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The following abstracts will be included in an upcoming issue of *In Vitro Cellular and Developmental Biology*:

P-3000

Influence of Added Nutrients in the Fermentation Process of Sugar Cane and Sweet Sorghum Varieties for Ethanol Concentration. L. PARKS. Tuskegee University, 808-B Phillips Dr., Tuskegee, AL 36088. Email: lparks3606@mytu.tuskegee.edu

The juice from five varieties of sweet sorghum, (MSIE, Della, Dale, Sugar Drip, and K. N. Morris) and three varieties of sugar cane, (TU Green, TU White, and TU Blue) were used in an experiment to determine the effect of the addition of nutrients during fermentation for ethanol yield. Juice from the two species was divided into two 200 ml aliquots with or without nutrient addition. Nutrients added consist of yeast extract, ammonium sulfate, magnesium sulfate, and sodium hypo-phosphate. Samples were autoclaved at 105°C for 15 minutes and rapidly cooled under fast running tap water to 30°C. The pH was checked and adjusted to 4.5 by adding 1M HNO_3 after which the nutrients were added. One (1g) yeast (*Saccharomyces cerevisiae*) was added and the samples were then fermented in a shaker bath at 100 rpm and 30°C for 96 hours. After fermentation, samples were centrifuged at 3000 rpm for 10 minutes for HPLC analysis. For sweet sorghum °brix in varieties ranged from 16.7% for MSIE to 21.9% for K.N. Morris). For sugar cane °brix ranged from 11% for TU White to 15.7 % for TU Green. Ethanol yield for sweet sorghum without added nutrients ranged from 16.7% to 28.6 % and with nutrients added, ranged from 11.6% to 33.3%. Samples from sugar cane are still being analyzed. These preliminary results suggest that nutrient addition enhances ethanol yield, and that there were differences among varieties. Research sponsored by USDA Evans- Allen, Auburn University- TU NSF Bioenergy Project, TU-GWCAES.

P-3001

Contamination Control in Yam (*Dioscorea Spp*) Tissue Cultures: Genotype Variations in Response to Disinfection Regime Indicates Different Endophytic Profiles. M. OLORUNTOYIN. BALOGUN[1,2], J. Augusto[2], and N. Maroya[2]. [1]Genetics Society of Nigeria and [2]International Institute of Tropical Agriculture PMB 5320, Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, 200216, NIGERIA. Email: kemtoy2003@yahoo.com

The production of yams, which are staples in West Africa is constrained by its relatively slow rate of vegetative propagation (1:6 per year compared to 1:300 in some cereals). Vegetative propagation has also caused a build up diseases, reported to cause up to 90% yield reduction due to scarcity of quality declared seeds. Plant regeneration from meristems takes 6 months to 2 years in yam. When rapidity is critical, nodal propagation is preferred but it is associated with high incidence of contamination, apparently clean plantlets showing contaminants in subsequent cycles. We tested the incidence of culture contamination and survival in 6 batches of collections from glasshouse-grown mother plants of 5 genotypes nested within 3 disinfection regimes: 1: 2% and 1 % sodium hypocloride (NaOCl) for 15 and 30 minutes respectively; 2: dipping in karate (lambda-cyhalothrin) + Team (63% mancozeb, 12% carbendazim) mixture, air exposure for 45 minutes before NaOCl; 3: dipping in (lambda-cyhalothrin) + Team (63% mancozeb, 12% carbendazim) mixture, exposing to clean air for 45 minutes, rinsing off followed by NaOCl. Nodes were cultured in Murashige and Skoog medium. After 2 weeks, cultures were scored for percentage contamination and survival. Microbes on contaminated plantlets were identified. The frequency of contamination varied significantly among genotypes within disinfection regime and batches, while percentage survival differed significantly for all the factors. Mean percentage contamination were 81.8, 10.0 and 8.0 respectively for regimes 1,2, 3 and 21.1, 46.7 and 62.1 for % survival. Regime 3 was optimum for TDa 98/01176, TDr 85/18544 and TDr Mecakusa while regime 2 was optiumum for TDr Pepa. The fungi were genotype specific, including *Cladosporium*, *Amerosporium*, *Verticillium* and others yet to be identified. Work is ongoing to identify the bacteria in some of the apparently clean plantlets. It will be useful to determine the endophytic profile of priority yam genotypes in a specific environment when planning to use nodal explants for rapid propagation.

P-3002

Developing Peach Orchard and Continuing *In Vitro* Studies for PTSL Evaluation. B. BISWAS[1], M. McKinney[1], T. Beckman[3], and S. Johnson[2]. [1]College of Agriculture and [2]MS Biotechnology Program, Fort Valley State University, 1005 State University Drive, Fort Valley, GA 31030 and [3]USDA-ARS, Byron, GA. Email: biswasb@fvsu.edu

Peach (*Prunus persica* (L) Batsch) is an important fruit crop for Georgia farmers. Tree survival and orchard longevity is a major problem for peach production. A combination of factors including *Armillaria* root rot, soil nematodes, peach tree borer, and cold stress reduce orchard longevity. The sudden collapse of peach trees in late spring is also known due to PTSL (peach tree short life) syndrome. This has been a major problem in the southeastern peach growing areas, more specifically in Georgia. This investigation was taken up to

screen locally grown peach tree variety and rootstocks for in vitro study to develop protocols for biotic and abiotic stress tolerant peach. Peach is a very recalcitrant species for in vitro responses. There is a very limited success found in literature on peach. Woody plant tissue culture is known for its slow response as well. We have enough success on various woody fruit tree tissue culture therefore we initiated our present study on peach in April 2012. Our goals were to find out suitable medium for in vitro culture of peach tissues for enough callus production, organogenesis and somatic embryogenesis. We have been successful for initial development of somatic embryogenesis from peach nucellus tissues, and we have also found adequate callus production from various peach somatic cells and tissues that will be used for protoplast fusion research to make somatic cybrid production. We have selected and used twelve different varieties and root-stocks in this study. We will be presenting our findings in this presentation.

P-3003

Characterization of *Fraser photinia* Associated Endophytic Plant Growth-promoting Bacteria Isolated from *in Vitro* Culture. M. DIVYAPICIGIL, Ö. Akkaya, M. Gül Şeker, H. 1 Akdemir, E. Kaya, E. Kırdök, and Y. Özden Çiftçi. [1]Gebze Institute of Technology, Istanbul Str., Istanbul, 41400, TURKEY. Email: mdivyapicigil@gmail.com

Evergreen Fraser photinia (*Photinia* X *fraser* Dress.) which has an impressive appearance with its bright-green leaves is a woody ornamental plant that has commercial significance. Microshoots of fraser photinia were maintained actively proliferating for several years and its shoot apices were routinely used for the optimization of medium and long-term conservation techniques. Generally, the presence of endophytic bacteria in plant tissue cultures is considered as contamination; however, a growth promoting endophytic bacteria from microshoots of fraser photinia has been identified during the tissue culture studies of this plant in Gebze Institute of Technology, Plant Biotechnology Laboratory. When the sequence analysis of 16S rDNA genes of isolated bacteria was compared with sequences previously entered to the National Center of Biotechnology Information (NCBI), it showed similarity to uncultured bacteria and proteobacteria, however, this similarity was not very high. Microbiological and biochemical characterization and application of preliminary molecular analysis were aimed in this project proposal to describe the endophytic bacteria in more detail and to determine its characteristics. For this purpose, the presence of various opine synthase genes and virD1 gene was determined in order to identify possible reasons for the positive effect of bacteria on the growth of plant microshoots. Moreover, the effects of endophytic bacteria on long-term *in vitro* conservation, rooting and acclimatization to greenhouse conditions of Fraser photinia were also be assessed and data were statistically evaluated.

P-3004

Engineering Designer Polymer as a Molecular Carrier for a Thermostable Endo-arabinase Expressed *in Planta*. C. ELMS, N. Zhang, B. Savary, and J. Xu. Arkansas Biosciences Institute, Arkansas State University, 504 University Loop East, Jonesboro, AR 72401. Email: christop.elms@smail.astate.edu

The *long term* goal of this project is to engineer emerging "energy beets" with *in planta* expressed glycosyl hydrolases for either *in vivo* or *post-harvest* modification of cell wall polysaccharides, facilitating the production of industrial sugars and recovery of functional oligosaccharides. Several varieties of "sugar beets" (*Beta vulgaris* L.), while being a main table sugar resource, are also being explored as alternative bioenergy crops. In order to make the use of sugar beets in biofuel production economically feasible, an effective method of recovering value-added products from the beet pulp byproduct needs to be developed. Functional oligosaccharides, such as feruloylated arabino-oligosaccharides (FAOs) can be extracted from the beet pulp with hydrolytic enzyme processing. FAOs may be used in food and feed applications for healthful colon functioning as implicated by prebiotic, anti-inflammatory and mucosal immunomodulatory activities. Our objective is to utilize an innovative strategy – hydroxyproline-*O*-glycosylation − to engineer novel "designer" polymer tags that can function as a "molecular carrier" for *in planta*-expressed glycosyl hydrolase. This will maximize enzyme function for post-harvest hydrolyzation of beet pulp polysaccharides. The current project aims to provide a proof of concept by engineering, in tobacco plant, a designer polymer consisting of 18 tandem repeats of a "Ser-Pro-Pro-Pro-Pro" motif and attaching this polymer to a thermostable enzyme- 1,4- endo-arabinase (ABN)- that can selectively cleave the arabinan chain of beet pulp polysaccharides to release FAOs. I predict that the designer polymer will help direct the accumulation of ABN in the plant cell wall matrix and stabilize ABN from proteolytic degradation.

