in Jamaica due mainly to the high cost of the process. Yet conventional production is inefficient due to high production costs and lack of clean planting material. This paper describes experiments aimed at improving the efficiency of the micropropagation process, both in culture and during the hardening process, in order to provide elite planting material at a reasonable and viable price to interested farmers. To optimise in vitro growth, various growth regulators (BAP, TDZ, Kinetin, IBA, and NAA) and minerals (copper and Iron additives) were tested. From these trials, suitable multiplication (BM, 2.0 mg/l BAP and 0.1 mg/l NAA) and rooting media (BM and 1.0 mg/l NAA or IBA) were identified. Increasing copper to 1.5 μM and iron to 250 μM further increased the multiplication rate. Various low-cost media substitutes (rain water, fertilizer, coconut water, pineapple juice, corn starch, yam starch) and a laminar flow substitute (called a mobile micropropagation unit [MMU]) were also tested. The MMU produced promising results while the multiplication rate further increased when distilled water and MS(1962) were substituted with rain water and 1/4 strength fertilizer (20:20:20 with micronutrients). Forty pineapple plantlets survived to fruiting in a farmer's field in Glengoffe, St. Catherine, Jamaica under rain-fed conditions. Of these plants, 31% fruited after 12 mo and were ready for harvest after 16 mo producing on average per plant, a 1.4 g fruit and 4.0 suckers for replanting.

P-2001

Macronutrient Optimization for In Vitro Growth of Turmeric (*Curcurma longa* L.). SEAN MICHAEL HALLORAN, and Jeffrey Adelberg. Department of Horticulture, Clemson University, Clemson, SC 29634. Email: shallor@clemson.edu

Most media for plant tissue culture is based on Murashige and Skoog 1962. This work maximized the fresh weight of tobacco callus on agar (one nutrient at a time) with few second order interactive effects. Our current work combined powerful statistical software designs to analyze the complex interactions of macronutrients in liquid culture micropropagation of turmeric (*Curcurma longa* L.), an important herbaceous perennial. The reasons for re-evaluating MS included: differences of water availability in agar and liquid media; media have not been defined for commercially micropropagated herbaceous perennials; differentiated tissues have greater nutrient requirements than callus; the limited potential of prior workers to conduct and analyze proper experiments to measure these interactions; modern designs save labor, space, materials, and increase visualization of multi-factor interactions. This experiment is a d-optimal design with 3 levels of sucrose (1.5 - 6%), plants per vessel (3 - 9 plantlets), volume (25 - 45 mL), nitrate (10 - 50 mM), and potassium and nitrogen expressed as a 2-component mixture. The full factorial of all these variables is an impractical experiment, while our d-optimal design space required only 55 vessels. Greatest plant multiplication in vitro was with 10 mM nitrate and 1:1 ammonium/potassium (25% of MS), 3 plants per vessel, and 6% sucrose. At higher nitrogen levels, like MS 1962, the sucrose and plant density are not apparent. This maximization shows the significance of analyzing the second order interactions to optimize factors involved in plant growth. The quality of plantlets to grow in the greenhouse likely has a different optimization. An evaluation of dissimilar combined responses will be made.

P-2002

Secondary Metabolism Inducing Treatments During In Vitro Development of Turmeric (*Curcuma longa* L.) Rhizomes. MATTHEW M. COUSINS[1], Jeffrey Adelberg[1], Feng Chen[2], and James Rieck[3]. [1]Department of Horticulture, Clemson University, Clemson, SC 29634; [2]Department of Food Science and Human Nutrition, Clemson University,

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Enhancement of phytochemical production in various in vitro systems has been studied significantly in the past. The objective of this series of studies was to develop a method for upregulation of secondary metabolite production in turmeric whole plant culture. Turmeric (*Curcuma longa* L.) plants were grown in 2.5 L vessels in MS liquid medium that was twice restored to 6% sucrose. Various methods were employed in attempts to upregulate secondary metabolism; antioxidant and total phenolic assays were utilized to characterize phytochemical activity. A first experiment (17 weeks) exposed four clones to phenylalanine and/or methyl jasmonate (MeJa) from week 12 to 17. In a second experiment (22 weeks) on one clone, 1.5 week exposure to proline, a natural proline-rich extract, MeJa, and chitosan began during week 20. A nitrogen stress treatment (weeks 16–22) was also included. The 5 week phenylalanine and MeJa treatments caused a genotype dependent decrease in biomass accumulation and antioxidant capacity. In the second experiment, only the low nitrogen treatment yielded an increase in phenolic content to 4.7% of dry weight (DW) compared to the control (4.1% of DW). Nitrogen stress decreased leaf growth while rhizome growth (63 g FW per vessel) was unaffected. None of the treatments had a significant effect on biomass or antioxidant capacity, though the low nitrogen treatment might have enhanced radical scavenging ($p=0.1207$). Results indicated that plants grown in a high nitrogen MS media (39.5 mM NO_3^-, 20.5 mM NH_4^+) were not responsive to elicitation. A subsequent study identified a reduced nitrogen medium (10 mM NO_3^-, 0 mM NH_4^+) that yielded DW similar to that of plants grown in nitrogen rich media. This should allow for simultaneous upregulation of metabolite and biomass.

P-2003

The Use of Silver Compounds to Direct Plant Tissue Culture Development. B. STEINITZ[1], Y. Tabib[1], N. Bar[1], and N. Bernstein[2]. [1]Department of Vegetable Research, Institute of Plant Sciences, ARO, The Volcani Center, Bet Dagan 50250, ISRAEL; [2]Department of Environmental Physics and Irrigation, Institute of Soil, Water and Environmental Sciences, ARO, The Volcani Center, Bet Dagan 50250, ISRAEL. Email: steinitz@volcani.agri.gov.il

Plant tissues cultures incubated in vitro emanate ethylene, and accumulation of the phytohormone in the culture vessel headspace may be unfavorable for culture growth. Undesirable impacts of ethylene can be avoided or reduced by either removing the gas from the headspace or by adding ethylene biosynthesis inhibitors or inhibitors of ethylene action to the culture medium. Silver ions (Ag+), known to inhibit ethylene action, may control cell and tissue development when culture media are supplemented with silver nitrate ($AgNO_3$) or silver thiosulfate (STS). However, we noticed that agar-solidified medium supplemented with $AgNO_3$ changes color and becomes darker than control medium. Silver is light sensitive, and $AgNO_3$ and other silver compounds change color or blacken upon exposure to light, and therefore media color changes presumably are due to photochemical alterations in silver addenda. Further, given that dissolved Ag+ from added $AgNO_3$ reacts with soluble salts such as $CaCl_2$, $CoCl_3$, thiamine hydrochloride and pyrimidine hydrochloride found in basal plant tissue culture salt mixtures (e.g. Murashige and Skoog 1962; Gamborg et al. 1968), the observed changes in medium color are possibly due to precipitates of insoluble $AgCl_2$. However, we have found no reference to color change or precipitate formation in media containing silver agents in reports on Ag+dependent modification in culture development. We shall demonstrate that chlorides from the basal salt mixtures are not the sole reason for color changes and that STS should be the preferred silver compound used to control in vitro tissue developmental responses.

P-2004

Obtaining Sethoxydim Resistance in Seashore Paspalum (*Paspalum vaginatum*) Via In Vitro Selection. D. L. HECKART[1], W. Parrott[1], and P. Raymer[2]. [1]University of Georgia, 111 Riverbend Rd Athens, GA 30602–6810 and [2]University of Georgia, Griffin Campus, Griffin, GA, 30223. Email: heckartd@uga.edu

Selective herbicide resistance has been a sought-after trait for turfgrasses, but recent attempts to commercialize herbicide resistant turfgrass obtained through GM technologies have been unsuccessful. Sethoxydim is a grass-specific herbicide, and resistance results from one of several single base-pair mutations. The most common mutation is an ILE to LEU substitution caused by an A to T mutation at position 1781 of ACCase. Research was initiated in efforts to develop a novel source of resistance to sethoxydim in seashore paspalum (*Paspalum vaginatum)*, a warm-season turfgrass. Our objectives were to develop in vitro selection and regeneration protocols and to use these protocols to efficiently and effectively select for naturally occurring mutations conferring herbicide resistance. An experiment to determine the growth response of callus tissue to sethoxydim dosage was performed to determine the optimum sethoxydim concentration for selection. Callus was induced from immature inflorescences excised prior to exertion. The callus was

then plated on callus induction medium containing 10 μM sethoxydim for selection. Resistant cell lines were placed on regeneration medium and green plants were recovered. The 1781 aa ILE to LEU mutation was confirmed by DNA sequencing in the first resistant cell line examined.

P-2005

Rice Promoters That Confer Organ-specific Transgene Expression. ROGER THILMONY, Mara E. Guttman, Meridith Cook, and James G. Thomson. USDA-ARS Western Regional Research Center, 800 Buchanan St., Albany CA 94710. Email: roger.thilmony@ars.usda.gov

We are investigating novel promoters from rice that confer organ-specific gene expression. Our goal is to identify a set of promoters that will allow precise control of transgene expression and become useful plant biotechnology tools for crop improvement. We have utilized microarray gene expression profiling, RNA blot hybridization, and analyses of publicly available gene expression resources, to identify candidate genes that exhibit distinct organ-specific patterns of expression. The corresponding upstream promoter sequences of validated candidates have been fused to a *gusA-enhancedGFP* bifunctional reporter gene in a binary vector (pGPro2) specifically designed for promoter characterization. These constructs have been transformed into rice, *Brachypodium distachyon* and/or *Arabidopsis thaliana*. Reporter gene expression is being documented throughout plant development in the transformed lines. The rice *LP2* promoter, derived from a leucine-rich repeat receptor-like kinase, controls light-responsive organ-specific expression in the aerial, vegetative parts of transgenic rice and *Brachypodium* plants, with the highest expression in the leaf blade. These *LP2::GUSeGFP* transgenic plants display little or no detectable expression in the mature seed, reproductive or root tissues. The rice *Root3* promoter, derived from a putative plastocyanin-like gene, exhibits expression only in the roots of the transgenic plants examined. Characterization of these two promoters and several others including candidates derived from anther/pollen-specific genes will be presented. We anticipate that our promoters will enable spatially-defined control of transgene expression in crop plants, reducing the unintended effects transgene products may have on plant physiology and the environment.

P-2006

Identification of Parameters Influencing *Agrobacterium*-mediated Transformation of Peanut. N. A. S. DIBY, K. Konan, and H. Dodo. Food Biotechnology Laboratory, Department of Food and Animal Sciences, Alabama A&M University, Normal, AL 35762. Email: nnandiby@gmail.com

Peanut (*Arachis hypogea* L) is an annual oil seed belonging to the Leguminosae family. It is a native from South America, and today, it is largely cultivated in many tropical and subtropical areas worldwide because of its high nutritive and economic values. Several peanut cultivars have been developed using plant breeding techniques. However, new technologies in genomics are poised to improve peanut for better productivity as well as to eradicate safety concerns due to peanut allergy. Efficient recovery of fertile transgenic peanut is one of the prerequisites to achieve peanut improvement via genetic manipulation. The objective of this research is to identify parameters susceptible to improve the transformation efficiency of peanut. Hypocotyl explants from 5–6-day-old seedlings of Georgia Green and Jumbo Runner were infected with *Agrobacterium tumefaciens* harbouring, an intron-containing β-glucuronidase uidA (gusA) gene, under the transcriptional control of CaMV35S promoter. After explant infection, followed by a 5-day co-cultivation period, kanamycin-resistant plants were regenerated on the selection medium. GUS assays, PCR and quantitative Real time PCR (qRTPCR) were used to assess the transgenic status of the plants. Higher number of transgenic plants was obtained when the application of the selection pressure was delayed to 2 weeks (14 plants) and 3 weeks (18 plants) compared to transferring explants immediately after the co-cultivation period (10 plants). Treatments of explants with different concentrations of antioxidants (L-cysteine, lipoic acid and silver nitrate) during co-cultivation resulted in significant increase of transformation frequency. Osmotic treatments and dark regimes also had positive effect on T-DNA transfer.

P-2007

Comparison Between Mannose and Kanamycin Selection Systems for Genetic Transformation of Citrus. M. DUTT, and J. W. Grosser. Citrus Research and Education Center, University of Florida/IFAS, 700 Experiment Station Road, Lake Alfred, FL 33850. Email: manjul@ufl.edu

Citrus cells are sensitive to the antibiotic kanamycin and the sugar monomer, mannose. Both of these have been successfully used in selection regimes following genetic transformation wherein transgenic cells expressing the neomycin phosphotransferase gene (NPTII, conferring antibiotic resistance to aminoglycoside antibiotics like kanamycin) or the phosphomannose isomerase gene (PMI, detoxifying mannose-6-phosphate into useable fructose-6-phosphate) are rendered resistant to the presence of kanamycin or mannose respectively in the tissue culture

medium. This provides a selective advantage allowing discrimination of the few transgenic cells from a much larger population of untransformed and therefore sensitive cells. Transformation of citrus cultivars "Duncan" grapefruit and "Carrizo" citrange were carried out using *Agrobacterium tumefaciens* EHA105. A pCAMBIA0390 based binary vector containing the PMI gene fused in frame to the enhanced green fluorescent protein (EGFP) gene or the EGFP gene fused in frame to the NPTII gene was used. Each fusion gene was under the control of a CaMV 35S promoter. Genetic transformation efficiencies were comparable on both systems while transgenic plants were quicker to regenerate in the mannose based selection system. Number of escapes was higher in the mannose based system, and plants rooted quickly, while those from the kanamycin based system did not. Several other transformation parameters were investigated and molecular analysis was performed to confirm the presence of the gene(s) in the citrus genome. Our results indicate that the mannose selection system can provide a viable alternative to antibiotic based selection systems.

P-2008

Site-specific Excision of Targeted DNA is Facilitated by Bxb1 in the *Arabidopsis* Genome. J. THOMSON[1,3], Y. Y. Yau[2,3], R. Blanvillain[2, 3], and D. Ow[1,2,3]. [1]Crop Improvement and Utilization Unit, Western Regional Research Center, USDA, Albany CA; [2]Department of Plant and Microbial Biology, University of California, Berkeley, CA; and [3]Plant Gene Expression Center, USDA-ARS, Albany, CA. Email: James.thomson@ars.usda.gov

We have designed a site-specific excision detection system in *Arabidopsis* to study the in planta activity of the large serine recombinase Bxb1. Using stable transgenic plant lines, we show that the Bxb1 recombinase is catalytically active and capable of performing site-specific excision of a chromosomally integrated target from the *Arabidopsis* genome. We further show that genomic excision by Bxb1 recombinase was detected in subsequent generations in the absence of the recombinase gene. The Bxb1 recombination system may be another useful tool for genome manipulation in transgenic plants such as for removing marker genes or other unneeded transgenic sequences.