P-3005

Cloning of the Caffeoyl Coenzyme a 3-*o*-Methyltransferase Gene from Egyptian Clover (*Trifolium Alexandrinum*). W. FOUAD, C. Ouma, and S. El-Shafie. The American University in Cairo, Egypt, AUC Avenue, P.O. Box 74, SSE Building, New Cairo, 11835, EGYPT. Email: wfouad@aucegypt.edu

The clover genus *Trifolium* has about 250 species, of which 20 species are used as forage plants in commercial agriculture worldwide. The most important of these species are white

clover (*T. repens*), red clover (*T. pratense*) and berseem clover (*T. alexandrinum*), Also known as, Egyptian clover, *T. alexandrinum* is an annual pasture legume that originated in the eastern Mediterranean region. Egyptian clover is an important winter-season annual forage legume cultivated in the Egyptian, Mediterranean basin, Indian subcontinent, South Africa, Australia and USA. The high value of the Egyptian clover as a forage crop is attributable to the long duration of green forage, its high green forage yield, good forage quality and high palatability. However, as the case in many other forage crops, lignin content significantly reduces its forage quality by limiting its digestibility. Genes encoding enzymes involved in lignin biosynthetic pathway have been identified, cloned and functionally characterized form several forage crops. However, not a single gene has been characterized form Egyptian clover. A key enzyme in the lignin biosynthetic pathway is caffeoyl Coenzyme A 3-*o*-methyltransferase (CCOMT). Degenerate primers based on CCOMT published protein sequences were used to specifically amplify partial sequences of *CCOMT* form Egyptian clover cDNA as confirmed by nested primer amplification of the initial PCR product and sequencing. The full-length cDNA cloning of the *TaCCOMT* was achieved using the 5' and 3' RACE kit. This report describes the cloning of CCOMT form Egyptian clover and its tissue specific expression.

P-3006

Comparative Analysis of PSII Transcript Expression as Harvest Index Indicator in Crop Under Different N Source. O. IDEHEN[1], L. Milad[2], S. Samuels[1], G. Benard[1], M. Egnin[1], D. Mortley[1], C. Bonsi[1], C. Lee[1], I. Ritte[1], and A. Grady[1]. [1]Tuskegee University, 104 Milbank Hall, Tuskegee Institute, AL 36088 and [2]University of Alabama, Tuscaloosa, AL. Email: megnin@mytu.tuskegee.edu

Photosynthetic activities in crop canopies are directly correlated to the source-sink potential of storage organs in plants. Photosynthetic activities can be used to measure plant development, which is influenced by available plant nutrient. In this experiment, we analyzed the harvest yield index of bell peppers, tomatoes, and sweetpotatoe grown in soils treated with different N fertilizers through direct analyses of the metabolic interaction between photosynthetic activities and nutrient input. Plants were subjected to 4 different treatments including: no fertilizer (P1), broiler liter (P2), conventional fertilizer (P4), and fish compost (P12). Total RNA was isolated from leaves collected from all treatments. Photosynthetic activities were compared between treatments by qRT-PCR utilizing the photosystem II (*PSII*) transcript as the harvest index. Evaluation of the genetic profiling analysis showed that plants treated with P12 demonstrated a CT value of 15.905 which was closest to the control *PSII* DNA (9.6), and lowest among all samples. There was steady level expression of housekeeping gene *b-actin*, as internal reference, in all the samples indicating high quality of mRNA extracted from all plant samples. The highest expression of PSII mRNA in both tomato and sweetpotato was in plants grown in soil with fish compost treatment. These results suggest a more prolific accumulation of *PSII* mRNA in plants cultivated in the fish compost amended soil in comparison to other fertilizer treatments. Results from this study indicate an increase in harvest index due to application of fish compost. Research supported by USDA-Evans-ALLEN, George Washington Carver Experiment Station, Plant Biotech and Genetics Research Lab, College of Agriculture Environmental Science and Nutrition Science, Tuskegee University, Tuskegee, AL.

P-3007

Skullcaps: Lesser Known Medicinal Genus from Lamiaceae. N. JOSHEE[1], P. Parajuli[2], and A. Rimando[3]. [1]Fort Valley State University, 1005 State University Dr, Fort Valley, GA 31030; [2]Wayne State University, Detroit, MI; and [3]USDA-ARS, Natural Products Utilization Research Unit, University, MS. Email: Josheen@fvsu.edu

Plants of the genus *Scutellaria* (common name skullcap or scullcap, Family Lamiaceae) are a part of Eastern as well as traditional American medicine. Skullcap is a North American perennial plant from genus *Scutellaria*. The genus is widespread in Northern Hemisphere represented by close to 400 species. We have a germplasm collection of twenty Scutellaria species at Fort Valley State University and the populations are maintained in the greenhouse and through micropropagation. Many species are rare, threatened, or endangered due to habitat destruction, urbanization, and poor seed set. Many skullcaps have showy, beautiful blooms with great potential as ornamental plants. Skullcap are used in alternative medicine as anti-inflammatory, antispasmodic, emmenagogue, nervine, sedative and strong tonic. Most of the research on the extracted pharmacological compounds has been on *S. baicalensis*, *S. lateriflora*, and *S. barbata*. We have made significant headway in the areas of micropropagation, transformation for desired gene transfer, extraction and HPLC analysis of targeted flavonoids, and clinical role of select flavonoids using glioma cell lines.

P-3008

Suppression of Lignin Biosynthesis in Sugarcane Following Intragenic Precision Breeding. J. JUNG, H. Dermawan, and F. Altpeter. University of Florida, 3085 McCarty Hall D, Gainesville, FL 32611. Email: jehyeong@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 bioethanol. Biofuel production from the abundant lignocellulosic sugarcane residues is expected to not only

improve the production rate per unit land area, but also promote green cane harvesting and minimize the open air burning of sugarcane leaf litter. However, bioconversion of lignocellulosic biomass to biofuel is highly limited due to the presence of lignin in plant cell walls. Down-regulation of lignin biosynthetic enzymes has been proven to be a promising strategy to increase the efficiency of bioconversion from lignocellulosic biomass. In the lignin biosynthetic pathway, 4-coumarate-CoA ligase (4CL) is one of the key enzymes, which catalyzes the formation of CoA thiol esters of 4-coumarate. In this study, 4CL gene was isolated and suppressed to reduce lignin content in sugarcane. In order to facilitate regulatory approval an intragenic approach was used to suppress 4CL. The entire DNA expression cassettes that were used for the generation of the lignin reduced sugarcane were derived from sugarcane and/or sexually compatible sorghum. A total of 60 intragenic sugarcane lines were generated suppressing the 4CL gene up to 97%. Intragenic sugarcane lines with high level of 4CL suppression exhibited brown coloration in the basal internodes and vascular bundle cells. Data describing the total lignin content in the stem biomass will be described.

P-3009

Genetic Transformation of Elite Cotton (*Gossypium hirsutum* L.) Cultivar for CLCuD Resistance. S. KHATOON and N. Sarin. JNU, M68, M Block, Abul Fazl Enclave, New Delhi, 110025, INDIA. Email: sameenahussain29@gmail.com

Transgenic cotton resistant against devastating cotton leaf curl disease (CLCuD) using RNAi- mediated technology were developed in *Gossypium* cultivars. CLCuD caused by CLCuV, a begomovirus, is transmitted by whitefly (*Bemisia tabaci*) vector. A binary vector pCAMBIA 2301 carrying IRRNAi gene construct was used. A very rapid and efficient protocol for *Agrobacterium*- mediated genetic transformation in an elite Indian genotype of cotton cv. Narsimha and Coker was developed. Hypocotyls and nodal explants were used for transforming with the RNAi cassette, and planted on selection medium containing kanamycin. The regenerated explants were further transferred to rooting medium, hardened and transferred to glass house. The putative transgenic plants were screened by PCR and the integration of the gene and its copy no. in genome was confirmed by Southern blot analysis. They were further screened for virus resistance following inoculation with viruliferous whiteflies.

P-3010

Insights into the Transient Leaf Expression of the Expression the Recalcitrant Cucumber Alpha-expansin Protein. T. SL. LAI[1], S. Florez[2], D. Durachko[3], D. Cosgrove[4], and W. Curtis[5]. Penn State University, [1]Wayne Curtis Lab and [2]Chemical Engineering Dept, 229 Fenske Laboratory University Park, PA 16802; [3]Dan Cosgrove Lab, 357 N. Frear and [4]Dept of Biology, 351 N Frear Lab, University Park, PA 16801; and [5]Dept of Chem Engineering, 226B Fenske Laboratory University Park, PA 16802. Email: tina.lai@curtislab.org

Expansins are an important family of plant proteins that are involved in many vital developmental processes such as cell growth, fruit softening, leaf abscission, root hair development, etc. This ubiquitous protein was discovered in 1993 where characterization is limited because native expression is low and purification has proven difficult. This is particularly true for the class of alpha expansins which have minimal expression and/or activity in platforms such as Pichia pastoris, E. coli, BY2 cells, and stably transformed tomato. Expansins derived their name from their ability to loosen plant cell walls and thus may be a potential tool for improving biomass pretreatment of cellulosic biofuels materials. Bacterial expansins have just recently (2014) demonstrated utility to improve cellulose degradation, plant expansins would be even more efficient if we had a means to produce them. This work aims to achieve higher expansin expression using a transient plant expression system. To show the utility of this expression platform, the Cucumber Alpha-Expansin (CuEXPA1) was chosen. Using viral components which include the hyper-translatable cowpea mosaic virus and the P19 gene silencing suppressor, CuExpA1 was transiently expressed in Nicotiana benthamiana via Agrobacterium leaf infiltration. Initial experiments showed active CuEXPA1 expressed based on creep assays relative to empty vector controls. Expression levels however, were variable and highly dependent on plant and growth conditions. To get more consistent expression levels and to optimize the efficiency of our system we took 3 approaches: 1) Optimized plant growth conditions, 2) Verified protein mRNA production, 3) Use apoplastic wash fluid (AWF) technique, a method previously developed to study apoplastic proteins, to extract more pure and concentrated CuEXPA1. Plant growth conditions were standardized by controlling temperature (25C), humidity (60%), and light levels (16 hour light cycle). CuEXPA1 mRNA levels quantified via qPCR were highly expressed, suggesting the variability is a result downstream of the gene transcription. The AWF technique has been utilized and the efficiency is still being tested, however it looks like a promising technique that could greatly simplify functional protein recovery from the transient leaf infiltration system. Work with this recalcitrant protein will provide additional insights into the use of transient expression as a means of rapidly testing genes and producing proteins for functional characterization.

P-3011

Molecular Profiling of Sugar Cane, Miscanthus, Gamagrass, and Sweetpotato for Cellulose Synthase. C. LEE[1], M. Samuels[1], S. Samuels[1], M. Egnin[1], D. Mortley[1], G. He[1], C. S. Prakash[1], C. Bonsi[1], and M. Tu[2]. [1]Tuskegee University, 104

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Petroleum dependence has raised political, economic, and environmental concerns in the United States. Developing an eco-friendly method of supplying energy would both decrease this dependence and improve the environment by mitigating CO_2 emissions. Research has suggested the utilization of biomass crops as an alternative energy source. This study was aimed at identifying potential energy crops that could adequately support bioenergy production through screening of the cellulose synthase specific gene. Leaf DNA was isolated from developing *Miscanthus*, Gamagrass, Bamboo, Sugar Cane, and Sweet Potato (DMOL1-158-204 and TU02), subjected to PCR with primers specific to sugarcane EST-SSR (E-30-1 and E-31-1) and *PSII*. The resulting PCR products revealed the presence of the internal reference gene, *PSII*, 400 bp fragment in all samples- indicating quality genomic DNA levels. EST-SSR30-1 resulting 400 bp cellulose synthase amplicon was present in all samples. However, three distinct amplicons (400bp, 250bp, and 300bp) were obtained with EST-SSR31-1, possibly due to nucleotide mutations occurring within the SSR motif and sequence flanking EST-SSR31-1. These sequences were used to search GenBank by BLAST for nucleotide sequence similarities and ClustalW for sequence alignment. The natures of these EST-SSR sequences were confirmed to be highly homologous to the cellulose synthase specific gene for the cellulosic characteristic. Thereby our results confirmed these crops as potential candidates for bioenergy feedstocks to be used for ethanol and potentially biofuel production. Research sponsored by: USDA-Evans-Allen, Tuskegee University CAENS-GWCAES and Plant Biotech Genomics Research Laboratory, Auburn-TU NSF Bioenergy Project

P-3012

The Relationship Between *PMI (manA)* Gene Expression and Optimal Selection Pressure in *Indica* Rice Transformation. X. LI, H. Gui, X. Li, and Y. Liu. Syngenta Biotechnology China Co. Ltd., No.25, Life Science Park Road, Changping Dist., Beijing, 102206, P. R. CHINA, Email: xianggan.li@ syngenta.com

This study was conducted to establish an efficient transformation system for *indica* rice, cultivar *IR58025B*. Four combinations of two promoters, rice Actin 1 and maize Ubiquitin 1, and two *manA* genes, native gene from E. coli (PMI-01) and synthetic maize codon optimized gene (PMI-09) were compared under various concentrations of mannose. Different selection pressures were required for different gene cassettes to achieve corresponding optimum transformation frequency (TF). Higher TFs as 54% and 53% were obtained when 5 g/L mannose was used for selection of prActin-PMI-01 cassette and 7.5 g/L mannose used for selection of prActin-PMI-09, respectively. TFs as 67% and 56% were obtained when 7.5 g/L and 15 g/L mannose were used for selection of prUbi-PMI-01 and prUbi-PMI-09 respectively. We conclude that higher TFs can be achieved for different gene cassettes when an optimum selection pressure is applied. By investigating the PMI expression level in transgenic calli and leaves, we found there was a significant positive correlation between the protein expression level and the optimal selection pressure. Higher optimal selection pressure is required for those constructs which confer higher expression of PMI protein. The single copy rate of those transgenic events for prActin-PMI-01 cassette is lower than that for other three cassettes. We speculate some of low copy events with low protein expression levels might not have been able to survive in the mannose selection.

P-3013

Establishment of Regeneration System in Big Bluestem (*Andropogon Gerardii)* Grass. P. PANTHA, S. Ponniah, and M. Manoharan. University of Arkansas at Pine Bluff, 1200 N. University Drive, Pine Bluff, AR 71601. Email: panthap5304@uapb.edu

Big bluestem (*Andropogon gerardii)* is a warm-season perennial grass (C4) native to North America and its huge biomass can be used as potential feedstock for biofuel production. However, lack of regeneration protocol has hindered the successful application of genetic engineering technologies for reducing lignin content in big bluestem. The objective of this study was, therefore, to develop efficient regeneration protocol that can be used for down-regulating lignin biosynthetic genes in big bluestem. Callus was induced from the seeds of cultivar Kaw on Murashige and Skoog (MS) medium with different concentrations (0.2, 0.5, 1, 2, 3, 5 mg/L) of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) at 28°C under dark and light conditions. The highest number of embryogenic calli were observed under dark on MS medium containing 0.5 mg/L 2,4-D, whereas under light condition, embryogenic calli failed to form. Subsequently, the embryogenic calli were cultured on regeneration medium (MS) with different concentrations (0.2, 0.5, 1, 3, 5 mg/L) of kinetin or 6-benzylaminopurine (BAP). Among different concentrations of kinetin or BAP tried, at least 3 shoots per calli were regenerated on MS medium containing 5.0 mg/L kinetin. Currently, we are screening other cultivars for their regeneration response.

P-3014

SSR Based Bulk Segregant Analysis for Tagging *Maize Chlorotic Mottle Virus* Resistant Genes on a Smaller Scale in Corn. I. RITTE, M. Egnin, C. Bonsi, S. Samuels, G. Bernard, D. Mortley, P. Kusolwa, A. Lorenz, T. Hoegemeyer, and G.

He. Tuskegee University, 1200 West Montgomery Rd., Tuskegee, AL 36088. Email: iritte8222@mytu.tuskegee.edu

Corn *(Zea mays)* is one of the most important crops in terms of usage, area under cultivation and annual yield. A significant percent of potential harvest yield of corn is lost each year due to diseases caused by viruses. Major problem facing corn production in Tanzania is the occurrence of corn lethal necrosis (CLN) caused by synergetic interaction between maize chlorotic mottle virus (MCMV) and any of potyvirus which infect cereal. Recent research in East Africa indicates no potential source of natural resistance for CLN is available. The purpose of this study is to further explore genes involved in resistance of corn to infection by MCMV through mapping mutations in corn plants that may be observed to display resistance to MCMV infection. Currently two bulked DNA samples are being generated from segregating population lines obtained from University of Nebraska Lincoln for bulk segregant analysis. The bulks will be screened for differences utilizing simple sequence repeat (SSR) markers. The expected future result of this study is to identify genomic region responsible for the MCMV resistance in corn and gain new insight of the genes involved. The findings may have potential impact on the development and introgression of MCMV-resistant genes in Tanzania maize varietal improvement programs. This research is supported by USAID Feed the Future (Ohio State University and iAGRI Project in Tanzania), TU-GWCAES, USDA-Evans-Allen and TU- Plant Biotech & Genomics Research Lab.

P-3015

Analysis of a Highly Expressed, Constitutive and Stress Inducible Full-length Transcript Promoter from *Dahlia Mosaic Virus* (DaMV). D. SAHOO[1], S. Sarkar[2], S. Raha[3], I. B. Maiti[1], and N. Dey[2]. [1]KTRDC, College of Agriculture, University of Kentucky, Lexington, KY 40546-0236; [2]Institute of Life Sciences, Chandrasekharpur, Bhubaneswar, Odisha, INDIA; and [3]Department of Radiation Oncology, Feinberg School of Medicine, Northwestern University, Ward-13-002, 303 East Chicago Ave., Chicago, IL 60611. Email: dipak.sahoo2@uky.edu

A 556 bp long full-length transcript promoter with enhanced activity was characterized from the *Dahlia mosaic virus* (DaMV). The strength of the DaMVFLt4- promoter has been evaluated in transient systems and in transgenic plants using two different reporter genes like *GUS* and *GFP*. The DaMVFLt4- promoter was found to be 4-fold and 5-fold stronger than the CaMV35S promoter in tobacco protoplast and transgenic tobacco plants, respectively. EMSA and super-shift analysis confirmed the binding of tobacco transcription factor TGA1a to the enhancer region of the DaMVFLt4- promoter. TGA1a specially interacted with the as-1 of DaMVFLt4- promoter as shown by DNaseI footprinting. UV crosslinking studies and SouthWestern blot analysis clearly demonstrated that the purified TGA1a specifically bound to as-1 element whereas in tobacco nuclear extract, two unknown transcription factors of about 41 kDa (putatively TGA1a) and about 67 kDa were bound to the as-1 sequence of the DAMVFLt4- promoter. Expression studies with the *DaMVFLt4-::β-glucuronidase* (*GUS*) genes in tobacco protoplasts co-transfected with *CaMV35S::TGA1a* showed that expression of TGA1a resulted in approximately 3.7 times elevated levels of GUS activity. The DaMVFLt4- promoter is a constitutive promoter and the expression level in tissues of transgenic tobacco plants was in the order: root>leaf>stem. In addition, the DaMVFLt4- promoter was regulated by a number of abiotic and biotic stresses as studied in transgenic Arabidopsis and tobacco plants. The newly derived DaMVFLt4- promoter would become an efficient tool for biotechnological application.