P-2009

Induction of *nptII* Exogene Removal from Transgenic Grape (*Vitis vinifera* 'Brachetto'). L. MARTINELLI, L. Dalla Costa, I. Vaccari, V. Poletti, and M. Mandolini. E. Mach Foundation - IASMA Research Center, Genetics and Molecular Biology Department, Via E. Mach, 1, 38010 San Michele all'Adige, ITALY. Email: Lucia.Martinelli@iasma.it

Marker gene removal is a relevant issue of gene transfer protocol, in particular when antibiotic resistance traits are involved. The objective of this work is to develop a suitable protocol to be applied in grape for removing the *nptII* exogene. We regenerated transgenics *V. vinifera* 'Brachetto' plantlets using the chemical-inducible site-specific *cre/loxP* pX6 vector (Zuo et al., 2001, Nature Biotechnol. 19, 157–161) where *nptII* removal is induced by the 17-β-estradiol. In this construct, *gfp* gene was replaced with a coat protein sequence of the Grapevine Virus A coding for a hairpin RNA. The β-estradiol supply was performed on buds during micropropagation and efficiencies of *nptII* removal were evaluated with Real-time PCR. For building the standard curve, two types of duplo-target plasmids carrying alternatively the sequences of a grape endogene (9-*cis*-epoxycarotenoid dioxygenase or chalcone isomerase) and the *nptII* transgene were tested as calibrators to simulate an ideal genetically modified plant carrying a homozygous single-copy exogene insertion. According to our results, hormone diffusion is the critical point of this protocol since we found a degree of exogene removal along the plant tissues, with the best results in the roots (0.01 mean copy numbers). For enhancing the β-estradiol diffusion into the plant tissues, we are testing the effectiveness of the hormone supply during shoot regeneration from transgenic leafs. This research is supported by the Autonomous Province of Trento, Project *EcoGenEtic.Com*. Thanks to Ivana Gribaudo and Pasquale Saldarelli (CNR, Italy) respectively for embryogenic calli and the pX6-pKcpGVA construct, and Nam-Hai Chua (Rockefeller University, New York) for the *cre/loxP* pX6 construct.

P-2010

Hypoallergenicity of Transgenic Peanut (*Arachis hypogea* L.) is Transmitted to the Progeny. ANTHONY ANANGA, Koffi Konan, and Hortense Dodo. Food Biotechnology Laboratory, Alabama A&M University, Department of Food and Animal Sciences, 4900 Meridian St. Carver Complex S. P. O. Box 1628, Normal, AL 35762. Email: Anthony.ananga@gmail.com

Peanut allergy affects a large population mostly in the Western world. Reactions to peanut allergy are often severe, and hypersensitivity persists throughout life for many. Ara h 1, Ara h 2, and Ara h 3 are the major allergenic proteins in peanut, judging from the fact that at least 50% of individuals allergic to peanut react to these proteins. Therefore, it can be expected that removal of these three proteins can significantly reduce the allergic potential of peanut seeds. Here we report that concomitant down regulation of Ara h 1, Ara h 2, and Ara h 3 using RNA interference is passed on to the progeny. The effects of

RNA interference were characterized at both the mRNA and the protein levels. Real time qRT-PCR revealed very low expression levels of the genes encoding the three proteins as compared to the wild type peanut. SDS-PAGE and 2D-PAGE showed absence of the three proteins in transgenic peanut samples. Immunological analysises (Western blots and ELISA) of proteins with allergen specific antibodies did not detect the major allergens in some transgenic seeds. Transgenic seeds of the second generation (T_2 seeds) showed significant reduction in allergenicity as reflected by a low IgE binding capacity of total protein extract as compared with that of wildtype control protein. The transgenic peanut plants in which Ara h 1, Ara h 2, and Ara h 3 gene expressions were inhibited showed normal phenotypic development

P-2011

Reducing Lignin Content in Bahiagrass (*Paspalum notatum* Flugge) by RNAi Suppression of 4-Coumarate-CoA Ligase. W. M. FOUAD, L. Martin, W. Vermerris, and F. Altpeter. University of Florida – IFAS, Agronomy Department, Plant Molecular and Cellular Biology, 3062 McCarty Hall, Gainesville, FL 32611. Email: altpeter@ufl.edu

Bahiagrass is the primary warm season forage grass in the south-eastern USA. In Florida alone it is grown on more than 5 million acres to support the beef cattle industry. However, the high lignin content of the bahiagrass biomass significantly reduces its forage quality. The objective of this study was to reduce the total lignin content of bahiagrass to improve its forage quality. A key enzyme in the lignin biosynthetic pathway is the 4-coumarate-CoA ligase (4CL). Reduction of 4CL and other lignin biosynthetic enzymes in various plant species resulted in reduced lignin content that was accompanied with increased digestibility of forage biomass. We cloned four 4CL cDNA's from tetraploid bahiagrass cv. 'Argentine'. An RNAi construct targeting a highly conserved domain in two out of the four genes was constructed using 200 bp of the coding sequences. The 4CL-RNAi construct was introduced independently to bahiagrass callus by biolistic gene transfer under transcriptional control of three alternative promoters: the constitutive e35S promoter, OsC4H promoter for xylem specific expression and the ZmdJ1 promoter for expression in the green tissue. Following regeneration of plants their transgenic nature was confirmed using PCR and Southern blot analysis. Significant reduction of 4CL gene expression was confirmed in several transgenic lines by Northern blot analysis. Klason lignin analysis conducted on transgenic lines growing in the green house in 2 m×1 m×0.6 m soil bins in a randomized block design of three replications showed significant reduction in total lignin compared to wild type plants. Data correlating the 4CL gene expression level with the lignin content and in-vitro digestibility analysis will be presented. The two genes targeted for silencing play an important role for lignin biosynthesis in bahiagrass. RNAi suppression of 4CL was more effective under transcriptional control of the xylem specific OsC4H promoter than under control of the e35S or ZmdJ1 promoters.

P-2012

Expression Analysis and Functional Characterization of Rice Genes Encoding *microRNA156*s and Their Application in Plant Genetic Improvement. DAYONG LI [1,2], Xue Liu [2], Qian Hu [1], Lihuang Zhu [2], and Hong Luo[1]. [1]Department of Genetics and Biochemistry, Clemson University, 110 Biosystems Research Complex, Clemson, SC 29634 and [2]State Key Laboratory of Plant Genomics and National Center for Plant Gene Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, PR CHINA. Email: lhzhu@genetics.ac.cn, hluo@clemson.edu

MicroRNAs (miRNAs) are small, endogenous RNAs that regulate gene expression in plants and animals. They repress gene expression at the transcriptional or posttranscriptional levels and have critical functions in plant defense, growth, development, disease and stress responses. Most of plant miRNAs arise from single-stranded primary miRNA transcripts (pri-miRNAs) encoded by miRNA genes. The miR156 family is one of the first characterized and conserved miRNA families in plants and it has been demonstrated to target *SQUAMOSA promoter-binding-like (SPL)* genes which encode plant-specific transcription factors. It was reported that there are 12 members of the miR156 family in rice (*Oryza sativa* L.). Little is known about the expression and function of the genes encoding the pri-miR156s. Transcript level analysis of the three *Osa-miR156* genes in various rice tissues and organs revealed different tempo-spatial expression patterns. We also found that the expression patterns of the three genes are different in differentiated and undifferentiated rice embryogenic calli, exhibiting intriguing expression patterns during the transition from undifferentiated to differentiated calli. Overexpression of the full-length cDNAs of *Os-miR156* genes in rice showed dramatic morphological changes, including significantly increased number of tillers, dwarfism, narrowed leaves, strongly reduced panicle size, and delayed flowering. The results of small RNA Northern blotting showed that the matured transcript of *Osa-miR156* (about 21nt) in the leaves of the transgenic plants was higher than that in the non-transgenic wild type. Over-expressing the rice *Osa-miR156* genes was also used in genetic modification of tobacco (*Nicotania tabacum* L.) and creeping bentgrass (*Agrostis stolonifera* L.). The results obtained point out the

great potential of microRNA genes for use in genetic improvement of plant architecture and biomass production.

P-2013

Biotechnology for Castor Oil Production. GRACE CHEN. Western Regional Research Center, USDA-ARS, 800 Buchanan St., Albany, CA 94710. Email: grace.chen@ars.usda.gov

Castor oil contains 90% ricinoleate that has numerous industrial uses. However, the production of castor oil is hampered by the presence of the toxin ricin and hyper-allergenic 2S albumins in its seed. *Lesquerella. fendleri* is a new oilseed crop whose seed contains lesquerolic acid derived from a 2 carbon elongation of ricinoleate. By suppressing the elongation step in *L. fendelri* through genetic engineering, it is possible to generate a *L. fendleri* crop producing ricinoleate. We conducted a series of seed development studies in castor and *L. fendelri*, including seed morphogenesis, oil and storage protein accumulation and lipid gene expression. In castor, synthesis of ricin, 2S albumin and ricinoleate/oil occurred during cellular endosperm development. We observed increased transcript levels of 12 lipid genes involved in synthesis of ricinoleate/oil, but with various temporal patterns and different maximal induction ranging from 4- to 43,000-fold. These results indicate that gene transcription exerts a primary control in ricinloeate/oil biosyntheses, and there are different transcriptional regulatory mechanisms involved. We have investigated the changes in seed size and color, seed weight and germination, lipid gene expression, and protein and lipid profiles during seed development of *L. fendelri*. Our results of seed studies provide integrative information for understanding their relationships during the seed development. In addition, we have demonstrated stable transformation in *L. fendleri*, providing tools for genetic engineering of *L. fendleri.* With the available sequences information from castor genome, we have identified castor lipid genes and their family members. We are conducting genome-wide analysis on gene expression profiles and promoter organizations. By characterizing co-expressed genes and conserved promoter motifs, we will be able to identify key genes, promoters and transcription factors as suitable targets for future metabolic engineering of ricinoleate production in transgenic oilseeds, as well as genetic suppression of ricin and 2S albumin in castor.

P-2014

Heterologous Expression of Arabidopsis H+-PPase Enhances Salt and Drought Tolerance in Transgenic Creeping Bentgrass (*Agrostis stolonifera* L.). ZHIGANG LI, Christian M. Baldwin, Qian Hu, Haibo Liu, and Hong Luo. Department of Genetics and Biochemistry, Clemson University, 110 Biosystems Research Complex, Clemson, SC 29634. Email: hluo@clemson.edu

The vacuolar H+-pyrophosphatase (AVP1), when overexpressed in transgenic Arabidopsis, can enhance plant resistance to salt and drought stress, and regulate root and shoot development via facilitation of the auxin fluxes. Here, we report that transgenic creeping bentgrass plants overexpressing heterologous AVP1 gene were much more resistant to salt and drought stress than the wild-type plants. The transgenic plants grew well in the presence of 100 mM NaCl and exhibited higher tolerance and faster recovery from damages of 200 mM and 300 mM NaCl exposure than the wild-type plants. Compared to the wild type plants, the tolerant performance of the transgenic plants were associated with the higher relative water content, Na+ concentration and lower solute leakage in the leaf tissues, and with the higher Na+, K+, Cl- concentration and total phosphorus in the root tissues. Under salt and drought stress, the proline content was increased in both wild-type and transgenic plants, but to a higher degree in transgenics. In addition, transgenic bentgrass plants exhibited greater biomass than wild-type plants under both normal and stress conditions. When subjected to salt stress, the fresh weight and dry weight of both the leaves and roots decreased much more in wild-type than in transgenic plants. Our results indicated that overexpression of AVP1 in creeping bentgrass can lead to increased leaf and root biomass, improved salt and drought tolerance, accelerated recovery from injury, and enhanced phosphorus uptake, pointing to the promising potential for future application of transgenic turfgrass in the salinized field and the barren area.

P-2015

Development of Procedures for Medium- and Long-term Storage of Coast Redwood (*Sequoia sempervirens*). EMRAH KIRDOK[1], Yelda Ozden-Tokatli[1], Maurizio Capuana[2], and Elif Aylin Ozudogru[1]. [1]Gebze Institute of Technology, Department of Biology, Istanbul cad., n°: 101, 41400 Gebze, (Kocaeli), TURKEY; and [2] IGV/Istituto Genetica Vegetale, CNR, via Madonna del Piano 10, 50019 Sesto Fiorentino (Florence), ITALY. Email: ekirdok@gyte.edu.tr, elif@gyte.edu.tr

Sequoia sempervirens is a conifer tree, belonging to the genus *Sequoia*. The lumber, valued for light weight and resistance to decay, makes the tree one of the most valuable timber species. However, urbanisation, fires and storms give rise to the loss of this valuable germplasm. Today, biotechnological approaches offer a powerful alternative for safe storage of such genetic resources. Among these, ‘slow

growth storage' enables the storage of plant germplasm from several months to several years (medium-term storage) by reducing the growth rate of in vitro cultures in modified conditions. Cryopreservation, i.e. storage of plant germplasm at ultra-low temperatures (*e.g.* in liquid nitrogen at -196°C), where the biological reactions in the cells are hampered, makes available the storage of plant material for theoretically unlimited periods of time (long-term storage). Present study investigated the possibility of applying slow growth storage and cryopreservation to *S. sempervirens*. Former was achieved by maintaining encapsulated buds (in 3% Na-alginate solution) and in vitro shoot cultures at 4°C, in darkness on four different media compositions (MS + 1 mg/l BA, MS 0, QL 1 mg/l BA, QL 0) for 3–15 mo, while latter was performed by pre-culturing the buds on sucrose-rich medium (0.12, 0.25 or 0.5 M for 24, 48 or 72 h), osmoprotection of the samples by PVS2 (15–120 min at 0°C) on aluminum foil strips (droplet freezing method) and direct immersion in liquid nitrogen. After at least 1 h of storage in liquid nitrogen, frozen samples were thawed by incubating them rapidly in 1.2 M sucrose solution and transferred to the recovery medium (QL + 20 gr/l activated charcoal). Outcomings of the study revealed the possibility of conserving in vitro shoot cultures at 4°C and in darkness for a minimum of nine months without losing growth potential. As regards cryopreservation, optimizing pre-culture conditions previous to storage in liquid nitrogen and using droplet freezing approach for the induction of osmoprotection led to a satisfactory long-term storage of the buds with high post-thaw recovery rates.

P-2016

In Vitro Propagation and Cryopreservation of South Florida Endangered Ferns: *Asplenium verecundum, Lomariopsis kunzeana, Thelypteris patens*, and *Trichomanes punctatum* var. *floridanum*. V. C. PENCE. Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220. Email: valerie.pence@cincinnatizoo.org

As part of the Endangered Plant Propagation Program at CREW, in vitro propagation methods for several endangered pteridophytes have been developed. *Asplenium verecundum* (*Av*), *Lomariopsis kunzeana* (*Lk*), *Thelypteris patens (Tp)*, and *Trichomanes punctatum* var. *floridanum* (*Tpf*), are all south Florida fern species that are rare due to habitat loss. In vitro propagation methods are being developed to contribute to the overall conservation strategy for these species, to provide material for outplanting, and for long-term germplasm storage. Spores of *Av, Lk* and *Tp* were germinated and sporophytes of *Tpf* were grown in a sterile soil-less potting mix in sterile plastic boxes. Sporophytes of *Tpf* initiated new growth and spores of *Av*, *Lk*, and *Tp* germinated into gametophytes after 7–10 weeks. *Tp* and *Av* initiated sporophytes after 10 weeks and 18 mo, respectively, although none have been observed from *Lk* gametophytes. In vitro cultures of gametophytes (*Lk)* and sporophytes *(Av, Tp, Tpf)* were initiated by surface sterilizing and culturing tissues on half-strength MS medium with 1.5% sucrose, 100 mg/L benlate (fungicide), plus 1 drop of antibiotic solution per culture. Tissues grew and multiplied on the same medium. Pieces of in vitro-grown *Tpf* sporophytes and *Lk* gametophytes were cryopreserved using the encapsulation dehydration method (Fabre and Dereuddre, 1990), with preculture on MS medium plus 10 μM ABA and 3M mannitol. *Lk* gametophytes showed regrowth in 67% of beads exposed to liquid nitrogen and *Tpf* sporophyte tissues showed regrowth in 36% of beads, and these species are being banked in the Pteridophyte Bank of CREW's Frozen Garden. These results demonstrate in vitro methods that can be used for the preservation and propagation of these species. They also provide additional evidence that tissues of pteridophytes are readily adaptable to cryopreservation for long-term germplasm storage. (This research was funded, in part, by grants from the Institute of Museum and Library Services and Fairchild Tropical Botanical Garden, in collaboration with FTBG and Marie Selby Botanical Gardens.)