P-3016

Remodeling Root Cell Walls with Thermostable Enzymes for Efficient Biomass Processing. J. XU, B. Savary, J. Tovar, and N. Zhang. Arkansas State University, P. O. Box 639, State University, AR 72467. Email: jxu@astate.edu

Sugar beets are targeted for expanded industrial sugar (sucrose) production beyond traditional growing regions to meet national needs for advanced biofuels, renewable chemical feedstocks, and conversion to value-added biobased products. Enzyme technologies may be useful in processing beets and for remodeling root cell walls. We are producing thermostable glycohydrolases and applying them to manipulate structural polysaccharides present in sugar beet pulp. We hypothesize that limited action by thermostable enzymes on pectin can reduce water-holding capacity for lowering energy inputs during drying and can solubilize arabinose-rich polysaccharides for improved recovery and utilization. Demonstrating these benefits will establish a rational basis to express directly these thermostable enzymes in sugar beet roots to obtain processing benefits in an economical viable and environmentally sustainable manner. We will present our recent results with developing a citrus pectin methylesterase and a *Bacillus denitrificans* endo-arabinanase towards these purposes.

P-3017

Biophysiochemical Analysis to Screen Salinity Stress Tolerance in Sugarcane. K. SENGAR[1] and R. Sengar[2]. [1]Sardar Vallabhbhai Patel University of Agri & Tech, Tissue Culture Lab, Meerut, 250110, INDIA and [2]SVP University, Modipuram Meerut, INDIA. Email: kalpana.sengar19@gmail.com

Sugarcane (*Saccharum* sp. Complex) being the most valuable commercial crop of India, not only sustains sugar and

distillery industries but also holds a key position in the national economy by earning foreign exchange. Sugarcane is a typical glycophyte exhibiting stunted growth or no growth under salinity and reducing yield. Salinity which affect crop productivity in one or more of the ways by adversely affecting yield per unit area and time, by eliminating some of the outstanding varieties from commercial cultivation. Molecular markers would provide reliable information about genetic diversity of crops. Since these molecular markers are numerous in number and thus, will have better coverage of whole genome especially for the traits associated with salinity tolerance. Nine varieties of sugarcane (Saccharum officinarum L.) were screened for their salinity stress tolerance in the experiment. Sodium ion (Na+) in all sugarcane varieties was enriched when plantlets were subjected to 200 mM NaCl, except Co 99004 and Co 87002. Chlorophyll a , chlorophyll b and total carotenoids in n the salt stressed leaves of all genotypes decreased significantly, but the extent of decrease was variable among different genotypes. Proline content in salt stressed plantlets of all sugarcane genotypes increased markedly, except in genotypes Co 419, Co 85036, Co 7704 and Co 775. Analysis of variance showed significant differences for five yield related traits among the nine varieties under control and salinity treatments. The results indicated that varieties Co 99004, Co 87002 and Co 94010 were the most tolerant while varieties Co 419, Co 85036 and Co 7704 were the most sensitive ones.

P-3018

Down-regulation of Genes Involved in Lignin Biosynthesis in Rice. Z. SHANG, S. Ponniah, V. Srivastava, and M. Manoharan. University of Arkansas, 1200 N University Drive, Pine Bluff, AR 71601 Email: shangzhenhua08 @gmail.com

The objective of this project was to reduce lignin by down reg ulating genes involved in lignin biosynthesis in rice. A strateg y of down-regulation of lignin biosynthetic genes regulation of lignin biosynthetic genes, cinnamate 4-hydroxylase (4H), hydroxycinnamoyl CoA: shikimate hycroxycinnamoyl transferase (HCT), coumarate 3 hydroxylase (C3'H), cinnamoyl CoA reductase (CCR), and cnnamyl alcholol dehydrogenase (CAD) has been to decrease lignin content in rice. A novel binary vector (TL) in which the trunctacted lignin gene (s) driven only by the promoter and no terminator was constructed and transferred to Agrobacterium tumefaciens for infecting rice calli. Putative transgenic rice plants were regenerated after selection in regeneration me dium (N6 medium containing 2.0 mg/L Kinetin, 0.02 mg/L N AA, 100 mg/L geneticin (G418) and 500 mg/L Carbenicillin) and confirmed by Polymerase Chain Reaction (PCR). Seeds were collected and germinated on MS medium containing 200 mg/L geneticin for segregation analysis. RNA was isolated fr om the segregated plants and Real time qPCR was conducted. The results indicated 50% reduction of some of the genes; (such as CAD) involved in lignin biosynthesis and may potentially lead to reduced lignin for efficient conversion of rice straw to cellulosic biofuel.

P-3019

Effect of Salinity on Morpho and Biochemical Parameters on Rice Germplasm Cultivated Under Saline Environment. A. SINGH[1] and R. Sengar[2]. [1]SVP University of Agri & Tech, Tissue Culture Lab, Meerut, 250110, INDIA and [2] SVP University Modipuram Meerut, INDIA. Email: ashubiot25@ gmail.com

Accumulation of salt in the soil has deleterious effects and leads to a reduction in crop production, including rice. Use of salt tolerant variety considered the most economical and most effective way of increasing crop production on saline soils. Therefore, development of salt tolerant varieties considered as one of the strategies to increase rice production in saline prone coastal areas. Salt tolerance in Ten rice genotypes were studied under salinized (EC=8.5 dSm-1) soil conditions after 90 days of transplanting. The aim of the present investigation is to provide information on the effect of salinity on chlorophyll concentration and yield and yield components of rice genotypes to elucidate the effect of salinity on these factors. The outcome showed that the yield per plant (which ranged from 26.955 to 50.59 %), number of primary braches per panicle, chlorophyll concentrations (which ranged from 4.165616 to 27.86813), and number of productive tillers, panicle length, and fertility percentage and of all the genotypes were affected by salinity. However the results also showed that, genotypes Pokkali Nonabokra CSR 30 & CSR 13 showed better salinity tolerance than others.

P-3020

Precise Excision of Plastid DNA by the Small Serine Recombinase ParA. J. THOMSON[1] and M. Shao[2]. [1]USDA-ARS, 800 Buchanan Street, Rm# 2122, Albany, CA 94710 and [2]UC Davis, CA, 800 Buchanan St., Albany, CA. Email: James.Thomson@ARS.USDA.GOV

Marker genes are essential for the selection and identification of rarely occurring transformation events generated in biotechnology. This includes plastid transformation, which requires that multiple copies of the modified chloroplast genome be present in order to obtain genetically stable transplastomic plants. However, the marker gene becomes dispensable when homoplastomic plants are obtained. Here we demonstrate the precise excision of *MRS* (recognition site) flanked DNA from the plastid genome mediated by the small serine recombinase ParA. We transformed the tobacco plastid genome with the pTCH-MRS vector containing the mCHERRY gene flanked by directly oriented non-

homologous *MRS* recombinase recognition sites. In the absence of the ParA recombinase, the transformed plastid genomes were stable and heritable. Nuclear transformed transgenic tobacco plants expressing a plastid-targeted ParA recombinase where crossed with transplastomic pTCH-MRS plants and the T1 hybrids exhibited efficient excision of the target sequence. The ParA/MRS system should prove to be a useful tool for site-specifically manipulating the plastid genome and generating marker-free transplastomic plants.

P-3021

Feedstock Modification by Down-regulation of Starch Degradation Enzymes in Maize- a C4 Plant. W. WANG, S. Basu, H. Moon, K. Markham, R. Whinna, M. Kim, D. Skalla, N. Zhou, H. Zhong, A. Prairie, S. Zhong, and M. Lee. Syngenta Biotechnology Inc., 3054 E. Cornwallis Rd., RTP, NC 27709. Email: wen.wang@syngenta.com

Down-regulation of transitory starch degradation enzymes in the C4 maize plant with double-stranded (ds) RNA interference approach was used to evaluate starch accumulation in green tissues (leaves and stems) and cobs. These non-seed biomass materials can be used not only for cellulosic bioethanol production but also for animal feed and other industrial applications. Increased starch content in these materials can add value to the feedstock. Three starch degradation enzymes [(a-amylase, b-amylase, and glucan water dikinase (GWD)] were targeted for expression with ds RNA by using a green tissues preferred phosphoenolpyruvate carboxylase (PEPC) promoter. Five enzymes [(a-amylase, b-amylase, GWD, phosphoglucan water dikinase (PWD) and chloroplastic a-amylase (Amy3)] were targeted with a rice MADS-box 13 (OsMADS13) promoter to manipulate starch accumulation in cobs. In addition, two GWD cassettes driven by cob-preferred promoters [tryptophan synthase alpha subunit (TrpA) and Zm015970] were also studied. Total 10 ds RNA binary vectors were constructed to transform corn via Agrobacterium-mediated transformation and T0/T1 green tissues and cobs were analyzed by iodine staining and for starch contents. The down-regulation of GWD and a-amylase in green tissues showed excess starch both in leaves of transgenic seedlings and mature plants, whereas b-amylase down-regulated plants accumulated starch only in young leaf tissues, and not in older leaves of mature plants. There was yellowish phenotype observed in some of GWD T0/T1 transgenic plants, the yellow pigmentation appeared to be caused by excess starch accumulation particularly in the bundle sheath. Starch accumulated in these leaves was heritable and could be stored at room temperature for months after air dry. More starch in the cobs was also observed in GWD suppressed plants than in a-amylase, b-amylase, PWD and Amy3. All three cob-preferred promoters driven GWD resulted in similar starch accumulation in the cob tissues, with TrpA promoter slightly higher. Fermentation of the leaf tissues from selected PEPC: GWD plants resulted in >3 fold of ethanol production (%, v/v), whereas fermentation of cob tissues from selected OsMADS13: GWD plants resulted in >2 fold of ethanol production compared with the null controls which were corroborated well with their starch contents. This study demonstrated the potential application of ds RNA interference approach for feedstock modification to increase starch accumulation in corn. Details in transgene expression, seed sets and plant phenotype will be presented.

P-3022

Engineering Hydroxyproline-O-glycosylated Designer Biopolymers in Plants and Their Applications. J. XU[1], N. Zhang[1], H. Fang[1], and M. Kieliszewski[2]. [1]Arkansas State University, PO Box 639, State University, Jonesboro, AR 72467 and [2]Ohio University, Department of Chemistry and Biochemistry, Athens, OH 45701. Email: jxu@astate.edu

Hydroxyproline-*O*-glycosylation involves post-translational hydroxylation of proline to hydroxyproline (Hyp) and subsequent glycosylation, a modification that is unique to higher plants and green algae. Our earlier work with synthetic genes encoding various Hyp-rich glycoproteins (HRGPs) expressed in plant cells elucidated the Hyp-*O*-glycosylation "code". Specifically, a Hyp-rich peptide sequence directs the Hyp-*O*-glycosylation; contiguous Hyp residues, as in X-Hyp-Hyp-Hyp-Hyp (where X is often Ser or Ala..), are sites of oligoarabinosylation; in contrast, clustered non-contiguous Hyp residues, as in X-Hyp-X-Hyp repeats, are mainly sites of highly branched arabinogalactan polysaccharide addition. These results demonstrated the feasibility of Hyp-*O*-glycosylation based biopolymer design in plants and triggered the applications in the following aspects: **1**) by introducing a Hyp-*O*-glycosylated designer biopolymer tag, e.g., 32 tandem repeats of "Ser-Pro" dipeptide motif or $(SP)_{32}$ for short, to recombinant proteins expressed in tobacco BY-2 cells, we dramatically enhanced the production of secreted proteins by up to 1500-fold; **2**) We significantly improved the yields of a $(SP)_{32}$-tagged recombinant protein transiently expressed in *Nicotiana benthamiana* by up to 16-fold; **3**) we engineered different Hyp-*O*-glycosylated designer biopolymer into plants to reconstruct the plant cell wall for improved biomass processability.

P-3023

Ex-situ Conservation of *Scutellaria havanensis*: A Potential Medicinal Plant. Y. ZAVALA- ORTIZ[1], J. Negrón-Berríos, and N. Joshee[2]. [1]Inter American University of Puerto Rico, Barranquitas Campus, Barranquitas, PR, 00794, and [2]Fort Valley State University, 1005 State University Dr, Fort Valley, GA 31030. Email: yarzav6165@br.uipr.edu

Scutellaria is a genus of about 400 species and many species have been used in traditional Native American medicine.

Pharmacological studies have confirmed that total extracts or flavonoids of the genus *Scutellaria* possess anti-lipoperoxidation, anti-platelet, anti-inflammatory, antitumor, hepatoprotective, antioxidant, antibacterial and antiviral activities. It is proposed to research the herb *Scutellaria havanensis*, only *Scutellaria* species reported from Puerto Rico. The objective pursued is the *ex situ* conservation of the herb using plant tissue culture by simplifying protocols to effectively micropropagate explants. This study seeks to optimize the rate at which multiple, viable shoots are induced reproducibly. We expect to achieve *ex-situ* conservation using tissue culture technology and optimized the time for shoot induction using various types (leaf, node, shoot tip, internode, and thin cell layer cultures) of explants. We hope that the antioxidant capacity of the plant provides basis for future phytochemical screening and clinical research. This work was supported by USDE Grant Number P031M090018.

P-3024

Seasonal Variation in Viability of Cryopreserved *Vaccinium* Dormant Buds. M. JENDEREK[1], J. Tanner[1], J. Postman[2], B. Ambruzs[3], and K. Hummer[2]. [1]NCGRP, USDA-ARS, Fort Collins, CO 80521; [2]NCGR, USDA-ARS, 33447 Peoria Road, Corvallis, OR 97333; and [3]retired. Email: maria .jenderek@ars.usda.gov

Berries of several *Vaccinium* L (Ericaceae) species have become an integral part of everyday diets of a vast American population. The most economically important are blueberry and cranberry. The USDA-ARS, National Plant Germplasm System maintains over 1,500 accessions of living plants and seeds at a genebank in Corvallis, Oregon. *Vaccinium* accessions are propagated clonally and only 2.7% are backed up as cryopreserved meristem (MS) shoots. Additional backup will help safeguard the collection from abiotic and biotic stress factors, including changes in climate that might lead to the irreversible loss of the berry germplasm. We investigated the possibility of backing up clonal *Vaccinium* genetic resources in liquid nitrogen (LN) as dormant winter buds (DB). The DB method has been successfully applied in preservation of a few temperate tree species but is not widely used in cryopreservation despite requiring less resources and being faster than preservation via MS. Our three year study on post-cryopreservation viability of the *V.* hybrid 'Northsky' identified an optimal DB harvest period that varied between years but was related to the mean daily temperatures preceding the harvest. The viability of DB harvested in the optimal period was 80 - 100%, whereas in the immediate pre- and proceeding time was from 25 to 45%. Studies on suitability of the harvest period to other genotypes are in progress.

P-3025

In Vitro Germplasm Conservation for Sugarcane in South Africa. S. SNYMAN. South African Sugarcane Research Institute, Private Bag X02, Mount Edgecombe, Durban 4300, SOUTH AFRICA. Email: sandy.snyman@sugar.org.za

Traditionally sugarcane germplasm collections comprising both commercial lines and ancestral breeding material are kept in the field. However these assemblages are costly to maintain, are vulnerable to environmental hazards and require large tracts of land. In vitro storage methodologies viz. slow growth and cryopreservation are feasible alternatives to field collections as cultures are aseptic environmental conditions can be controlled and space requirements are minimised. In vitro plantlets of cultivar NCo310 were maintained in slow growth conditions at two temperatures (18 and 24°C) and on four semi-solid media: (1) Murashige and Skoog (1962) salts and vitamins and 20 gl^{-1} sucrose; (2) as in (1) with 1 mgl^{-1} abscisic acid; (3) half strength MS with 10 gl^{-1} sucrose; and (4) as in (3) with 1 mgl^{-1} abscisic acid. After 8 and 12 months, survival and shoot multiplication rates were recorded, when single shoots were removed from the storage media and sub-cultured bi-monthly on MS shoot multiplication medium [medium (1) with 0.015 mgl^{-1} kinetin and 0.1 mgl^{-1} benzyl aminopurine] for 2 months. At 24°C, no green shoots could be recovered on media 1, 3 and 4 after 12 months, whereas at 8 months, storage on all four media supported recovery and multiplication of shoots with plants stored on medium (3) resulting in significantly more shoots (1041 ± 262) than on the other media. At 18°C, less shoot multiplication was evident but storage for 12 months was successful, with 88 ± 15 to 121 ± 18 shoots being recovered after 2 months of multiplication. Currently, cryopreservation of in vitro-derived shoot meristems is underway encompassing both encapsulation-dehydration and droplet vitrification. Work in this area is ongoing as cryopreservation is a good long-term storage option for valuable germplasm.