P-2017

Somatic Embryogenesis from Young Leaves of Safed Musli, *Chlorophytum borivilianum* - An Important Medicinal Plant of India. A. SABITHA RANI. Department of Botany, Osmania University College for Women, Koti, Hyderabad-500095, INDIA. Email: sabitaammana@yahoo.com

Somatic embryos were induced from in vitro regenerated leaves of safed musli. Callus initiated within two weeks of inoculation from the both abaxial and adaxial surface of the leaves. Embryogenic and granular callus was initiated on MS with 1.5-mg/l 2,4-D concentration. However, in all the concentrations of 2,4-D, the further differentiation of callus was not observed. Somatic embryogensis was observed after 20–25 d of culture on MS_1 media with various concentrations of 2,4-D (0.25 to 4.0 mg/l) along with Kn (0.25 and 0.5 mg/l). Among all the concentrations, 2.5 mg/l 2, 4-D with 0.5 mg/l Kn resulted in the production of high frequency of somatic embryos (36.9%). However, further development of embryos was not observed on same media. Reduction in the concentrations of 2,4-D and Kn to 0.25 mg/l (MS_2), favoured the maturation of somatic embryos. Histological studies of callus confirmed the presence of

somatic embryos at various stages of development. Matured somatic embryos germinated with a frequency of 14.6% on MS basal medium (MS_3) without any growth hormones. Plantlets with well-developed roots were transferred to pots and established in the field with 90% survival rate.

P-2018

Experiments on Camphor (*Cinnamomum camphora*) Micropropagation by Shoot Culture. K. VOLBRECHT, and M. J. Bosela. Department of Biology, Indiana University-Purdue University at Fort Wayne, 2001 E. Coliseum Boulevard, Fort Wayne, IN 46805. Email: lorkk01@ipfw.edu

As a prelude to beginning micropropagation research on *Sassafras albidum*, we evaluated the response of shoot cultures of camphor, a related tree species, to culture variables that have been traditionally shown to have significant effects on the growth response of plants in vitro such as differences in nutrient formulation, gelling agent, and cytokinin type. Cultures were initiated using explants (shoot tips and nodal cuttings) from a greenhouse stockplant. The explants were surface sterilized with bleach and transferred vessels of culture media. Eight culture media were employed in total representing all possible combinations of two media types (Murashige and Skoog [MS] vs. Lloyd and McCown's woody plant medium [WP]), two gelling agents (Gerlite vs. agar), and two cytokinins (benzyladenine [BA] and zeatin, at 10 μM concentration). The vessels were cultured under a 16 hr photoperiod (23–25°C) and transferred to fresh medium every 3–4 weeks. The degree of growth and health of the cultures was better on agar medium than on Gelrite and for MS than WP as visually evaluated. Shoot multiplication via axillary branching was reliably induced by BA but not ZEA, but two to three culture periods were required for the axillary shoots to reach 'harvestable' size (> 1 cm) and the axillary shoots generally had slender stems and small, narrow leaves and did not appear 'sturdy' enough for rooting and ex vitro transplanting. However, these shoots could be induced to resume a more desirable growth pattern with thicker stems and larger leaves when they were cultured on MS medium with ZEA. In more recent tests, ZEA has proven effective for the induction of axillary proliferation when used in combination with a third nutrient formulation (Driver and Kuniyuki Walnut medium, DKW). Rooting experiments have recently been initiated and the results from these experiments will also be included our presentation.

P-2019

Optimization of Technique for Micropropagation of an Elite Cultivar Banana "Malbhog". LAKSHAMI NARAIN SHUKLA, Tulilka Kumari, Kumari Anjali, Archana Kumari, Pratibha Kumari, Moni Kumari, and Kumari Shalini. University Department of Botany, B. R. Ambedkar Bihar University, Muzaffarpur-842001(Bihar), INDIA. Email: Shukla_ln@rediffmail.com

We have optimized an efficient, simple Micropropagation technique for the vegitatively propagated an elite cultivar of banana "Malbhog" (Musa Sapientum, group AAB),using suckers in semi solid medium, containing basal salts of Murashige and Skoog's(MS)medium (Murashige and Skoog, Physiol.Plant 15:473–497,1962) supplemented with different concentrations and combinations of plant growth regulators, 3%Sucrose, 0.8%Agar, 15 ml/1pre-boiled coconut milk. The culture medium supplemented with 6-Benzyl aminopurine (0.5–2.5 mg/l), Kinetine (0.5 mg/l), 0.5 mg/l Napthaline acetic acid, revealed different response, with particular reference to shoot bud initiation. All supplementary induced- shoot proliferation, but the optimal results was on the medium, supplemented with 2.0 mg/l 6-Benzyl aminopurine+0.5 mg/l Kinetin+15 ml coconut milk +0.5 mg/l naphthalene acetic acid, which induced 6.5 shoots per explant.Browning of the medium and blackening of the explants was observed during incubation of the explants. This was due to exudation of phenolics and its oxidation. Addition of 100 mg/l of each of ascorbic acid and citric acid followed by sub culturing reduced phenolic exudation. Excised shoots with well developed leaves were rooted on half strength Murashige and Skoog's basal salts supplemented with different concentration and combinations of Indole butyric acid and naphthalene acetic acid. Maximum no. of roots was initiated in the plantlet inoculated with 1.0 mg/l Indole butyric acid +0.5 mg/l Napthalene acetic acid. The regenerated plantlets were acclimatized and successfully transferred to the soil in pots and then in garden.

P-2020

Plant Regeneration from Sugarcane Leaf-roll Explants in Response to Different Growth Regulators. Y. TAPARIA, W. M. Fouad, M. Gallo, and F. Altpeter. University of Florida-IFAS, Agronomy Department, Plant Molecular and Cellular Biology, Laboratory of Plant Molecular Physiology, 3062 McCarty Hall, Gainesville, FL 32611. Email: altpeter@ufl.edu

In vitro culture plays a crucial role in the conservation, creation and utilization of genetic variability of sugarcane, including cryopreservation, in vitro selection, genetic engineering and commercial mass production of disease-free sugarcane. Young meristematic tissues such as immature leaf, immature inflorescence or basal shoot meristems are required in sugarcane to induce regenera-

ble tissue cultures. In this study, the culture response of sugarcane leaf-roll-cross sections from the commercially important sugarcane cultivar CP-88–1762 was examined on media differing in auxin type and cytokinin concentration. Auxins 1-naphthalenacetic acid (NAA; 10 μM); 2,4-dichlorophenoxyacetic acid (2,4-D; 22.6 μM), 4-amino-3,5,6-trichlorophyridine-2-carboxylic acid (Picloram; 40 μM), 4-chlorophenoxy acetic acid (CPA; 10 μM) plus naphthalenacetic acid (NAA; 10 μM) were evaluated alone or in combination with the cytokinin 6-benzylaminopurine (6-BAP) at 0.4 or 4.0 μM in a 3×4 factorial design with 10 replications. Differences in callus induction, callus morphology, necrosis and regeneration pathways were observed. Data on the quantity and quality of callus and the plant regeneration efficiency of the explants on the different media will be reported.

P-2021

Production of Yam (*Dioscorea spp*) Minitubers Using In Vitro Culture Techniques. O. A. KOUADIO[1,2], Koffi Konan[1], and H. W. Dodo[1]. [1] Food Biotechnology Lab, Department of Food and Animals Sciences, School of Agricultural and Environmental Sciences, Alabama A & M University and [2]Laboratoire de Biochimie et des Sciences des Aliments, UFR Biosciences, Université d'abidjan- Cocody (Cote d'Ivoire), FRANCE. Email: amarin_olivier@hotmail.com, amarin_olivier@yahoo.fr

Yams (*Dioscorea* spp.) are major food crops in many tropical countries where they contribute up to half the total calorific intake of the population. However, yams are facing many constraints for efficient production among which, the utilization of 25–30% of the production is used for planting material. Developing alternative propagules such as minitubers could prevent yam shortage an improve food security in many rural areas. The objective of the study was to develop a protocol to produce yam seeds (minitubers), which can be utilized by farmers as planting material. Minitubers were produced either via microtubers or plantlets. Microtubers were first produced from 8 weeks old plantlets explants which were cultured either on MS medium or T-medium containing 8% (w/v) sucrose and Jasmonic acid. Microtubers were then treated using T-medium containing JA or GA_3 solution to break the dormancy. Minitubers were produced by transferring treated microtubers to soil. Alternatively, single nodal explants from in vitro plantlets were cultured for 8 weeks on MS medium supplemented with sucrose (2 to 8%) to initiate root development. Jasmonic acid was added to the rooting medium to stimulate the tuberization signal. Rooted plantlets were then transferred to soil and minitubers ranging from 1.5 to 15 g were produced. The yield of production was ranging from 1 to 3 tubers per planlets. This approach, which is still under improvement, seems to be the most reliable and the most efficient in producing yam minitubers.

P-2022

Enhanced Accumulation and Simultaneous Determination of Betulinic Acid, Oleanolic Acid, and Ursolic Acid in Cell Cultures of *Lantana Camara L.* Using RP-HPLC. RAKHI CHATURVEDI , and Priyanka Srivastava. Department of Biotechnology, Indian Institute of Technology- Guwahati, Guwahati - 781039, Assam, INDIA. Email: rakhi_chaturvedi @yahoo.co.uk, rakhi_chaturvedi@iitg.ernet.in

Lantana camara (Verbenaceae) has attracted considerable attention worldwide for several reasons. It is a cause of concern to some due to its ever-spreading nature; at other times it is prominent because of numerous bioactive compounds it harbors in its various parts. Although few reports have described chemical constituents of *Lantana* growing in wild, biotechnological side is still unnoticed. For commercial utilization, it is important to obtain stable in vitro lines for uniform qualitative and quantitative production of secondary metabolites. Leaves, being the reservoir of metabolites, were used to establish callus cultures, in the present study. Further, the cultures were checked for presence of pharmacologically active compounds. Leaves, taken from plants bearing pink-yellow flowers, were surface sterilized with 1% (v/v) Tween-20 for 15 min and 0.1% (w/v) mercuric chloride solution for 10 min, followed by three rinses in sterile water. Basal media in all experiments on callus induction and proliferation, consisted of MS (Murashige and Skoog, 1962) medium enriched with 30 g/l sucrose and solidified with 0.8% agar along with various growth regulator combinations. Constantly growing cell biomass was obtained from leaf-discs cultured on MS medium containing 6-benzylaminopurine (5 μM), 2,4-dichlorophenoxyacetic acid (1 μM) and α-naphthaleneacetic acid (1 μM). Cell lines, thus obtained, were extracted in methanol following sonication and then subjected to liquid-liquid partitioning in ethyl acetate and water. HPLC of the ethyl acetate extract (yield 0.17%) on a RP C_{18} column with 80:20 (v/v) acetonitrile:water, flow rate of 1 ml/min and a detection wavelength of 209 nm revealed the co-occurrence of three powerful anticancerous pentacyclic triterpenoids: Betulinic acid (3.1%), Oleanolic acid (1.88%) and Ursolic acid (4.12%) per gram dry weight. No in vitro source has earlier been identified for Betulinic acid so far. Furthermore, efficacy of the above culture extract and, thus, the cell cultures has been demonstrated in a cytotoxic assay against cancerous HeLa cell lines.

P-2023

AGPs Secretion in Cell Cultures of *Beta vulgaris* by Osmotic Stress. J. CAPATAZ-TAFUR, G. Trejo-Tapia, M. Rodríguez-Monroy, and G. Sepúlveda-Jiménez. Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional. P. O. Box 24, Yautepec, Morelos, 62730, MEXICO. Email: jcapataz@ipn.mx; gsepulvedaj@ipn.mx

Arabinogalactan-proteins (AGPs) are involved in cellular growth control and differentiation process. Biotechnological interest of AGPs is owed to their emulsificant properties and immunostimulant activity. Also, its addition improves somatic embryogenesis and organogenesis which are key steps in plant micropropagation. *Beta vulgaris* L. cell cultures secrete AGPs into the medium representing an advantage for product recovery. The effect of osmotic stress on cell growth and AGPs secretion was investigated in *B. vulgaris* cell cultures. Osmotic stress was achieved by changing initial sucrose (suc) concentration and feeding mannitol (man), a non-metabolic osmotic agent. Initial sucrose concentration significantly affected cell growth and AGPs secretion. At initial suc of 43.8 mM (equal to 87.6 mOsm), cultures reached a maximum growth of 10.33 g/L and secreted 13.49 mg AGPs/L. In contrast, at 87.6 mM (175.3 mOsm) and 131.4 mM suc (262.9 mOsm), maximum biomass was 15.97 and 23.68 g/L, respectively. AGPs content was 59.12 and 67.80 mg/L with 175.3 and 262.9 mOsm, each one. When man was added at the beginning of the culture (47.9, 95.9 and 143.9 mM) instead of suc, growth reduced significantly and AGPs were not secreted. To understand the role of AGPs in cell response to osmotic stress, experiments were performed feeding suc and man. Cultures growing with initial suc at 43.8 mM (87.6 mOsm) were fed at day 7 of culture with suc or man to reach 175.3 mOsm. Sucrose feeding increased AGPs secretion to 78.24 mg/L and growth reached 21.56 g/L. After man feeding, cells secreted 84.0 mg AGPs/L without changes in growth compared to non-feeding cells (10.33 g/L). AGPs secretion by *B. vulgaris* cells was almost 6-fold higher than that of non-feeding (13.49 mg/L). Moreover, cells developed with initial 87.6 mM suc were fed with suc or man to 175.3 mOsm; cell growth and AGPs were enhanced. Maximum AGPs secretion of 84.05 and 113.07 mg/L were obtained at day 18, respectively. In this case, maximum growth was 23.35 and 20.56 g/L, for each one. Stimulated AGPs secretion was part of *B. vulgaris* cell response to osmotic stress.