P-3026

Development of Sonication Assisted *Agrobacterium*-mediated Transformation Protocol of Cotton (*Gossypium hirsutum* L.). S. BARAMPURAM, G. Allen, and S. Krasnyanski. North Carolina State University, Department of Horticultural Science, Campus Box 7550, Partners II Bldg, Room 1208, Raleigh, NC 27695-7550. Email: sbaramp@ncsu.edu

Cotton is an economically important crop that is grown throughout the world and considered the backbone of the textile industry. The economic importance of cotton makes it a major target for improvements through genetic transformation. Successful genetic transformation depends on the efficiency of plant regeneration and stable transgene integration and expression. The existing protocols for regenerating cotton transgenic plants based on somatic

embryogenesis are lengthy and genotype-dependent. Published genotype-independent protocols based on the transformation of pollen or ovaries are not practical because of very low efficiency along with the difficulty to reproduce them. Development of alternative cotton transformation protocols that are more efficient and genotype-independent would be of high practical application. Currently, we are investigating various parameters of an *Agrobacterium*-mediated cotton transformation protocol based on the use of embryonic axis explants. Our protocol involves two consecutive treatments of sonication and vacuum infiltration. The age of embryonic axis explants, sonication and vacuum infiltration exposure, and co-cultivation time were studied as factors affecting transformation efficiency. We used an *Agrobacterium tumefaciens* strain GV3101 carrying a plasmid containing a green fluorescent protein (GFP) gene driven by 35S CMV promoter. Stable and transient GFP expression in explants was visualized by fluorescence microscopy. The level and localization of stable GFP expression in the embryonic axis explants and emerging shoots was scored 2-3 weeks after each treatment tested. Rooted shoots are currently being screened by PCR for the presence of the GFP transgene, and the best treatments will be incorporated into our optimized cotton transformation protocol.

P-3027

Effects of Promoters Driving the Selectable Marker Gene *aad-1* in Transformation of Maize Cultivar B104. J. BERINGER[1], M. Beck[1], S. Bennett[1], W. Chen[1], M. Fitter[1], S. Foulk[1], R. Garton[1], M. Gupta[1], J. Komnick[1], S. Kumar[1], A. Love[1], T. Minnicks[1], H. Robinson[1], N. Sardesai[1], H. Schoon[2], J. Torrence[1], H. Wang[2], S. Weaver[1], and H. Wu[1]. [1]Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN 46268 and [2]Kelly Scientific Resource, 9330 Zionsville Road, Indianapolis, IN 46268. Email: jrberinger@dow.com

The efficient production of transgenic events is a prerequisite for the development of new transgenic traits in corn. A major factor in the efficiency of transgenic event production is the choice of promoter to drive the selectable marker gene. The promoter can influence transformation frequency, copy number, and even the expression of neighboring genes in the expression cassette. We analyzed the effects of a set of different promoters driving the selectable marker gene *aad-1* in transgenic events of the maize cultivar B104. The promoter(s) was inserted upstream of the *aad1* coding sequence in otherwise identical *Agrobacterium* vectors which also carried a *yfp* visual marker gene driven by a maize ubiquitin promoter. T_0 plants of these transgenic events were sampled at the V5 growth stage and analyzed for *aad-1* and *yfp* gene copy numbers, *aad-1* and *yfp* relative transcript levels, and AAD-1 and YFP protein levels. Promoters were then evaluated for their ability to produce high quality transgenic events in future projects.

P-3028

Gene Stacking in Cisgenic Wheat. A. ISMAGUL[1], E. Maltseva[2], G. Iskakova[3], N. Yang[3], S. Lopato[3], S. Eliby[3], and P. Langridge[3]. [1]Research Officer, University of Adelaide, PMB 1, Glen Osmond, SA, Adelaide, 5064, AUSTRALIA; [2]Aytkhozhin Institute of Molecular Biology and Biochemistry, IMM Almaty, Kazakhstan; and [3]ACPFG, The University of Adelaide, Australia, PMB 1,Glen Osmond, Adelaide, AUSTRALIA. Email: ainur.ismagul@acpfg.com.au

Bread wheat (*Triticum aestivum* L.) biotechnology research, such as reverse genetics and functional genomics, accelerates the breeding of new advanced cultivars but requires efficient genetic engineering tools for the production of transgenic plants. The Plant Transformation Group of the Australian Centre for Plant Functional Genomics (ACPFG) conducts research in high throughput cereal genetic transformation and is developing a cisgenic approach to improve plants by modulating expression of their own genes. Cisgenic plants are produced using genetic engineering techniques, however, in contrast to transgenic organisms, modifications in this case are carried out only with the genes from related crops, which may crossbreed naturally. We define cisgenics as genetically enhanced plants (cells) that express the genes derived from the same (Cis-) genus. For cisgenic wheat this means that the introduced genes are only derived from the *Triticum* genus. In the present research we have co-transformed three wheat genes – Acetohydroxy acid synthase (AHAS, *als*), Chitinase I and DREB3 in Australian wheat cultivar Gladius, and four Kazakh spring wheat cultivars Saratovskaya 29, Kazakhstanskaya 19, Astana 2 and Tselinnaya 3C. In total, 5 stable transformation experiments were performed. PCR analyses of 74 herbicide- resistant regenerants identified 71 cisgenic plants with the AHAS gene, 61 plants with the Chitinase I and 53 transformants with the DREB3 gene; all three genes were present in 45 regenerants. The highest co-transformation frequency (CTF) 67.8 % was in cv. Saratovskaya 29, where 40 plants out of 59 regenerants were shown to contain all three genes. In cv. Kazakhstanskaya 19, the number of regenerated plants were 3 of which 2 carried all 3 genes (CTF = 66.7%). And in cv. Gladius, only 3 plants out of 10 contained all 3 genes (CTF = 30.0%). Molecular and expression analyses of the cisgenic wheat are in progress.

P-3029

Elimination of Unnecessary Protein Production of Transgenes in Transgenic Plants. S. ÖZCAN[1], E. Anayol[2], A. Bakhsh[3], S. Özcan[2], M. Aasım[1], B. Gürbüz[1], and L. Ünlü[4]. [1]Department of Field Crops, Faculty of Agriculture, University of Ankara, Ankara, 06110, TURKEY; [2]Ministry of Food, Agriculture and Livestock, Central Research Institute for Field Crops, Ankara, TURKEY; [3]University of Niğde, Department of Agricultural Genetic Engineering, Niğde, TURKEY; and

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Transgenic crops around the world have been transformed with the genes driven by constitutive promoters such as CaMV 35S. Under the control of these promoters, the transgenic plants keep on synthesizing the foreign gene's protein at high concentration in all plant parts like leaf, fruit, roots and seed irrespective of the requirement. To ensure the expression of foreign genes at required time and in desired organs, use of tissue, organ or expression-specific promoters is necessary. The expression of GUS gene driven by wound inducible AoPR1 promoter, isolated from *Asparagus officinalis* mesophyll cells suspension showed higher GUS activity only in wounded regions, but did not show any significant activity in plant parts like leaves, stem, roots, tubers, seed and pollen. In this study, cry genes under the control of wound inducible AoPR1 promoter were transferred to cotton by *Agrobacterium tumefaciens* using different explants. The regenerated putative primary transformants were transferred to green house and confirmed using standard molecular techniques. Insect bioassay experiments revealed that transgenic plants have significant resistance against targeted insect pests. The transgenic plants under the control of AoPR1 promoter showed restricted protein expression in insect wounded sites only. This work was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK)

P-3030

Agrobacterium Tumefaciens-mediated Transformation of *Asclepias*, a Model for Plant-insect Interactions. J. VAN ECK[1], P. Keen[1], A. Picard Hastings[2], and A. Agrawal[2]. [1]The Boyce Thompson Institute, 533 Tower Rd., Ithaca, NY 14853 and [2]Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853. Email: jv27@ cornell.edu

There are over 130 species in the genus *Asclepias*, commonly referred to as milkweeds because of the latex they produce. The latex contains cardenolides (steroids), which provide a defense to limit insect damage and the content differs in various species. Efforts to develop *Asclepias* as a model for plant-insect interactions include the need for transformation methodology to identify genes and networks that play a role in defense mechanisms. We focused our efforts on 4 species (*Asclepias syriaca*, *A. tuberosa*, *A. hallii*, and *A. curassavica*) based on their cardenolide content and evolutionary relationships. Before attempting transformation, we needed to develop robust plant regeneration methods. We started with attempts to establish *in vitro* plants that could be propagated and provide a continuous source of clean material for plant regeneration experiments. Unfortunately, *in vitro* rooting was problematic for all species except *A. curassavica*, which readily rooted *in vitro*. As a result, leaves and stem internode segments from young seedlings grown in soil were used for regeneration studies of *A. syriaca*, *A. tuberosa*, and *A. hallii*. Stem internode segments were more responsive than leaves for all species. However, media components and culture conditions required for efficient plant regeneration differed and results will be presented. We were successful in development of plant regeneration methods for all species except *A. curassavica*, for which work is still in progress. As for transformation, to date, we have recovered transgenic lines of *A. tuberosa* and *A. hallii* following infection of stem internode segments with LBA4404 that contained the pCambia 2301 vector. This vector contains the plant selectable marker gene, *nptII*, and the GUS reporter gene. Transformations of *A. syriaca* are in progress with the goal to knock down expression of the coronatine insensitive 1 gene (*coi1*), which encodes an F-box protein similar in structure to jasmonate. The *coi1* knockdown lines will be evaluated for effects on insect feeding and on the overall interaction with herbivores commonly found on milkweeds.