P-2024

DMSO Stimulates Production of Artemisinin and Also Suggests That the Sesquiterpene May Function as a ROS Sink in *Artemisia annua*. P. J. WEATHERS[1], A. Mannan[2,4], C. Liu[3,4], M. J. Towler[1], D. Vail[1,4], and A. Lorence A[4]. [1]Department Biology/Biotechnology, Worcester Polytechnic Institute, Worcester, MA 01609; [2]Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad 54320, PAKISTAN; [3]National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100080, CHINA; and [4]Arkansas Biosciences Institute, Arkansas State University, State University, AR 72467–0639. Email: weathers@wpi.edu

The antimalarial sesquiterpene, artemisinin, is in short supply and the role of artemisinin in the plant is not well established. Prior work suggested that addition of dimethyl sulfoxide (DMSO) increases artemisinin in shoots. When in vitro cultured *Artemisia annua* plantlets were fed different amounts of DMSO (0–2.0% v/v), artemisinin levels increased up to 12 fold in shoots and showed biphasic optima at 0.25 and 2.0% DMSO. Maximum response was observed 3 d after DMSO treatment. However, only rooted shoots responded to DMSO; there was no stimulation of artemisinin production in DMSO treated unrooted shoots. The first gene in the artemisinin biosynthetic pathway, amorphadiene synthase (ADS), showed no increase in transcript level in response to DMSO compared to controls. In contrast the second gene in the pathway, CYP71AV1 did response to DMSO but at a level of transcripts inverse to artemisinin levels. When *A. annua* shoots were stained for the reactive oxygen species (ROS), H_2O_2, ROS increased with increasing DMSO concentration. Both the increases in DMSO-induced ROS response and corresponding artemisinin levels were inhibited by addition of vitamin C. Together these data show that artemisinin production and ROS appear to correlate and that when ROS is reduced, so also is artemisinin suggesting that ROS plays an important role in artemisinin production in *A. annua.*

P-2025

Scaling Up a New Version of the Mist Bioreactor for Hairy Root Cultures. P. J. WEATHERS[1,2], G. Sivakumar[2], C. Liu[2,3], M. J. Towler[1], and F. Medina-Bolivar[2]. [1]Department Biology/Biotechnology, Worcester Polytechnic Institute, Worcester, MA 01609; [2]Arkansas Biosciences Institute, Arkansas State University, State University, AR 72467–0639; and [3]National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100080, CHINA. Email: weathers@wpi.edu

Transformed (hairy) roots can produce high amounts of secondary metabolites and engineered proteins. A real

bottleneck, however, exists in the production of products from hairy roots, especially secondary metabolites, for two main reasons: (1) many of the relevant biochemical pathways and their regulation are inadequately understood; and (2) cost effective scalable production systems are not yet well developed. Here we report on successful scale-up to 4 L of two hairy root cultures, *Artemisia annua* and *Arachis hypogea* in a mist bioreactor. The reactor used was a newly designed version of the older mist reactor and has a flexible wall, disposable culture bag for a growth chamber and a high throughput sonic misting nozzle to deliver culture medium. Similar to earlier observations in our lab, the growth rate between days 0–6 in the mist reactor and in shake flasks was higher at 0.17 and 0.16, respectively. Growth in the mist reactor continued at a higher rate than in the shake flasks. Compared to our earlier studies, *A. annua* growth rates nearly doubled in the new reactor from 0.07 to 0.12 after 21 d. Furthermore, during fed batch mode, prior results showed only a slight increase in growth rate from 0.07 to 0.08, while studies with the new reactor showed a greater increase, from 0.12 to 0.14. These data demonstrate for the first time that at a 1 L working volume, early root growth in particular in the new mist reactor could be sustained at a higher rate than in shake flasks, and that the concept of the flexible wall growth chamber is functioning as anticipated to enhance the long term growth rate of roots compared to earlier studies. When compared to growth in shake flaks, peanut hairy root cultures also grew faster in the 1 L mist reactors with overall growth rates of 0.12 and 0.13, respectively, after 28 d of culture (Figure 4). When *A. hypogea* was subsequently scaled to 4 L, the growth rates continued to improve even beyond that observed at 1 L. These results suggest that scale-up of the mist bioreactor is quite feasible to at least 4 L for culture of hairy roots. Future work is aimed at larger volumes in order to determine the limit of scalability of the mist reactor.

P-2026

The Expression of Glutamine Synthetase in Senescing Soybean Leaves. FERNANDO SOLORZANO and Champa Sengupta-Gopalan. Molecular Biology Program, New Mexico State University, Las Cruces, NM 88003. Email: fsolorza@nmsu.edu

Glutamine synthetase (GS) is a key enzyme in nitrogen metabolism. It catalyzes the ATP-dependent biosynthesis of glutamine from glutamate and ammonia. Plant GS exists as two major isoforms: located either in the chloroplast/plastid (GS2) or cytosol (GS1). GS2 is the major form found in the mesophyllic cells of young and mature photosynthetic leaves, where it assimilates ammonia produced by the reduction of nitrate and re-assimilates ammonia produced by photorespiration. GS1 is part of a multi-gene family, existing as various isoforms. It is located in the phloem generating glutamine for nitrogen transport. The goal of this research is to elucidate the regulation of GS in soybean leaves during senescence and profile the carbon and nitrogen metabolites that may play a role in regulating GS expression during leaf senescence. Physiological parameters such as photosynthesis rate, stomatal conductance, transpiration rate for different age leaves have been recorded using the LI-COR 6400. Northern blot analysis using gene-specific probes along with 2D gel western blot analysis of different age leaves have shown an increase in the expression of the GS1 *Gmgln*β form and a drop in GS2 as the leaves senesce. Analysis of tobacco transformants with the *Gmgln*β gene driven by the constitutive CaMV 35S promoter have shown that the increased expression of *Gmgln*β in senescing leaves is a transcriptional and not a posttranscriptional function. GS enzyme activity, measured by the transferase activity assay shows an increase as the leaves age. Based on these results, we conclude the GS1 isoform *Gmgln*β whose expression shifts to the mesophyll cells plays a key role in the re-assimilation and remobilization of nitrogen released from the disintegration of cellular structures.

P-2027

Induced Tetraploidy in *Hedychium bousigonianum* via Treatment of Embryogenic Callus with Antimitotic Agents. HAMIDOU F. SAKHANOKHO[1], and Rowena Y. Kelley[2]. [1]USDA-ARS, Thad Cochran Southern Horticultural Laboratory, P.O. Box 287, 810 Hwy 26 West, Poplarville, MS 39470 and [2]Department of Biochemistry and Molecular Biology, Mississippi State University, P.O. Box 9650, Mississippi State, MS 39762. Email: Hamidou.Sakhanokho @ars.usda.gov

The genus *Hedychium* belongs to the Gingiberaceae family and includes both diploid and tetraploid species. *H. bousigonianum* is a diploid (2n=34) species with fragrant flowers, a desired and targeted trait in *Hedychium* breeding. The objective of this study was to induce tetraploidy in this species. A protocol for this purpose was developed by treating embryogenic callus with various concentrations of colchicine (2.5, 5, and 10 mM) or oryzalin (30, 60, 120 μM) for 24, 48, and 72 h. Ploidy level of regenerated plants was determined using flow cytometry and stomatal frequency. Triploid, tetraploid, and mixoploid regenerated plants were obtained with both antimitotic agents. The most efficient treatments for inducing tetraploidy were the 2.5 mM colchicine for 24, 48, or 72 h and the 60 μM oryzalin for 72 h.

P-2028

Mutation Breeding for Improved Turf and Forage Properties in Apomictic Bahiagrass (Paspalum notatum Flugge). P. LOMBA, and F. Altpeter. Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida - IFAS, Gainesville FL-32611. Email: altpeter@ufl.edu

Bahiagrass (*Paspalum notatum* Flugge) is the predominant forage grass and a popular turf in the southeastern United States. Bahiagrass' popularity is attributed to its strong persistence under low input conditions. However, the quality of bahiagrass is limited due to its open growth habit and prolific production of tall seedheads, coarse leaf texture, light green color and poor nutritive value. Improvement of bahiagrass cultivar 'Argentine' by conventional breeding is very difficult due to its apomictic reproduction mode. Our objective was to explore the potential of chemical and tissue culture derived mutagenesis for genetic improvement of apomictic bahiagrass for generation of uniform mutagenized seed progeny with improved turf and forage quality. Scarified and surface sterilized bahiagrass seeds were treated with the mutagen sodium azide at various concentrations and exposure times. Callus was induced from these seeds and regenerated via somatic embryogenesis to obtain uniformly mutagenized plants. 2,000 of the 20,000 regenerated seedlings were selected based on their vigor or morphological characteristics and transferred to soil. Plants exhibiting desirable traits of shorter stems and more vegetative tillers were moved to the greenhouse for further observations. Under controlled environmental conditions plants were evaluated by counting tillers, measuring tiller length and monitoring seedhead emergence. A total of 46 independently mutagenized lines with reduced stem length, higher tiller density or reduced or delayed seedhead formation were moved to the field for further evaluation. Plants were established in 1.2 m×1.2 m plots in a randomized block design with 4 replications. Field performance was evaluated by comparing establishment, density, leaf texture, tiller length, color, growth pattern, biomass, seedhead and seed production. Several plants with improved characteristics have been identified will now be evaluated in larger plots. Greenhouse and field data from selected mutagenized lines in comparison to non-transgenic bahiagrass will be presented.

P-2029

Enhancing Resistance to Common Scab Disease in Potato (*Solanum tuberosum*) Cultivars Through Callus Selection. A. TRAHAN DUCHARME[1], C. Beaulieu[1], D. Michaud[2], and N. Beaudoin[1]. [1]Boul. De l'Université, 2500 Boul. de l'Université, Dept. of Biology, University of Sherbrooke, Sherbrooke, Qc, CANADA, J1K 2R1 and [2]Dept. of Phytology, Pavillon des Services, Laval University, Québec, Qc, CANADA, G1K 7P4. Email: Audrey.trahan.ducharme@usherbrooke.ca

Potato is one of the major food crops grown all around the world. Common scab is an economically important disease affecting potato and other root-vegetables. This bacterial disease is caused by *Streptomyces scabiei* through the production of thaxtomin A (TA), a phytotoxin essential for the induction of common scab symptoms. It has been shown that TA inhibits cellulose biosynthesis in plant cells and triggers the formation of necrosis and lesions characterizing the disease. Nonetheless, our laboratory has been able to progressively adapt plant cells to grow and divide in the presence of lethal concentrations of TA. It was also found that potato cultivars that are more resistant to common scab are also more resistant to TA. Hence, TA represents a good selecting agent to isolate potato lines that are more resistant to common scab. Based on these findings, our laboratory is using two approaches to enhance resistance to TA in different potato cultivars. First, potato calli are adapted to TA by growing them on medium containing increasing concentrations of TA starting at 0.2 μM up to 1.0 μM for a period of 6 to 8 mo. The second method involves a direct selection of resistant callus cells with a high TA concentration of 4.0 μM in liquid medium. For both methods, callus cells are then transferred to plant regeneration medium. Resistance in regenerated plants will be evaluated in leaves, micro-tubers and tubers using either TA or *S. scabiei*.

P-2030

In Vitro and In Vivo Investigation of Salt Tolerance in Two Oriental Tobacco (*Nicotiana tabacum* L.) Species. ÖZGE ÇELİK, and Çimen Atak. Istanbul Kultur University, Faculty of Science and Letters, Department of Molecular Biology and Genetics, 34156, Ataköy, İstanbul, TURKEY. Email: ocelik@iku.edu.tr

Nicotiana tabacum L. have ability to adapt to the extreme environmental conditions. Akhisar 97 and İzmir Özbaş are oriental species. They are salt tolerant varieties in tobacco species. The effects of salt stress on the activity of antioxidant enzymes and lipid peroxidation were studied. The experimental studies were carried out both in vitro and in vivo. Tobacco seeds were surface sterilised and germinated on modified MS medium. In in vitro study, 1 mo-old seedlings were transferred to fresh medium which contains 0, 50, 100, 150, 200, 250, 300 and 350 mM NaCl in modified MS medium. After 15 d, leaves of seedlings were

used for the determination of antioxidant enzyme analysis. In vivo study was performed in growthchamber conditions with 25°C and 16 h/8 h light/dark regime. Salt treatments with 2 mo-old seedlings which grown in perlit were started by adding the same concentrations of NaCl with the in vitro experiment. This treatment was continued through 15 d. In both experimental conditions, malondialdehyde (MDA) level significantly increased according to the increasing NaCl concentrations in both tobacco species ($p<0.05$). Ascorbat peroxidase (APX; EC 1.11.1.11) activities signficantly increased after 200 mM NaCl concentration in both groups. Glutathione reductase (GR; EC 1.6.4.2), SOD (EC 1.15.1.1) activities increased in all experimental groups ($p< 0.05$). CAT (EC 1.11.1.6) activity significantly decreased in all groups ($p<0.05$). Guaiacol peroxidase (GPX, EC 1.11.1.7) activity showed a significant increase after 250 mM NaCl concentration ($p<0.05$). Salt tolerance index was calculated as total chlorophyll concentration obtained from in vitro and in vivo experiments. İzmir Özbaş variety was found more resistant to salt stress than Akhisar 97 variety ($p<0.05$).

P-2033

Agrobacterium-mediated Transformation of *Fraxinus pennsylvanica* Hypocotyls. N. Du[1], and P. M. PIJUT[2]. [1]Purdue University, Dept. of Forestry and Natural Resources, Hardwood Tree Improvement and Regeneration Center (HTIRC), 715 West State St., West Lafayette, IN, 47907 and [2]USDA Forest Service, Northern Research Station, HTIRC, 715 West State St., West Lafayette, IN 47907. Email: ppijut@purdue.edu

A genetic transformation protocol for green ash (*Fraxinus pennsylvanica*) hypocotyl explants was developed. Green ash hypocotyls were transformed using *Agrobacterium tumefaciens* strain EHA105 harboring binary vector pq35GR containing the neomycin phosphotransferase (*nptII*) and β-glucuronidase (GUS) fusion, and an enhanced green fluorescent protein (EGFP) gene. Pre-cultured hypocotyl explants were transformed in the presence of 100 μM acetosyringone with 90 sec sonication plus 10 min vacuum-infiltration. Kanamycin at 20 mg/l was used for selecting transformed cells. Adventitious shoots regenerated on Murashige and Skoog (MS) medium supplemented with 13.3 μM 6-benzylaminopurine (BA), 4.5 μM thidiazuron (TDZ), 50 mg/L adenine sulfate, and 10% coconut water. GUS- and PCR-positive shoots from the cut ends of hypocotyls were produced via an intermediate callus stage. Presence of the GUS and *nptII* genes in GUS-positive shoots were confirmed by polymerase chain reaction (PCR) and copy number of the *nptII* genes in PCR-positive shoots was determined by Southern blotting. Three transgenic plantlets were acclimatized to the greenhouse. This transformation and regeneration system using hypocotyls provides a foundation for *Agrobacterium*-mediated transformation of green ash. Studies are underway using a construct containing the Cry8Da protein of *Bacillus thuringiensis* for genetic transformation of green ash.

P-2034

The Effect of *Agrobacterium* Growth Phase and Density on Citrus Transformation. R. P. NIEDZ, and T. J. Evens. USDA-ARS-U.S. Horticultural Research Laboratory, 2001 South Rock Road, Ft. Pierce, FL 34945–3030. Email: Randall.Niedz@ars.usda.gov

The effect of *Agrobacterium* growth phase and density on transformation of citrus rootstock US-812 (*Citrus reticulata* x *Poncirus trifoliata*) epicotyl explants was determined. In the first experiment, *Agrobacterium* EHA105 containing pBINGUSint was grown in YEP medium to an OD_{600} of 1 and glycerol stocks made and stored at -80C. The three factors incubation time (18 to 24 h), stock dilution (1/10, 1/100, 1/1000), and temperature (26C or 28C) were simultaneously varied and a response surface for OD_{600} generated. The resulting model was highly significant with a R^2 of 0.99 and was suitable for predicting growth throughout the design space. In a second experiment, transformation of juvenile epicotyl explants was assessed by fluorometric analysis of GUS activity from treatments in a 2×2 factorial arrangement of *Agrobacterium* culture OD_{600} (0.1 or 1) and inoculation density OD_{600} (0.1 or 1). Because the *Agrobacterium* density in the inoculating solution affects transformation efficiency, it is important to know both the initial growth and inoculation densities to achieve both consistent and optimal transformation levels.

P-2035

Vacuum Infiltration of Multiple Shoot Meristems of Indian *Musa* spp. *cv Matti* (AA) for Rapid and Highly Efficient Transformation. ANJANA CHAURASIA[1,2], Vishnu Bhat[1], and Neera Bhalla Sarin[2]. [1]Department of Botany, University of Delhi, Delhi, INDIA; and [2] Jawaharlal Nehru University, New Delhi, INDIA. Email: anjana.chaurasia@gmail.com

Banana and plantains are major horticultural crops and form the staple food of the mankind in the developing world. Most of the edible bananas being triploid and highly sterile are not amenable to breeding, therefore genetic engineering offers an alternative approach to their improvement. A rapid and highly efficient transformation system has been developed for the production of transgenic dessert

variety of *Musa* spp. cv *Matti* (AA). Compact clusters of multiple shoot meristems which are used as explants were obtained by culturing the apical shoot tip meristems in MS media supplemented with BAP and IAA. The explants were vacuum infiltrated with *Agrobacterium* strain EHA 105 harbouring the binary vector pCAMBIA 1301 containing the *hptII* gene as the selectable marker and *GUS-INT* as the reporter gene. The explants were co-cultivated with *Agrobacterium* and selected on medium containing hygromycin. The GUS expression was observed not only in the germline cells but throughout the plants. Southern blot confirmed stable integration of the transgenes in the *Musa* genome. This study is based on transformation of rapidly regenerating compact multiple shoot meristems which can be micropropagated for the homogeneous population in a short span of time. A transformation frequency of 18% was achieved in this study. This system is currently being exploited for developing abiotic as well as biotic stress tolerant *Musa* transgenics.

P-2036

Genetic Transformation in the Medicinal *Echinacea* Plants. KIN-YING TO, Hsin-Mei Wang, and Shin-Yun Hsu. Agricultural Biotechnology Research Center, Academia Sinica, Taipei 11529, TAIWAN. Email: kyto@gate.sinica.edu.tw

Echinacea species (also known as coneflowers) are important plants in both pharmaceutical and ornamental markets. There are nine varieties of *Echinacea* plants but those most commonly used medicinally and in research are *Echinacea angustifolia, E. pallida* and *E. purpurea* for the prevention and treatment of common cold and wound healing. Currently, extracts and whole plants made from these three species comprise one of the largest sectors of the herbal medicine market in North America and in Europe. Moreover, purple coneflower (*E. purpurea* L.) is widely cultivated in gardens and grows in wild in some countries. Here, we reported the optimal conditions for callus induction and plant regeneration from in vitro explants of *E. purpurea*; subsequently, we transformed the plasmid carrying a chimeric *Petunia* chalcone synthase construct by *Agrobacterium*-mediated method. The obtaining transgenic *Echinacea* plants were then characterized extensively. Chalcone synthase is a key enzyme in synthesizing anthocyanins/flavonoids, a large class of secondary metabolites. In parallel, optimal conditions for tissue culture and regeneration of two *Echinacea* species (i.e., *E. angustifolia* and *E. pallida*) were also determined. As an initiative, transformation is carrying out into these *Echinacea* species with few selected genes (i.e., aureusidin synthase) in aurone/anthocyanin biosynthetic pathway. Preliminary data demonstrated that antibiotic selection marker and foreign transgene were integrated into genomes of these putative transformants. Thus, we believe that we will establish the transformation system for these economically important *Echinacea* species, and will lead practical applications for metabolic engineering among these herbal plants. Furthermore, transgenic *Echinacea* plants carrying different flower colors may provide an extra value from health protection to ornamental market. Currently, a patent regarding the method of producing transgenic *Echinacea* plants has been issued, supporting the novelty and important value of this research.

P-2037

Light Intensity Affects the Production of Hybrid Aspen Transgenic Lines. Robert Thomas, Maud Hinchee, and SHUJUN CHANG. Arborgen, P.O. Box 840001, Summerville, SC 29485. Email: SXCHANG@ARBORGEN.COM

The hybrid aspen genotype Ca-2–75 has high growth potential and is widely adapted for growth in the United States. This makes it an excellent candidate for biotech improvements directed toward improving its ability to produce woody biomass for industrial applications including the production of pulp and bioenergy. An efficient transformation method has been developed for this genotype and several hundred transgenic lines have been produced. In the process, the effect of light intensity on transgenic line production was investigated to see if lower intensity would work. Low light intensity has the benefit of lower electricity load to the growth chamber to avoid over heating issues. Routinely, cultures receive ~ 35 μE light supplied via four T15 fluorescent tubes hanging 10 inches above tissue in Magenta Boxes. When the light intensity was decreased to ~ 17 μE by covering the boxes with three layers of shade cloth, transgenic line production in the shaded boxes was 79 percent lower than standard boxes. This dramatic result has led to further light intensity comparisons, which will be discussed.

P-2038

Factors Effecting *Agrobacterium tumefaciens*-mediated Gene Transfer to Sainfoin (*Onobrychis viciifolia* Scop.). C. Sancak[1], S. Cocu[2], S. Avci[1], S. Uranbey[3], and S. OZCAN[1]. [1]Department of Field Crops, Faculty of Agriculture, University of Ankara, 06110, Dışkapı, Ankara, TURKEY; [2]Department of Field Crops, Seyrani Faculty of Agriculture, University of Erciyes, Kayseri, TURKEY; and [3]General Directorate of Agricultural Research, Ministry of Agriculture and Rural Affairs, Ankara, TURKEY. Email: ozcan@agri.ankara.edu.tr

Leguminosae plants are more resistant to *Agrobacterium* infections compared to many other plant species. This study reports effects of explant type, co-cultivation period and acetosyringone on efficiency of *Agrobacterium*-mediated transformation of sainfoin using leaflet and petiole explants by inoculating with *Agrobacterium tumefaciens* strain GV2260:: p35SGUS INT. Transformation frequency increased significantly by pretreatment of acetosyringon. The highest transformation frequency was achieved from the leaflet explants, inoculated with *A. tumefaciens* in presence of 100 μM acetosyringone in inoculation and co-cultivation medium by maintaining co-cultivation periods of 2 d. The GUS positive plantlets were successfully acclimatized and presence of NPT-II was confirmed by Polymerase Chain Reaction (PCR). Transgenic T1 plants were obtained and selected by germinating the seeds on kanamycin. Heredity of the transgene in T1 progeny was also confirmed.

P-2039

Development of a Rapid *Agrobacterium*-mediated Genetic Transformation of Yam. F. N. OGUNKUNLE, N. K. Konan, and H. W. Dodo. Department of Food and Animal Sciences, Alabama A&M University, Normal, AL 35762. Email: Felicia.anike@gmail.com

A fast, simple, and reproducible protocol for yam genetic transformation has been developed, a premier to open an avenue to yam improvement using modern techniques of genetic manipulation. Edible yam serves as a staple food and plays an important role as a source of carbohydrate for over 300 million people globally. Despite its importance, yam has not yet benefited from advances in genetic manipulation technologies. Genetic transformation studies were carried out using disarmed *Agrobacterium tumefasciens* strain EHA 101 harboring the binary vector pIG121-Hm. An intron-containing glucuronidase *uidA* (gusA) under the transcriptional control of the CaMV 35S promoter served as the reporter gene. Parameters which significantly affected yam transformation frequencies were plant genotype, the co-cultivation time, acetosyringone treatments, and the time of application of the selection pressure. Shoots emerged along petiole segments after 7–10 d of infection with *Agrobacterium tumefaciens*. Putative transformants were initially identified by kanamycin selection and GUS assays. PCR, RT-PCR and Southern blot analysis confirmed the stable transformation. Transgenic yam plants were produced 6–8 weeks after *Agrobacterium* inoculation.

P-2041

Stable Genetic Transformation of Energy Cane. W. M. FOUAD, Y. Xiong, C. Steeves, H. Oraby, S. Sandhu, M. Gallo, and F. Altpeter. Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida – IFAS, Gainesville FL-32611. Email: altpeter@ufl.edu

Energy cane is recognized as one of the most efficient converters of sunlight into biomass and is a prime target for biofuel production in the southern U.S. Energy canes are varieties in the *Saccharum* genus developed specifically for utilization as a biofuel resource. These can be distinguished from conventional sugarcane cultivars by higher biomass production and persistence. Genetic transformation protocols for energy cane genotypes still need to be developed. Two energy cane genotypes H0-OO-961 and L 79–1002 were selected to establish a regeneration and stable transformation system in energy cane. Embryogenic callus was initiated from leaf-roll or immature inflorescence transsections on callus induction media (CI3) supplemented with 3 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D). The neomycin phosphotransferase (npt-II) selectable marker gene under the constitutive maize ubiquitin promoter was introduced to the embryogenic callus using biolistic gene transfer. Selection of transgenic events occurred in the presence of geneticin during callus subculture and paromomycin during rooting of regenerated shoots. The transgenic nature of the regenerated energy cane plants was confirmed using the NPTII-ELISA assay. Southern blot analyses evaluating the transgene integration pattern will also be presented.

P-2042

Transgene Integration and Expression Following Biolistic Gene Transfer of Different Quantities of Minimal Expression Cassettes into Sugarcane. J. Y. KIM, M. Gallo, and F. Altpeter. Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida, Gainesville, FL. Email: altpeter@ufl.edu

Sugarcane (*Saccharum sp.* hybrids) is a highly productive C_4 grass used as the main source of sugar and more recently to produce ethanol, a renewable transportation fuel. Biolistic gene transfer is superior to alternative methods in gene stacking or pathway engineering, when multiple expression cassettes need to be co-expressed. However, biolistic gene transfer is sometimes associated with complex transgene integration that may cause gene silencing. This study investigated the integration complexity following biolistic gene transfer of minimal transgene expression cassettes without vector backbone and the effects on transgene expression. Cross sections of immature inflorescences from sugarcane (cultivar CP-88–1762) were placed on callus induction medium (CIM) and

subcultured biweekly to induce embryogenic callus. Embryogenic callus was used as a target for biolistic gene transfer of an expression cassette consisting of *npt*II under the control of the constitutive 35S promoter with HSP70 intron and NOS 3′ UTR. Prior to gene transfer, the vector backbone was removed by restriction digestion followed by gel electrophoresis and gel extraction of the *npt*II expression cassette. The minimal *npt*II expression cassette was precipitated on 1.0 μm gold particles and the precipitation equivalent of 50 ng, 25 ng or 12.5 ng of the expression cassette were delivered to the target tissue per shot. Two independent experiments of 10 shots were performed for each DNA quantity. Transgenic plants were regenerated following selection on geneticin or paromomycin containing media. PCR screening suggested that more than 80% of the regenerated plants were transgenic. Ten plants were randomly selected per experiment (total of 60 plants) and analyzed for transgene expression by NPTII - ELISA and transgene integration by Southern blot analysis. Data will be presented describing transgene expression and complexity relative to the amount of DNA used during bombardment.

P-2043

Expression and Validation of Zinc Finger Nucleases in Plant Cells. A. Tovkach, V. ZEEVI, and T. Tzfira. Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI. 48109. Email: vz11@hotmail.com

Zinc finger nucleases (ZFNs) are a new type of artificial restriction enzymes that are custom-designed to recognize and cleave specific DNA sequences, producing double-strand breaks (DSBs) in living cells. DSBs can be repaired by homologous recombination or nonhomologous end-joining, which can lead to gene replacement, site-specific mutagenesis and targeted DNA integration at the repair site. The deployment of ZFN technology for gene-targeting experiments in plant cells is hindered by the lack of versatile tools for the assembly, validation and expression of novel ZFNs. To overcome these technological barriers, we have recently designed a set of constructs and procedures for the cloning, biochemical analysis and in-planta analysis of newly designed ZFNs. Cloning begins with de-novo assembly of the DNA-binding regions of new ZFNs from overlapping oligonucleotides containing modified helices responsible for DNA-triplet recognition, and fusion of the DNA-binding domain with a *Fok*I endonuclease domain in a dedicated plant expression cassette. Following the transfer of fully assembled ZFNs into *Escherichia coli* expression vectors, proteins can be easily expressed, purified on nickel columns and used for in-vitro digestion analysis of palindromic target sequences. We have also developed a collection of three *in-planta* activity assays suitable for validating the nucleic activity of ZFNs in plant cells. The assays are based on the reconstruction of a mutated *uidA* gene, its transient or stable delivery into plant cells, and monitoring for GUS activity in target tissues. We have further demonstrated the use of our tools for the analysis of various ZFNs and the recovery of mutated *Arabidopsis* seedlings.

P-2044

Plant Genetic Transformation by Large Multigene Binary Vectors. V. Zeevi, Z. LIANG, A. Tovkach, and T. Tzfira. Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI 48109. Email: zbliang@umich.edu

The use of transgenic technologies for the genetic manipulation of plant species has had a profound impact on basic plant research and biotechnology. The ability to control integration, inheritance, and expression of multiple transgenes is a prerequisite for analyzing and manipulating complex metabolic pathways and agronomic characteristics attributed to multigene traits in plants. We previously described the construction of a basic, yet modular set of satellite (pSAT) vectors useful for the assembly of up to six expression cassettes onto a single *Agrobacterium* binary plasmid using a set of rare-cutting enzymes. To overcome the problem of the very small number of commercially available rare-cutting enzymes, a set of zinc finger nucleases (ZFNs) and compatible plasmids were developed, allowing the expansion of our current cloning system beyond seven independent cassettes. Using a combination of commercial rare-cutters and home-made artificial ZFNs, plant binary vectors carrying seven and eight functional units and gene expression cassettes have been constructed. Transgenic *Arabidopsis* plants, carrying seven cassettes consisting of ca. 21-kb-long artificial T-DNA molecule, have been produced. PCR analysis confirmed the presence of the entire transgene in selected plants. Phenotypical analysis indicated that all seven cassettes are functional in these plants and that the transgene is stably inherited by successive generations. Production of longer constructs and generation of transgenic plants with eight expression cassettes is underway. Our data show that extremely large multigene transformation vectors can be assembled and used for plant genetic transformation, creating a novel route for the possible application of this system to multi-trait engineering in plants.

P-2045

3′UTR Mediated Transcript Turnover may be a General Regulatory Step in the Expression of Some Isoforms of

Cytoplasmic Glutamine Synthetase in Legumes. BINDU VASUDEVAN SIMON, Jose Luis Ortega, Suman Bagga, and Champa Sengupta Gopalan. Department of Molecular Biology, New Mexico State University, Las Cruces, NM. Email: bsimon@nmsu.edu

Glutamine synthetase (GS) is an enzyme that plays a key role in N assimilation by catalyzing the condensation of glutamate and ammonia to form glutamine. There are two major isoforms of GS in plants, the chloroplastic isoform (GS2), and the cytosolic isoform (GS1). While GS2 functions in the assimilation of ammonia produced by photorespiration or nitrate reduction, GS1 assimilates ammonia produced by all other physiological processes. Both GS1 and GS2 are known to be transcriptionally regulated, however, some recent work from our laboratory has shown that a soybean GS1 (Gmglnβ1) gene is posttranscriptionally regulated at the level of transcript turnover, the process being mediated by its 3′UTR. The main objective of this paper is to determine if the 3′UTR mediated transcript turnover exhibited by Gmglnβ1 applies to other GS1 genes. To accomplish this objective, we have made gene constructs consisting of the GUS reporter gene engineered individually with the 3′UTR of the two alfalfa GS1 genes (MsGS100 and MsGS13), the Gmglnβ1, or the nopaline synthetase gene (NOS), all driven by the constitutive CaMV 35S promoter. The constructs have been introduced into alfalfa by *Agrobacterium tumefaciens* mediated transformation and the putative transformants have been confirmed for the integration of the transgene. The putative transformants will be tested for the expression level of the transgene in the different organs and under different N and C regimens. Preliminary analysis based on agroinfiltration of tobacco leaves of gene constructs with the different 3′UTRs behind the GUS coding regions indicates that the 3′UTR of the MsGS100 but not MsGS13, like the 3′UTR of the Gmglnβ1 gene plays a role in transcript turnover.