P-3031

Evaluation of Cefotaxime for the Prevention of Microbial Contamination in Immature Maize Embryos. M. WELTER, N. Botimer, N. Arnold, M. Simpson, T. Strange, L. Rowland, and S. Webb. Dow AgroSciences, 9330 Zionsville Rd, Bldg 314/2B, Indianapolis, IN 46268. Email: mwelter@dow.com

Bacterial contamination can have a serious impact on transformation frequency for any system dependent on greenhouse grown plant donor material. Cefotaxime is an antibiotic that is an inhibitor to bacterial cell wall bio-synthesis and is known to be highly effective against Gram-negative bacteria. It also has been widely reported for the use of elimination of *Agrobacterium* after infection Cefotaxime was evaluated for its abiliy to control epiphytic bacterial contamination in immature embryos of maize. Surface sterilization of the maize ears prior to isolation of embryos is generally used to eliminate surface contaminants. However, this method is not always successful and the loss of material 1-2 weeks after initiation of experiments is costly and can jeopardize project timelines. A series of dose-response studies examined the effect of cefotaxime concentration (1) on inhibition of bacterial growth for the first 12 days of tissue culture, (2) the impact on maize transformation via microparticle bombardment, (3) the stability of cefotaxime in tissue culture media as measured by zone of clearing in a bacterial pour plate assay. A dose of 100 mg/L cefotaxime was determined to eliminate bacterial contamination and maintain stability over 12 days while not impacting transformation frequency.

P-3032

Floral Dip Transformation of Wisconsin Fast Plants (*Brassica rapa*). M. YOUNG and D. Sprouse. Elizabeth City State University, 1704 Weeksville Rd, Elizabeth City, NC 27909. Email: mmyoung2@mail.ecsu.edu

Wisconsin Fast Plants (*Brassica rapa*: WFP) is a widely used model system for K-16 education in plant sciences, and research. It has a very short generation time (seed-to-seed in less than 40 days), many mutants are commercially available and physiological distresses are easily observed. However, there are very few studies on transformation of this plant. Seeds of WFP were sown in soil-less media, and unopened floral buds were dipped in *Agrobacterium* containing the *nptII* and *gus* genes. Flowers were cross-pollinated with pollen from the control (uninoculated plants), and seeds were collected. These seeds were germinated on MS medium containing kanamycin. T1 plants were recovered that were positive for the *nptII* and *gus* genes. T2 and T3 progenies were generated, and were also positive for both genes. Optimization of additional parameters including age of floral buds, the use of a self-fertilizing variety, and selection of transgenics in vitro or ex vitro will also be discussed.

P-3033

Understanding Storage Root Formation in Sweetpotato Through Comparative RNA-seq Analysis. S. PONNIAH[1], V. Kalavacharla[2], K. Bhide[3], J. Thimmapuram[3], and M. Manoharan[1]. [1]University of Arkansas at Pine Bluff, 1200 N University Dr., Pine Bluff, AR 71601; [2]Delaware State University, Center for Integrated Biological and Environmental Research, Dover, DE 19901; and [3]Purdue University, Bioinformatics Core, Cyber Center, Discovery Park, West Lafayette, IN 47907. Email: ponniahs@uapb.edu

The storage roots (tubers) of sweetpotato (*Ipomoea batatas)* provide high levels of digestible nutrients and fiber. Although morphological data indicate that tuberizing results from the activation of the cambium followed by cell proliferation, detailed molecular information on genes controlling tuber formation is lacking in sweetpotato. In order to understand the mechanisms of tuberization, we have carried out RNA-Seq analyses of both cultivated hexaploid (tuber forming cultivated species-Sp1) and non-cultivated, ancestor - (non-tuber forming *Ipomoea trifida* root tissues; Sp2). Transcriptome sequencing using Illumina (RNA-Seq) generated 416 (Sp1) and 400 (Sp2) millions of short sequence reads. A reference transcriptome was developed by *de novo* assembly of these sequences, as there is limited sequence information that exists in sweetpotato. We report progress on the annotation of the contigs using BLASTx, separately against TAIR, the cassava protein database, the non-redundant protein database (nr) from NCBI and the sweetpotato gene index. A unique list of tuber-forming genes, transcriptome-derived microsatellites, and single nucleotide polymorphisms (SNPs) were identified from the transcriptome data. The annotated contigs 21,777 (Sp1) and 57,415 (Sp2) were used for downstream processes to find orthologs between Sp1 and Sp2 and paralogs within the species. A total of 4,702 (Sp1) and 4,670 (Sp2) orthologs that were identified between the species may possibly help in understanding tuber formation. Similarly, 60 (Sp1) and 2,286 (Sp2) paralogs that were identified may help in understanding speciation events within the species. Therefore, this dataset will serve as a valuable resource for sweetpotato genetics and genomics research and as an important tool for training undergraduate and graduate students.

P-3034

Engineering of Genistein Production in Rice by Overexpressing Isoflavone Biosynthetic Genes. S. POKHREL, S. Ponniah, and M. Manoharan. University of Arkansas at Pine Bluff, 1200 North University Drive, Pine Bluff, AR 71601. Email: pokhres9036@uapb.edu

Isoflavones are secondary metabolites produced predominately in leguminous plants such as soybean. Isoflavones play an important role in enhancing human health and plant disease resistance response. The objective of this study was to express isoflavone genistein in rice, a major staple food that does not naturally produce isoflavones. Expression of isoflavone in rice may enhance its nutritional value as well as pathogen resistance. Chalcone synthase (CHS) gene from soybean was cloned into plant expression vector pH2GW7 downstream of 35S promoter. Similarly, soybean isoflavone synthase (IFS) and a chimeric transcription factor containing maize C1 and R coding regions, called CRC, were cloned individually into another plant expression vector pCAMBIA-1304 by replacing reporter gene fragment (mgfp5:gus). Ten days old scutellum-derived calli, cultured on N6D medium containing 2.0 mg/L 2,4-D, were infected with *Agrobacterium tumefaciens* strain EHA 105 harboring plant expression vector with desired genes (CHS, IFS, CRC). Putative transgenic rice plants were regenerated on regeneration medium (REIII) containing 2.0 mg/L kinetin, 0.02 mg/L NAA, 50 mg/L hygromycin, and 500 mg/L carbenicillin. A total of sixteen, fifteen, and ten independent transgenic CHS, IFS, and CRC lines respectively were confirmed by polymerase chain reaction (PCR) and Southern blotting, and the expression was confirmed by reverse transcriptase-PCR (RT-PCR). Stacking of all three independent transgenes (CHS, IFS, CRC) into a single plant through crossing is in progress. Isoflavone content from seeds will be analyzed by gas chromatography-mass spectrometry (GC-MS) and disease resistance of transgenic lines will be tested against rice blast (*Magnaporthe grisea*).

P-3035
Control of Germination in Ornamental Nymphaea Water Lily Seeds. N. HOANG, M. E. Kane, and H. Perez. University of Florida, PO Box 110675 Gainesville, FL 32611-0675. Email: nhhoang@ufl.edu

Understanding the germination process of Nymphaea water lilies is fundamental for efficient aquatic plant production. Effects of light and temperature conditions on germination of different ornamental Nymphaea cultivars (*N*. Blue Panama, *N*. Shirley Brine, *N*. Ganna Walska, and *N*. Mayla) was studied in sterile and unsterile conditions. Light intensity (0 and 35 $\mu mols^{-1}m^{-2}$) and temperature conditions (20, 23, 26, 30, and 35ºC) maintained on a thermal gradient table had a significant effect on seed germination of both hardy and tropical water lilies. Dried tropical water lily seeds exhibited no dormancy following rehydration. Hardy water lily seeds exhibit dormancy and must be stored in water for germination to occur. Hardy water lily embryos were isolated and successfully cultured in liquid half-strength MS media supplemented with 10% coconut water. The inhibition effects of excised seed coats and endosperm tissue, cultured in the presence of excised embryos, was tested in scintillation vials containing different liquid medium volumes (1, 3, and 5 ml). Excised embryo growth was significantly inhibited with the addition of excised seed coats into liquid media. However, co-culture of excised endosperm tissue was not inhibitory to excised embryo growth. These results suggest that hardy water lily seed germination may be physiological dormant, imposed by the seed coat.

P-3036
Modification of Spinner Flasks for Orchid Seed Bioreactor Culture. B. HUGHES and M. E. Kane. University of Florida, 2043 IFAS Research Dr, Rm 109, Gainesville, FL 32611. Email: benhughes@ufl.edu

Germination of orchid seeds on solid media often results in uneven germination and seedling development resulting in reduced propagation efficiency. Seeds often fuse together when germinated on solid media and makes separation into individual seedlings difficult. Application of bioreactor technology for orchid seed culture offers the potential for reduced culture space requirements and labor while increasing synchronization of orchid seedling development. Bioreactors were constructed from Bellco Bel-Flo 250mL spinner flasks and modified to provide aeration. Silicone tubing with in-line bacterial air vents was connected to each sidearm of the flask to deliver and exhaust sterile air through the media. Enhanced medium aeration was achieved using a silica airstone, attached to the inside flask wall with silicone adhesive. All materials used were autoclavable. Aeration was provided by a Pondmaster aquarium pump delivered through a PVC manifold that allowed aeration for up to 20 bioreactors. The design of this bioreactor will be fully described. This culture system is being optimized for orchid seed liquid culture which may be scale able to commercial levels.