P-2046

Expression of *cry1Ac* Gene is Detrimental to Regeneration and Development of Transgenics: A Study in Tobacco and Cotton. PREETI RAWAT, Krishna Ray, Amarjeet Kumar Singh, Deepak Pental, and Pradeep Kumar Burma. Department of Genetics, University of Delhi South Campus, Benito Juarez Road, New Delhi-110 021, INDIA. Email: prawat2k@gmail.com

Achieving high levels of *cry1Ac* expression in transgenic plants has been difficult, despite several modifications made till date to improve its expression. Our group has been working to develop insect resistant transgenic lines in cotton using the *cry1Ac* gene, wherein we have developed over 350 independent transgenic events. However, a large proportion of these lines (~80%) showed no or extremely low levels of *cry1Ac* expression. The few lines which showed appropriate levels of expression had abnormal vegetative and reproductive morphology. Southern analysis of the developed transgenics revealed that ~30% of the transgenics which showed the presence of the marker gene (*nptII*) did not show the presence of the linked *cry1Ac* gene. These observations suggested that Cry1Ac protein had a negative influence on plant regeneration and growth. As designing directed experiments in cotton is cumbersome, we carried out transformation experiments in tobacco with different constructs of *cry1Ac* gene to critically analyze the above observations. We compared transformation and regeneration process in tobacco using constructs with and without the *cry1Ac* gene. Two different codon optimized *cry1Ac* genes under different promoters were tested. Several independent experiments showed that the initial stages of regeneration were adversely affected when transformations were carried out with binary vector expressing *cry1Ac* gene. Further, a larger majority of transgenics developed did not show any detectable levels of Cry1Ac protein. Few lines that showed high levels of *cry1Ac* expression were observed to have abnormal phenotype. In order to circumvent this problem, we targeted the protein to the chloroplast. This led to an improvement in the regeneration and also resulted in a larger number of transgenics showing expression of *cry1Ac* gene, with higher level of expression than the lines wherein the protein was cytosolic in nature. The results of these experiments and the future goals will be discussed in the light of our work and a few earlier reports commenting on similar observations.

P-2047

Expression of a Synthetic *Bt cry* Gene in Seashore Paspalum Confers Fall Armyworm Resistance. F. ALTPETER[1], I. Neibaur[1], H. Zhang[1], R. L. Meagher[2], and M. Gallo[1]. [1]Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida - IFAS, Gainesville FL-32611 and [2]USDA, ARS, CMAVE, Gainesville, FL 32608–1069. E-mail: altpeter@ufl.edu

Seashore paspalum (*Paspalum vaginatum* Swartz) is a salt tolerant, fine textured turfgrass used on golf courses in coastal, tropical and subtropical regions. Fall armyworm [*Spodoptera frugiperda* (J. E. Smith)] is a devastating pest of seashore paspalum. Therefore, insect resistance is a prime target for genetic engineering of seashore paspalum. However, a genetic transformation protocol for seashore paspalum was lacking. Here we report the development of a

genetic transformation protocol for this commercially important turfgrass species as well as the generation of fall armyworm resistant seashore paspalum expressing a synthetic, codon optimized *Bt cry* gene. Biolistic gene transfer of embryogenic callus with a linearized plasmid carrying constitutive hygromycin and *Bt cry* expression cassettes was followed by selection with hygromycin and regeneration of plants. Transgene integration and expression of the regenerated plants was confirmed by PCR, Southern blot, RT-PCR, and ELISA respectively. Resistance of transgenic plants to fall armyworm was evaluated in comparison to wildtype and correlated well to *Bt cry* expression levels.

P-2048

Transformation of Poplar Trees with the PAP-Y Gene for Disease Resistance. W. DAI[1], V. A. Magnusson[1], and J. A. Walla[2]. [1]Department of Plant Sciences, North Dakota State University, Fargo, ND 58108; and [2]Department of Plant Pathology, North Dakota State University, Fargo, ND 58018. Email: wenhao.dai@ndsu.edu

Ribosome-inactivating proteins (RIPs), found in many plant species, are believed to function as defense proteins by inhibiting pathogen protein translation. Pokeweed antiviral protein (PAP), an antiviral protein from pokeweed (*Phytolacca americana*), is an RIP that has been used to protect transgenic plants from various viral and fungal diseases. An aspen hybrid (*Populus* × *canescens* × *P. grandidentata*) was transformed with the PAP-Y gene which is a mutant form of PAP with reduced toxicity but still retains antiviral activity. In this study, *Agrobacterium tumefaciens* strain EHA105 that harbored a binary vector (pND0401, constructed based on pBI121) carrying the *npt*II gene under the nos promoter and tandem the PAP-Y-*uid*A (GUS) genes with the CaMV 35S promoter was used. Transformation was primarily confirmed by histochemical β-glucuronidase assay (GUS) and polymerase chain reaction (PCR). Transgenic plants with the PAP-Y gene were also identified and a few plants showed the insect (spider mites) resistance in the greenhouse. These transgenic lines are being propagated using softwood cuttings. Expression of the PAP-Y gene in transgenic plants will be determined using RT-PCR. The disease resistance of transformed aspen plants will be further evaluated under both greenhouse and field conditions. This research shows a great potential to improve disease resistance of poplar trees which is an important tree species for the forestry industry and biomass production.

P-2049

RNAi Mediated Viral Resistance in Transgenic Wheat. LUISA CRUZ[1], John P. Fellers[2], and Harold N. Trick[1]. [1]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506 and [2]USDA-ARS Plant Science and Entomology Research Unit, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506. Email: luisac@ksu.edu

Significant yield losses in Wheat (*Triticum aestivum* L) occur throughout the major wheat producing countries of the world due to viral diseases. Wheat Streak Mosaic Virus (WSMV) and Triticum Mosaic Virus (TriMV), are two of the major viruses in the Great Plains of the United States. Cultural practices and mite vector control are the primary methods of disease management; however they are not fully effective. Resistant varieties are also deployed, although none of these varieties are totally effective. Interference RNA has been recognized as a natural defense mechanism against viral infection and is a biotech approach that may provide resistance to these wheat viruses. RNAi expression vectors were independently created from the genetic sequences of the coat proteins (CP) of both WSMV and TriMV. Immature embryos of the wheat cultivar 'Bobwhite' were independently co-transformed via particle bombardment with these RNAi expression vectors and pAHC20, which contains the bar gene for glufosinate selection. After tissue culture, putative transformed plants were generated and analyzed for the presence of the appropriate RNAi CP gene via PCR analysis. Transgenic T_1 seeds were collected and each line was tested for transgene expression through RT-PCR. In order to determine viral resistance, T_1 progeny was mechanically inoculated with the corresponding virus. Viral presence was determined by ELISA. siRNA presence will be monitored on the virus resistant transgenic lines. Tests will continue with later generations of the transgenic wheat plants.

P-2050

Resistance to Cucumber Mosaic Virus in *Gladiolus* Plants with the Coat Protein or Replicase Genes. K. KAMO, H. T. Hsu, R. Jordan, and M. A. Guaragna. Floral and Nursery Plants Research Unit, 10300 Baltimore Ave., Bldg. 10A Room 122, U.S. National Arboretum, USDA, Beltsville, MD 20705. Email: Kathryn.Kamo@ars.usda.gov

Cucumber mosaic virus (CMV) is one of the most important plant viruses because it infects approximately 1000 plant species, including food crops and ornamentals. Plant viruses are, and CMV in particular, is a major problem in many flower bulb crops, including *Gladiolus*. The viruses result in streaking of the flowers making them unmarketable, and infected plants have decreased vigor resulting in a poor bulb yield. It is very difficult to eliminate the viruses from infected materials because they

are propagated each year within the flower bulb. In addition, few species have any genetic basis for resistance to these viruses. Transgenic-based forms of resistance have been shown to be a useful means of plant protection. Transgenic *Gladiolus* plants that contain either the CMV serotype I coat protein, CMV serotype II coat protein, CMV replicase, a combination of the CMV serotypes I and II coat proteins, or a combination of the CMV serotype II coat protein and replicase genes were developed. In vitro grown plants were challenged using a hand-held gene gun to inoculate plants with purified CMV isolated from *Gladiolus*. Three plant lines expressing the replicase gene, under control of the duplicated CaMV 35S promoter, were resistant to CMV. None of the plant lines containing the CMV serotype I coat protein gene, under control of the duplicated CaMV 35S promoter, were found to be resistant. Three plant lines with the CMV serotype II coat protein gene, under control of the duplicated CaMV 35S promoter, were resistant to CMV. This work will facilitate the evaluation of virus resistance in transgenic gladiolus plants to yield improved floral quality and productivity.

P-2051

Modified Lipid Content, Enhanced Seed Viability, and Increased Freezing Tolerance on Transgenic Soybean with Attenuated Phospholipase Dα Activity. J. LEE[1], H. N. Trick[1], and W. T. Schapaugh[2]. [1]Department of Plant Pathology, Kansas State University, Manhattan, KS 66502 and [2]Department of Agronomy, Kansas State University, Manhattan, KS 66502. Email: churkie@gmail.com

Reduction of phospholipase Dα (PLDα) activity in *Arabidopsis* results in the alteration of fatty acid profiles and the physiological alteration to temperature stress and seed viability. We attenuated soybean PLDα enzyme activity by RNAi and antisense constructs using the PLDα gene sequence. Transgenic soybean lines were established by particle inflow gun co-bombarding PLDα-suppressing constructs and pHG1, and were evaluated for the presence and expression of transgenes through the T_5 generation. PLDα-suppressed soybean lines were characterized by decreased PLDα mRNA and PLDα enzyme activities. The PLDα-attenuated transgenic lines contain 36 and 49% oleic acid in the field and greenhouse evaluations, respectively, which are equivalent to the mid-oleic acid soybean lines improved by conventional breeding and mutagenesis. Germination rates appear to improve in PLD-attenuated lines compared to non-transgenic control seeds. Soybean cultivar, Fayette, seed stored for two months exhibited about 95% viability but become nonviable if stored at room temperature and uncontrolled relative humidity for 33 mo. PLD-suppressed transgenic seeds stored in the same condition had germination rate 30 to 50%. PLD attenuated lines were also more resistant to extreme temperatures. The early growth of PLDα-attenuated soybean seedlings were recovered from freezing treatments (-8°C). The disruption of the plasma membrane and organelles was observed in freeze-stressed non-transgenic control seedlings. On the other hand, the structures of the plasma membrane, oil bodies, and cell organelles in transgenic seedlings were partially sustained after enduring freezing and thawing stresses.

P-2052

Manipulation of Sucrose Phosphate Synthase in *Medicago sativa* (Alfalfa); Enhancing our Understanding of Carbon Metabolism and Its Influence on Nitrogen Metabolism in the Root Nodule. MARK SEGER[1], Jose Luis Ortega[2], and Champa Sengupta-Gopalan[1,2]. [1]Graduate Program in Molecular Biology and [2]Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces, NM 88003. Email: mseger@nmsu.edu

Sucrose-phosphate synthase (SPS), a key enzyme controlling the flux of carbon into sucrose biosynthesis, converts UDP-Glucose and Fructose 6P to Sucrose 6P, which is subsequently hydrolyzed by Sucrose phosphate phosphatase (SPP) to release sucrose. Sucrose produced in photosynthetic tissues is the main carbohydrate transported into the root nodule, where sucrose is metabolized initially by sucrose synthase (SuSy). Sucrose provides the carbon source for nodules and its metabolism in the nodules ultimately supports bacteroid growth and fuels the N_2 fixation and NH_4+ assimilation processes. Our objective is to determine how the constitutive increase of SPS activity in alfalfa will affect nodule function (N_2 fixation and NH_4+ assimilation), the interactions between the carbon and nitrogen metabolic pathways, and the physiology of the transformants. To date we have introduced a maize SPS B family gene driven by the CaMV35S promoter into alfalfa. Initial observations show improved performance of the transformants with regards to growth and chlorophyll content. We will present data on the expression of the transgene and the endogenous SPS genes, along with other key genes in sucrose metabolism in leaves and nodules. To determine changes in carbohydrates and amino acid content, metabolite analysis using UPLC-MS methods on leaf and nodule tissue of the same plants will be presented. The activity of nitrogenase (N_2 fixation) and glutamine synthetase (NH_4+ assimilation) will be assayed in the nodules to determine the effect on nitrogen metabolism. Data on several physiological parameters such as net

photosynthetic rates and total protein content, chlorophyll content, and C/N content will be presented.

P-2053

Heterologous Expression of *galE* Changed the Soluble Carbohydrate Composition and Facilitated the Growth and Development in Tobacco. A. F. YANG, H. Y. Yin, Y. F. Zhang, Z. Chen, and J. R. Zhang. School of Life Science, Shandong University, 27 Shanda South Rd, Jinan 250100, CHINA. Email: nihaohua@263.net

galE gene, encodes UDP-galactose-4-epimerase (EC 5.1.3.2), catalyzes the reversible conversation of UDP-Glucose (UDP-Glc) to UDP-Galactose (UDP-Gal). In this study two homogenous transgenic tobacco lines E54 and E41 that overexpress *galE*, together with line E23 that containing antisense *galE*, and a wild type line (WT) were analyzed for the growth and development responses during the seedling, vigorous growing, and maturing periods, and the soluble carbohydrate composition during vigorous growing period was analyzed. All the experiments were carried out in green house with five replicates. Results suggested that heterologous overexpression of *galE* facilitated both growth and development of the transgenic plants of line E54 and E41, which showed longer root system in seedling period, much leaf area in vigorous growing period due to longer and wider leaf blade, and higher plant height in maturation period. Compared to the WT, the initiation of blossom was earlier, and an obvious increase in net photosynthesis rate was observed in these two lines. However, the growth potential, initiation of blossom, and net photosynthesis rate of the antisense transgenic plants were not significantly different from the WT. The GalE activity in the transgenic plants was active between pH7 to pH9, with peak activity at pH7.5–8, and the optimal reaction temperature of the enzyme was 20 degree. The yield of UDP-Gal of the two sense transgenic lines was significantly higher (1.5–1.6 fold) than the antisense line and the WT. The content of total soluble carbohydrates in E54 and E41 was significantly higher than that of the WT and the levels of glucose, fructose, and sucrose in these two lines were all increased in considerable degrees. The content of UDP-Gal and UDP-Glc in E54 and E41 were significantly higher than that of the WT, the content of UDP-sugars, total soluble carbohydrates, and other carbohydrate composition in the antisense transgenic line E23 were not significantly different compared with the WT.

P-2054

Field Evaluation of Turf-type Bahiagrass (*Paspalum notatum* Flugge) Expressing *ATHB16* a Repressor of Cell Expansion. P. LOMBA, H. Zhang, K. Kenworthy, and F. Altpeter. Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida - IFAS, Gainesville FL-32611. Email: altpeter@ufl.edu

Bahiagrass (*Paspalum notatum* Flugge) is a low-input turfgrass widely grown in the southeastern United States and other subtropical regions around the world. However, its turf quality is limited by its coarse texture, prolific production of long seedheads and low turf density. *ATHB16* represses cell elongation independent of GA signal transduction. We recently reported improved turf characteristics in bahiagrass expressing *ATHB16* a repressor of cell expansion. Here we describe a field evaluation of turf quality and drought tolerance of these transgenic bahiagrass lines. Transgenic bahiagrass and wildtype plants were established in 1 m×1 m plots under USDA-APHIS permit 06–219–01r in a split-split-plot design. Following establishment plants were evaluated under two different mowing environments (weekly and biweekly) and three different irrigation regimes (full, moderate and no irrigation) in four replications. Turf was evaluated by comparing establishment, persistence, turf density, number of inflorescences, clipping weights, root and rhizome weight under different mowing and irrigation conditions. Statistical analysis was performed according to the randomization structure using the MIXED-procedure of SAS. Transgene expression under field conditions was evaluated with RT-PCR. Some of the bahiagrass lines expressing *ATHB16* produced shorter stems, finer leaves and more tillers than wildtype. Several transgenic plants also showed delayed flowering or reduced seed-head formation. The low-input characteristics of bahiagrass, including drought tolerance were maintained in the transgenic plants and root and rhizome biomass did not differ from wildtype.

P-2055

Optimized *Agrobacterium*-mediated Transformation of Soybean and Expression Analysis of Floral Transition Genes in Transgenic Plants. WAN-JUN ZHANG[1, 2], Prem Bhalla[1], and Mohan Singh[1]. [1]The University of Melbourne, Plant Molecular Biology and Biotechnology Laboratory, ARC Centre of Excellence for Integrative Legume Research, Parkville, Victoria, AUSTRALIA, 3010 and [21]Crop Science Department of North Carolina Sate Univesity, Campus Box 7287, 1200 Partners II, 840 Main Campus Dr. Raleigh, NC, 27606. Email: wzhang12@ncsu.edu

The development of shoot apical meristem (SAM) into floral meristem was precisely timed for plants to ensure reproductive success. Expressions of some SAM genes,

which are highly up- or down- regulated during floral transition stage, may be vital to soybean flower formation and productivity. To explore the function of these genes in soybean, an efficient genetic transformation system is highly desirable. By modifying the medium components, optimizing the hormones concentrations and culture process, a highly efficient *Agrobacterium*-mediated transformation protocol was developed. *Agrobacterium tumefaciens* AGL1, which harbors a binary vector derived from pCAMBIA1301 containing a hygromycin resistant *hpt* gene, was used for the transformation. The wounded cotyledon-node from 7-day-old seedling of soybean [*Glycine max* (L.) Merr] cv. Bragg was dipped into AGL1 suspension (OD_{600} about 0.5, pH5.4) for 2 sec, then node-down embedded into solid co-culture medium, followed by 5 d of co-culture at 25°C in the dark. The explants were then transfered to a recovery culture medium for 7 d. Later, the explants/shoots were subject to 5 $mg{\cdot}l^{-1}$ hygromycin selection. The regenerated shoots were rooted on B_5 medium containing 0.2 $mg{\cdot}l^{-1}$ IBA and 2 $mg{\cdot}l^{-1}$ hygromycin. Using this protocol, the differentiation rate of explants ranged from 52%-100%. Two to ten putative transgnic plants were obtained from an explant. The transgenic plants were transplanted to soil about four months later. With this optimized protocol, six promoter-*gus* constructs and two genes' over-expression and anti-sense vectors had been used in soybean transformation. The transgenic events were confirmed by PCR, Southern-blot analysis, and leaf-disk test on agar plate containing 5 mg/l hygromycin. GUS histochemical assay of T1 plants showed the inheritance and stable integration of transgene. The result indicated the tested highly–up regulated genes of SAM during soybean flower transition stage may also have important function in pollen development.

P-2056

Development of a Model System to Test Flowering Control Genes in Trees. SHUJUN CHANG, Eric Gulledge, Robert Thomas, Chunsheng Zhang, and Maud Hinchee. Arborgen, P.O. Box 840001, Summerville, SC 29485. Email: SXCHANG@ARBORGEN.COM

Controlling reproduction in transgenic trees can have several benefits, including the acceleration of flowering to facilitate tree breeding, and the elimination or reduction of pollen, or seed production to improve vegetative biomass and to prevent undesired gene flow. Most trees flower only after several years of growth, however, *Eucalyptus occidentalis* has been reported to be a species that flowers precociously. Seed from wild provenances of *E. occidentalis* were germinated and grown in the greenhouse to screen for flowering time. Genotypes of *E. occidentalis* were selected that flowered at five months in the greenhouse, and in vitro shoot cultures of these early flowering genotypes were established as tissue culture stocks. Leaf explants were screened for regeneration and transformation. The best responding clones were then transformed with a marker gene construct or a pollen control gene construct. Transgenic and non-transgenic plants in the greenhouse showed flower initiation at three months. Seventy-five percent of plants had flowers at five months and 100 percent at nine months. After nine months, 69 percent of plants had a second round of flowering. Transgenic plants showed the same flowering phenology as the non-transgenic controls, but the transgenic plants expressing the pollen control construct produced no viable pollen. One of the early flowering clones, Eo129 has been used consistently for testing gene constructs targeting flower control in tree species.

P-2058

Populus deltoides Transformation to Identify Genes That Contribute to Recalcitrance in Conversion of Lignocellulosics to Bioethanol. SHUJUN CHANG[1], Kim Winkeler[1], Cassandra Collins[1], Robert Thomas[1], Jessica Johnson[1], Lindsey Wood[1], Will Rottmann[1], Lee Gunter[2,3], Jerry Tuskan[2,3], and Maud Hinchee[1]. [1]ArborGen, LLC; [2]Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831; and [3]BioEnergy Science Center at http://bioenergycenter.org. Email: SXCHANG@ARBORGEN.COM

Cellulosic biomass is the most abundant polymer on earth, and it is one of the few sustainable energy resources available. However, the use of cellulose as an energy source has been limited due to the recalcitrance or difficulty of extracting cellulose from cell walls or converting cellulose to usable energy molecules such as sugars. ArborGen is a partner in the U.S Department of Energy's BioEnergy Science Center (BESC) in a joint effort to discover genes that overcome the inherent recalcitrance of woody biomass. Genes being evaluated are predicted to affect plant cell wall biosynthesis ultimately allowing efficient release of cellulose and conversion of cellulose to sugars. ArborGen has developed a high-throughput transformation system with an elite *Populus deltoides* genotype and is using this system to test more than 200 genes a year to support the BESC initiative. The *Populus* transformation pipeline has multiple components: 1) gene identification by the BESC cell wall biosynthesis team that involves Oak Ridge National Laboratory (ORNL), University of Georgia, Nobel Foundation, University of Tennessee and ArborGen, 2) gene cloning and vector assembly at ORNL and ArborGen, 3) *Populus* transformation and transgenic plant production at

ArborGen, and 4) cell wall phenotype analysis by the National Renewable Energy Laboratory (NREL) and the gene candidate nominating institutions. All experimental information is tracked via a BESC LIMS system. We will describe our status in identifying important genes and modifications that could lead to an increase in the efficient release of sugars in the conversion processes and/or an increase in cellulose content. Ultimately, the technologies are expected to lead to improved plantation trees as feedstock for more efficient production of biofuels to support a significant portion of future energy consumption.

P-2059

High Throughput Transformation of Switchgrass (*Panicum virgatum)* and Its Application for Lignin Reduction. RUYU LI, and Rongda Qu. Crop Science Department, North Carolina State University. Campus Box 7287, 1200 Partners II, 840 Main Campus Dr., Raleigh, NC 27606. Email: ruyuli@gmail.com, rongda_qu@ncsu.edu

Switchgrass is an important energy crop for biofuel production. Efficient genetic transformation is a prerequisite to improve switchgrass through genetic engineering. In this research, selected embryogenic calli induced from mature seeds were infected with *Agrobacterium tumefaciens* strain EHA105 harboring plasmids pTOK47 and derivatives of pCAMBIA1300. Up to 50% transformation efficiency was achieved when GFP was used as a marker. In addition, RNAi strategy was employed to suppress the 4-coumarate:coenzyme A ligase (4CL) gene expression in switchgrass to reduce lignin content. Approximately 200 transformation events have been obtained and analysis of the putative 4CL-RNAi transgenic plants is on the way.

P-2060

RNAi Suppression of 4-Coumarate-CoA Ligase (4CL) in Sugarcane. Y. XIONG, C. Steeves, W. M. Fouad, S. Sandhu, M. Gallo, W. Vermerris, and F. Altpeter. University of Florida-IFAS, Agronomy Department, Plant Molecular and Cellular Biology, Genetics Institute, 3062 McCarty Hall, Gainesville, FL 32611. Email: altpeter@ufl.edu

Sugarcane (*Saccharum sp.* hybrids) is a highly productive C_4 grass used as the main source of sugar and more recently to produce ethanol, a renewable transportation fuel. Typically, farmers reduce the sugarcane post-harvest leaf residue by open air burning, which negatively impacts air quality. Fuel grade ethanol can be made from sugarcane leaf litter residue. However, a major constraint for economic ethanol production from hemicellulosic sugarcane residues is lignin which acts as a physical barrier to enzyme hydrolysis. Thus, down-regulation of lignin biosynthesis pathway enzymes is a promising strategy to increase the efficiency of bio-ethanol production from hemicellulosic sugarcane residues. Therefore, the objective of this study is to reduce lignin content in sugarcane by altering 4-coumarate-CoA ligase (4CL), a key enzyme in lignin biosynthesis. Two 4CL partial sequences were isolated from the genome of sugarcane. Two RNAi constructs targeting a conserved region in the two genes were constructed using 200 bp from each of the two genes. One or both Sc4CL-RNAi constructs, under the control of the xylem specific OsC4H promoter, were introduced into sugarcane callus along with a selectable nptII expression cassette by biolistic gene transfer. Following selection on medium containing geneticin and regeneration, 49 independent transgenic lines were generated. The transgenic nature of these lines was confirmed using NPTII ELISA analysis. Data describing the level of 4CL suppression and its effect on lignin content will be presented.

P-2061

Developing Sugarcane as a Viable Biofactory for High Value Proteins. M. B. DAMAJ[1], J. J. Molina[1], M. T. Buenrostro-Nava[1], H. Park-Kang[1], D. Rossi[1], D. Odokonyero[1], J. L. Jifon[2], Z. Nikolov[3], S. White[3], and T. E. Mirkov[1]. [1]Department of Plant Pathology and Microbiology, Texas AgriLife Research, Weslaco, TX 78596; [2]Department of Horticulture, Texas AgriLife Research, Weslaco, TX 78596; and [3]Department of Agricultural and Biological Engineering, Texas A&M University, College Station, TX 77843. Email: mbdamaj@ag.tamu.edu

Sugarcane (*Saccharum spp.*) has a great potential for the production of protein-based therapeutics. It has a fast growth cycle and an efficient carbon fixation pathway, produces a large biomass, and offers the prospect of inexpensive biopharmaceutical production. A specific goal of this project is to develop sugarcane as a recombinant expression system for the production of a mammalian enzyme that has a broad-spectrum antimicrobial activity and a potential use in food, cosmetics and agriculture. Expression of this mammalian gene was enhanced in sugarcane by modulating transcription, transcript stability and translation. Expression vectors were generated using a synthetic gene that was codon optimized for expression in a monocot system. A single promoter as well as a multiple promoter system was used to drive expression. The 5′ and 3′ untranslated regions of a virus that infects sugarcane were fused to the coding region of the gene to enhance translation. Embryogenic calli and leaf rolls of two commercial sugarcane varieties were transformed biolistically, and the phosphinothricin acetyl transferase (BAR) gene was used as a selectable marker. Immunoblot analysis

as well as enzymatic activity assays of stably transformed sugarcane plants revealed the presence of an intact mammalian enzyme that accumulated at levels as high as 0.36 mg/kg in stalks of plants expressing it from a single promoter vector, and up to 0.60 mg/kg in stalks transgenic for co-expression from three different promoters in separate vectors. Each vector did not adversely affect the others as shown by copy number, steady-sate mRNA levels and the presence of the functional enzyme. These results suggest that transcriptional synergism resulted through additive promoter activities and increased gene expression. A growth cycle study for an 11-month period showed a substantial increase in enzyme accumulation over time in the transgenic lines. This study suggests the commercial feasibility of producing a stable recombinant enzyme in transgenic sugarcane, and developing sugarcane as a biofactory for high value proteins.

P-2062

Devitalized Reference Seeds as Reliable Standards for Biotech Events. J. Seurinck, J. Aelvoet, H. Ronse, D. De Wispelaere, S. Haelterman, R. SHILLITO, E. Bates, and E. van der Biezen. BioAnalytics Dept., Bayer CropScience, BioScience N.V. Technologiepark 38, B-9052 Gent, BELGIUM. Email: jan.aelvoet@bayercropscience.com

Certified reference materials (CRM) are produced according to international standards and are available for commercial Biotech events. Reference materials are used for the development and validation of analytical detection methods, for use as controls in the application of detection methods, for the calibration of analytical equipment, for use in laboratory proficiency testing, and are provided for regulatory purposes to government agencies. In the framework of stewardship policies for containment and confinement measures of Biotech seeds, protocols for the production of reference seeds impaired in germination capacity were developed. This seed devitalization project aimed to realize a procedure that prevents seed germination, thereby eliminating seed propagation while retaining the seed detectability by DNA-based analytical methodologies. Performance requirements were defined as quality standards for the production of devitalized reference seeds; the criterion was defined as less than 0.1% germination (95% confidence level). The DNA detectability criteria were determined at qualitative detection of more than 95% of individually tested seeds (95% confidence level), and in addition, accurate quantitation in devitalized seed bulks using real-time PCR. This paper presents the results of technical approaches applying heat treatments under defined durations and conditions, and the subsequent germination and detectability analysis. These standardized protocols are being used for production of devitalized corn, cotton, canola, soybean, and rice reference seeds.

P-2063

Generation of Large-scale Insertion Mutants in *Medicago truncatula* Using the *Tnt1* Retrotransposon. H. K. LEE[1], M. Tadege[1], J. Wen[1], J. He[1], M. Hartwell[1], J. Gallaway[1], D. Vanthana[1], X. Cheng[1], M. Wang[1], C. Pislariu[1], J. Murray[1], P. Zhao[1], M. Udvardi[1], P. Ratet[2], and K. Mysore[1]. [1]Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK 73401 and [2]Institute des Sciences du Vegetal, CNRS, 91198 Gif sur Yvette Cedex, FRANCE. Email: hklee@noble.org

Retrotransposons are retrovirus like elements, which encode functions required for their own replication and transposition. Retrotransposons can be activated to transpose in tissue culture, can transpose long distances and show preference for gene rich regions. We are using tobacco retrotransposon *Tnt1* to mutagenize and tag the whole genome of model legume *Medicago truncatula*. We show that *Tnt1* is very active and transpose into, on average, 25 different locations during *M. truncatula* tissue culture. Mutations induced by *Tnt1* insertion are stable during seed- to-seed generation. We have generated over 10,000 independent *Tnt1*-containing lines encompassing approximately 250,000 insertion events. Over 11,000 *Tnt1* flanking sequence tags (FSTs) have been recovered. We have pooled genomic DNA from 9,500 lines for customized reverse-genetic screening, and the frequency of insert identification in this pool for average-sized-gene is approximately 90 percent. The range and diversity of mutant phenotypes suggest that *M. truncatula* offers a great opportunity to dissect symbiotic and developmental pathways for a comprehensive understanding of legume biology.

P-2064

Wild Relatives of Wheat as Sources of Useful Genes. N. HOVHANNISYAN[1], M. E. Dulloo[2], A. Yesayan[1], M. S. Röder [3], H. Knüpffer [3], A. Amri[4], and A. M. Danielian [5]. [1]Alex Manoogian St., No 1, Dept. of Ecology and Nature Protection, Yerevan State University, 0025, ARMENIA; [2]Via dei Tre Denari St., No 472a, Bioversity International, 00057, ITALY; [3]Correns St., No 3, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), 06466, GERMANY; [4]Tel Hadya St., International Center for Agricultural Research in the Dry Areas (ICARDA), 5466, SYRIAN ARAB REPUBLIC; and [5] Charents St., No 45, UNEP/GEF "In-situ Conservation of Crop Wild Relatives Through Enhanced Information Management and Field Application" Project, ARMENIA. Email: bionellibiotech @yahoo.com

Crop wild relatives, represent a source of useful genes for improvement of wheat to meet the food needs of the present and future generations. In the past, the majority of the information on plant genetic resources was based on phenotype, geographic origin, social history and parentage. The development of DNA markers has provided new powerful ways of assessing genetic relationships and diversity, identification of useful genes in crop wild relatives. The aim of this work was to investigate the presence of some genes for resistance to *Puccinia recondita* (*Lr28*) and *Erysiphe graminis* (*Pm1*) in *Triticum boeoticum* and *T. urartu* accessions collected from Armenia and acquired from the gene banks at ICARDA and IPK, by using PCR-STS marker sets. DNA from young seedlings was extracted by application modified by us CTAB method. STS marker sets for *Pm1* gene (Hu et al, 1997) and *Lr28* gene (Naik et al.,1998) were used. As a positive control hexaploid cultivar "Bezostaja 1" with location of *Pm1* gene on 7A chromosome, and *Lr28* gene on 2A chromosome was used. Products corresponding to two STS markers of resistance gene were amplified successfully from DNA of studied accessions of *T. boeoticum* and *T. urartu.* The sizes of marker fragments were: *Pm*1–420 bp., *Lr28*–380 bp. The *Pm1* marker of resistance gene to powdery mildew was found in all studied accessions. Markers for gene *Lr28* was identified in four accessions, of the 11 tested of *T. urartu,* but not in *T. boeoticum*. The study showed that accessions of *T. boeoticum* and *T. urartu* can contribute useful sources of genes for disease resistance which will contribute to prevent wheat production losses and improve livelihood and food security of people. Acknowledgements. The study was supported by the IFAR Ravi Tadvalkar Memorial Scholarship program and UNEP GEF Crop Wild Relatives Project in Armenia.

P-2065

A Novel Receptor Like Kinase Plays a Role in Root Architecture in *Arabidopsis*. H. NAJAFI ZARRINI[1, 2], J. T. M. Elzenga[1], and F. C. Lanfermeijer[1]. [1]Laboratory of Plant Physiology, CEES, University of Groningen, NETHERLANDS and [2]Plant Breeding Department, Sari University of Agricultural Sciences and Natural Resources, IRAN. Email: H.Najafi.Zarrini@rug.nl

Receptors like Kinases (RLKs) genes constitute a large family in *Arabidopsis* (approximately 600 genes). However, the function of most of them is unknown. Based on the known function of the few that are characterized, it can be speculated that they play important roles in plant development, morphology or (a)biotic stress resistance. Therefore, it is important to assign a function to the unknown RLKs. This undertaking is complicated because 1) possible redundancy, 2) the absence of clear phenotypes in mutants, either as a result of the wrong physiological conditions chosen or of the scale at which the phenotype is visible. Recently we identified a large number of RLKs of the *Arabidopsis* leave plasma membrane using an optimized proteomic approach. For many of those identified RLKs, there is no functional annotation. In this study, a reverse genetic approach was used to test ten high ranking identified RLKs under normal growth condition and a range of abiotic stress in order to assign them a role. In addition, available gene expression data was analyzed. Of the studied RLKs, one RLK's T-DNA insertion mutant showed a clear phenotype. This phenotype is typified by primary root length difference in a later stage. The reduction of root length is exaggerated under osmotic stress. We present data on the phenotype of this mutant and a second T-DNA mutant. Additionally, gene expression analysis was used to define a role of this RLK. The obtained data indicate that this RLK plays a role in the root elongation zone.

P-2066

Characterization of Sugarcane 4-Coumarate-CoA Ligase (4CL) and Caffeic Acid 3-O-Methyltransferase (COMT). J. H. JUNG, J. Y. Kim, W. M. Fouad, W. Vermerris, M. Gallo, and F. Altpeter. Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida – IFAS, Gainesville FL-32611. Email: altpeter@ufl.edu

Sugarcane is the highest yielding biomass producer. Typically, farmers reduce the sugarcane post-harvest leaf residue by open air burning, which negatively impacts air quality. Fuel grade ethanol can be made from sugarcane leaf litter residue following acid hydrolysis pre-treatments to remove lignin which acts as a physical barrier to enzyme hydrolysis. Thus, down-regulation of lignin biosynthesis pathway enzymes is a promising strategy to increase the efficiency of bio-ethanol production from hemicellulosic sugarcane residues. In the lignin pathway, 4-coumarate-CoA ligase (4CL) and Caffeic acid 3-O-methyltransferase (COMT) are key enzymes that catalyze the formation of CoA thiol esters of 4-coumarate and other hydroxycinnamates and the methylation of 5-hydroxyconiferaldehyde to sinapaldehyde, respectively. However, sugarcane has a complex polypoid genome and these genes belong to a large gene family. Their broad substrate specificities have made it difficult to identify orthologs that are specifically involved in lignin biosynthesis. We have isolated two 4CL genes, Sc4CL-M and Sc4CL-L, and two COMT genes, ScCOMT1 and ScCOMT2 from a commercially important

sugarcane cultivar by a PCR-based strategy. Results from RT-PCR expression analysis in different tissues including leaves, internodes, nodes, and roots, will be presented. Since substrate specificities of COMTs cannot be accurately predicted on the basis of sequence similarity, in vitro substrate preferences are currently being determined from purified enzymes.

P-2067

Transitory Polymorphisms Are Observed in Cryopreserved Rubus Shoot Tips Using AFLP Markers. B. M. REED[2], N. R. F. Castillo[1], S. Wada[1], and N. V. Bassil[2], [1]Department of Horticulture, Oregon State University, Corvallis, OR and [2]USDA-ARS National Clonal Germplasm Repository, 33447 Peoria Road, Corvallis, OR 97333–2521. Email: Barbara.Reed@ars.usda.gov

Questions often arise concerning the genetic stability of plant materials stored in liquid nitrogen for long time periods. This study followed the genetic stability of cryopreserved shoot tips of *Rubus* germplasm that were stored in liquid nitrogen for over 12 years, then rewarmed and regrown. We analyzed the genetic stability of *R. grabowskii*, two blackberry cultivars ('Hillemeyer' and 'Silvan') and one raspberry cultivar ('Mandarin') after regrowth in vitro, transplanting to the greenhouse and transfer to the field. No phenotypic differences were observed in greenhouse-grown cryopreserved plants when compared to the control mother plants. In the field, cryopreserved plants were more vigorous than mother plants as is common for plants derived in vitro. SSR and AFLP analyses were performed on shoots immediately after recovery from cryopreservation and on shoots subcultured for seven months before analysis. No SSR polymorphisms were observed between cryopreserved shoots and the corresponding mother plants irrespective of subculture. AFLP revealed polymorphisms in three of the four *Rubus* genotypes subcultured for seven months while no polymorphism was detected in those analyzed immediately after recovery from cryopreservation. Greenhouse and field-grown plants from the polymorphic shoot tips of *R. grabowskii* and 'Silvan' displayed the same AFLP fingerprints as their corresponding mother plants. Only long-cultured in vitro shoot tips displayed polymorphisms and they were no longer detected when the plants were grown ex vitro. The transitory nature of these polymorphisms should be carefully considered when monitoring for genetic stability.

P-2068

Using of Molecular Markers for Genetic Characterization of Some Local Populations of *Phytophthora infestans*. C. BOTEZ, D. Pamfil, A. Taoutaou, and Erika Balazs. Manastur St., No. 3–5, Dept. of Biotechnology, Cluj-Napoca University of Agricultural Sciences and Veterinary Medicine, 400372, ROMANIA. Email: constantinbotez@yahoo.com

Revealing the genetic variability of local populations of *Pytophthora infestans* is a crucial step for the development of plant breeding programs for resistance to this pathogen as well as for an efficient potato late blight control. At the moment it is difficult to perform an accurate identification of intraspecific variation of *P.infestans* by morphological features. RAPD molecular markers could be beneficial for revealing genetic variation in this species. In spite of its simplicity, the method has several limits. On account of these limits we followed three main objectives: increasing of molecular polymorphism in order to better characterize different Romanian *P.infestans* accessions, conversion of RAPD markers into SCAR markers in order to increase the identification efficiency of *P. infestans* in the infected material and correlating of molecular analysis with reaction of *P.infestans* accessions to fungicide (Metalaxyl) and with the mating type. Specific primers (ITS3-PISP, SSR primers like Pi56, and primers for mitochondrial DNA, like H4) did not give any monolocus molecular polymorphism among the analysed *P.infestans* accessions. RAPD primers gave a significant polylocus molecular poplymorphism in relation with the used primers and with restriction enzyme used for digestion of RAPD amplification products. Some RAPD amplification products were converted into SCAR markers. Secondary molecular polymorphism, based on digestion of mitochondrial amplification products with Eco RI restriction enzyme, has differentiated one accession as Ia haplotype. The others belong to IIa haplotype. The mating type of *P.infestans* accessions, determined by PCR, was correlated with reaction to Metalaxyl and molecular polymorphism. The possibility to characterize, at molecular level, different *P.infestans* accessions which have specific reaction to Metalaxyl and specific mating type, could be very useful for potato resistance to *P. infestans* breeding program and from practical point of view.

P-2069

Effect of 6-Benzylamino purine on the In Vitro Germination of *Orchis italica* Poiret and *Orchis punctulata* Steven ex Lindl (Orchidaceae). K. A. HAMDAN[1], and S. N. Talhouk[2]. [1]Simon Fraser University, Department of Geography, Burnaby, British Columbia, CANADA, V5A 1S6 and [2]American University of Beirut, Faculty of Agricultural and Food Sciences and IBSAR, Nature Conservation Center for Sustainable Futures. Beirut, LEBANON, PO Box 11–0236, Riad El Solh 1107–2020. Email: kah13@sfu.ca

Benzylamino purine (BA), also known as Benzyladenine, is a cytokinin that was reported to enhance the in vitro seed germination of several terrestrial orchids. The Effect of 6-Benzylamino purine (BA) was tested in vitro on native Lebanese terrestrial orchids *Orchis italica* Poiret. and *Orchis punctulata* Steven ex Lindl. Two media were used, terrestrial orchid medium-1 (BM-1, does not contain BA used as a control), and terrestrial orchid medium-2 (BM-2, containing BA). Ten weeks following seed inoculation in the two media, 13.1% (±2.3) and 2.0% (±1.4) of *O. italica* seeds germinated in BM-2 and BM-1 respectively. These showed a significant difference according to *Student t*-test. Surprisingly, *O. punctulata* showed a very similar germination percentage of 17.3% and 17.00% on BM-2 and BM-1 respectively. These findings suggest that BA may play a role in the initiation of seed germination of *O. italica* but has no influence on the initiation of seed germination of *O. punctulata.* This conforms with the notion of differential response of orchid species to cytokinins, and supports the fact that the response to exogenous hormones vary from genus to genus and even from species to species.

P-2070

Antioxidative Metabolism Response Induced by PPV (Plum Pox Virus) in Micropropagated Plantlets of Peach GF305: Effect of Treatments with BTH and OTC. A. PIQUERAS, P. Díaz-Vivancos, J. A. Hernández, and M. J. Clemenente-Moreno. Dept. of Tree Breeding, Biotechnology Group, CEBAS-CSIC, PO Box 164,30100 Espinardo-Murcia, SPAIN. Email: piqueras@cebas.csic.es

For the present work micropropagated shoots cultured were established from peach GF305 plants control and infected with Plum pox virus (PPV, Sharka), this cultivar is highly susceptible to PPV infection and has been widely used as a model host for virological studies with PPV. This plant material has been used to study the effect of the application of BTH (benzothidiazole) and OTC (1,2-oxothiazolidine-4-carboxilic acid on PPV infection and antioxidant metabolism. Both BTH and OTC stimulated shoot development in micropropagated shoots with a slightly higher effect for BTH. Infected non treated plants presented reduced levels of glutathione (GSH), almost one third, compared to control uninfected plants. In micropropagated infected shoots the treatments with 10 and 20 μm OTC raised GSH levels and a negative correlation could be observed between GSH and the level of PPV infection. The infected cultures also showed increased activities of APX (Ascorbate peroxidase), GST (Gluthathione-S-transferase) and SOD (superoxide dismutase) compared to controls. In control cultures BTH treatments reduced APX and CAT (Catalase) activities but increased GST and G6PDH (Glucose-6-phosphate dehydrogenase) activities while under OTC treatments a reduced activity of MDHAR (Monodehydroascorbic acid reductase), GR (Gluthathione reductase), CAT and increase of DHAR and GST were detected. The application of BTH to the infected cultures decreased the activity of APX, MDHAR, GST, SOD, and CAT, with an increased DHAR and G6PDH activities compared to infected untreated cultures. However, the same material treated with OTC brought about a decrease in APX, DHAR, SOD and GR with an increase in GST activity at the higher concentration (20 μ) compared to untreated infected cultures. In the infected cultures, the application of BTH and OTC could favor the accumulation of H_2O_2 at the sub cellular level unbalancing the internal redox state towards an activation of the antioxidant system able to modulate the response to the infection by PPV.

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Optimizing the Expression of *Eisemia fetida* Lumbrokinases in Alfalfa (*Medicago sativa*) Plant. Zhaorong Wei[1], Gangqiang Li[1], Pei-Lan Tsou[2], Dehu Liu[1], and YUEJU WANG[3]. [1]Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, PR CHINA; [2] Cell and Molecular Biology Department, Grand Valley State University, Allendale, MI 49401; and [3] Biology Department, Macon State College, Macon, GA 31206. Email: yueju.wang@maconstate.edu

Lumbrokinase is a fibrinolytic enzymes that could be a potential treatment for thrombosis. In this study, two of lumbrokinases were transferred to alfalfa. The lumbrokinases cDNA were obtained from *Eisemia fetida* by RT-PCR. In order to increase expression level of the gene in alfalfa, the 5′ and 3′ untranslated region of the alfalfa mosaic virus (AMV) coat protein mRNA were fused to the two ends of the lumbrokinases genes respectively. CaMV 35S promoter was used for genes expression. 10 μg/ml kanamycin was used for screening transformants. Timentin was used to inhibit *Agrobacteria* growth in alfalfa transformation. Transformation frequency can be as high as 30%. PCR and Southern blot analysis showed that the lumbrokinase genes were successfully transferred into alfalfa plants. Western blot and fibrin assay showed lumbrokinase expression in transgenic alfalfa plants but at a low level. We plan to further optimize the construct in order to achieve higher production levels of lumbrokinase in alfalfa plant.