P-3037
Manifestations of Cobalt on Growth and Development of *Erythrina Variegata*: An In Vitro Study. S. JAVED and M. Anis. Aligarh Muslim University, Plant Biotechnology Laboratory, Department of Botany, Aligarh, 202 002, INDIA. Email: saadjaved84@gmail.com

Unlike the well-established essentiality of cobalt across the animal kingdom its role in the plant kingdom is not quite as articulate. It is considered essential for the lower plant while in higher plants it is considered semi essential for nodulated legume due to its requirement by the symbiont, while beneficial for other higher plants. Many of the earlier workers have reported the influence of cobalt on morphogenetic processes in higher plants. Cobalt appears to be having a more intricate role in the nitrogen metabolism of the higher plant, as enhanced nitrogen content in plants exposed to higher cobalt concentrations has been observed. Enhanced growth in excised plant tissues has also been reported in the presence of cobalt. Although cobalt has not been ascribed to any specific role in the plant metabolism of higher plants, it continues to be an integral part of most of the culture media proposed for tissue culture of plants. Even with the wide spread conjecture about the role of cobalt in plant metabolism it has remained largely ignored. *In vitro* conditions such as in plant tissue culture provides a good model to assess the effect of a particular factor as it is free from environmental contingencies. Moreover the time required for the study can also greatly reduced through the use of these biotechnological tools. Therefore, we evaluated the effect of cobalt on morphogenic potential, chlorophyll content and antioxidant enzyme activity of cobalt chloride ($CoCl_2$) across a concentration gradient (0 to 200µM). A 50 µM concentration was found to be favourable for growth and development of *Erythrina variegata*. Number of shoots produced by the nodal segment and chlorophyll content increased at the optimum concentration of cobalt. Above the optimal level stress symptoms were evident with increased activity of SOD, APX and GR, while CAT was inhibited limiting its activity. Attrition in number of shoots and chlorophyll content was also observed at the above optimum level, secondary metabolites as to provide adequate raw materials for pharmaceutical industries.

P-3038
Micropropagation of Caribbean Grape Varieties. A. JIMENEZ-ORTIZ and J. Negrón- Berrios. Inter American University of Puerto Rico, Barranquitas Campus, PO Box 517 Barranquitas, PR 00794. Email: arljim7459@br.uipr.edu

Grape is a plant that has been cultured since ancient times. Grapes belong to the genus *Vitis*, family *Vitaceae*, and native from the Mediterranean, Central Europe and Southwest Asia. The grapes global trade generates around a billion dollars in exports of thousands of tons of grapes. Due to the tropical climate, in the Caribbean grapes can produce two to three crops in the year, making this fruit a potential source of commercial value as table fruit, vinaigrettes, wines, juice and more. A limitation to accomplish this goal is the need to identify grapes species with good adaptation to the tropical climate, standardization of propagation techniques, and in general, lack of research applied to grapes in the Caribbean. There are a limited number of grapes varieties that have demonstrated adaptability to the Caribbean. Vegetative propagation has been used extensively in grapes cultivars. Micropropagation offers faster and more efficient methods for the production of pathogen free stocks and to amplify genetic variation in selected cultivars. We have applied micropropagation to two varieties from ***Muscat*** grape that have shown adaptability to the tropical climate. Our main goal is to improve productivity and add value to these grape varieties through micropropagation. Shoot-tip media was prepared; the explants were cultured in this media with growth regulators to generate roots and shoots. Different concentrations of auxins and cytokinins were used to promote cell division. Callus formation was induced by cytokinin and auxin. This work was supported by USDE Grant Number P031M090018.

P-3039

Dormancy and Sprouting of *In Vitro* Produced *Sagittaria latifolia* Corms. P. QUIJIA and M. E. Kane. University of Florida, P. O. Box 110675, Gainesville, FL 32611. Email: paulinaquial@ufl.edu

Wetland restoration projects require extensive use of plant species for reestablishment of native plant communities. Plant material has been traditionally obtained from different sources, such as bare-root transplants, mulch containing seeds, rhizomes, corms and/or tubers from natural donor population. These common practices could cause environmental damage by over collection. Application of in vitro plant propagation is a viable solution that could facilitate the production and storage of wetland plants. *Sagittaria latifolia* L. commonly called arrowhead or duck potato, is a native monocotyledon wetland species, widely distributed in southeastern Canada and in the eastern half of the United States. During the growing season vegetative growth occurs via production of rhizomes terminating in leafy shoots. Toward the end of the growing season corms are produced at the rhizome tips. Corms production allows survival during unfavorable winter conditions. In vitro corm production could be used as efficient method to produce, store, and handle propagules used for wetland restoration projects. *S. latifolia* has been successfully *in vitro* propagated using shoot culture. Effects of temperature, photoperiod and growth regulators on corm production have been reported as well. However, to our knowledge, there have been no studies assessing degree of dormancy and sprouting capacity of in vitro produced corms. Optimal culture interval, temperature, photoperiod, and plant growth regulator supplementation for induction of corm formation in vitro was evaluated Plants cultured under a 10-hr photoperiod at 20^0C, in liquid MS medium containing either 10 or 25 µM ABA and 190 mM sucrose promoted corm formation. The corms produced were used to determinate their degree of dormancy, under optimal growth condition for vegetative growth (forcing conditions: 16-hr photoperiod, 25 ^{0}C). Longitudinal and lateral excision of corms or the effects of different plant growth regulator concentrations on intact corm sprouting were evaluated.

P-3040

Effect of Pruning on Root Regrowth of In Vitro Cultured *Dendrophylax Lindenii* Seedlings. R. RODENIUS and M. E. Kane. University of Florida, P. O. Box 110675, Gainesville, FL 32611. Email: floragp26@gmail.com

Dendrophylax lindenii, the ghost orchid, is a leafless epiphytic orchid. The species ranges from southern Florida, Cuba and the Caribbean and is noted for its striking white flowers. Morphologically, plants consist of photosynthetic roots attached to a reduced stem having highly reduced scale-like leaves. Plantlets have been observed to regenerate from broken or damaged roots but this is not well documented. Likewise, little is known of the root regeneration from the reduced stem of the ghost orchid. An experiment was designed to: 1) examine the effects of root pruning on subsequent root regrowth of *Dendrophylax lindenii* seedlings; 2) examine the anatomical origin of regenerated roots; and 3) compare the effects of two orchid media PhytoTechnology Laboratories P723 supplemented with 3% (w/w) banana powder and PhytoTechnology Laboratories P748 on root regrowth following pruning. Roots of the seedlings were pruned to 0.5 or 1.0 cm in length and cultured on the two media for 14 weeks. Ghost Orchid seedlings displayed the capacity to regenerate new roots from the central stem following pruning with maximum root regeneration and length observed on P723 medium. The results are useful for producing smaller roots on older seedlings which would facilitate root attachment to substrates during greenhouse acclimatization.

P-3041

Micropropagation of Two Breadfruit Cultivars. J. ROUSE-MILLER, F. Solomon Jr., and L. Roberts-Nkrumah. The University of the West Indies, Department of Food Production, Faculty of Food and Agriculture, St. Augustine

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In the Caribbean, breadfruit production is constrained by a number of factors which limit its potential as a food security crop. One of the factors identified is inadequate planting material. The overall goal of the study was to develop a protocol for micropropagation of two breadfruit cultivars 'yellow heart' and 'Ma'afala'. The study specifically investigated the most appropriate donor material and treatments for establishment of aseptic culture and medium consistency for multiplication. Shoot tips and nodal explants were isolated from mature field grown plants and adventitious shoots formed on root cuttings in the greenhouse or in a 35 ^{0} C growth room. All explants were initiated on solid medium and multiplication rate was compared on agar solidified or liquid MS 1962 medium supplemented with benzylaminopurine at 1 mg L $^{-1}$. The most appropriate donor plant material and treatment for establishment of both cultivars were adventitious shoots grown at 35 ^{0} C in a growth room. Multiplication was achieved on both solid and RITA liquid cultures systems however liquid culture resulted in a 2.5 – 4:0 fold increases in multiplication rate for both cultivars. Microshoots were rooted on MS 1962 medium and plantlets were successfully hardened to greenhouse conditions. Application of this protocol for rapid multiplication of breadfruit propagules would eliminate one of the factors which limit the crop by increasing the amount of planting material available for orchard production.

P-3042

Histological Analysis of Somatic Embryos Derived from Mature Zigotic Embryos of *Passiflora alata*. S. RODRIGUES DA SILVEIRA and A. Martinelli. University of São Paulo, Avenida Centenário, 303, São Dimas, Piracicaba, São Paulo, BRAZIL. Email: sylviarsilveira@ gmail.com

Somatic embryogenesis can be a model for studying the morphological, physiological, biochemical and molecular events occurring during embryogenesis in plants, since somatic embryos resemble zygotic embryos in many aspects. There are reports on successful systems of somatic embryogenesis from a few *Passiflora* spp, such as *P. giberti*, *P. cincinnata* and the economically important *P. edulis*, the yellow or sour passionfruit. *P. alata*, the sweet passionfruit, shows commercial importance not only for the production of fruits, but also as an ornamental vine, that produces large, colorful flowers, and for its potential as a rootstock for *P. edulis*. As part of a larger project that aims to characterize embryo and aril development in *Passiflora* spp, somatic embryogenesis has been induced from mature zygotic embryos of *P. alata* cultivated in the dark at 27±2ºC, in MS medium supplemented with 2,4-diclorophenoxyacetic acid and 6-benzyladenine. In the present work we describe the morpho-anatomical characteristics of the somatic embryos, observed by light and scanning electron microscopy. Somatic embryo formation was indirect, from callus that proliferated from cotyledons and embryo axis, and asynchronous, with different developmental stages observed simultaneously in culture. Somatic embryos showed normal developed cotyledons and at the time of the cotyledonary stage histodifferentiation of protoderm, procambium, ground meristem, shoot and root apical meristems was clearly observed. (Acknowledgements: FAPESP, CNPq).

P-3043

Establishment of Adventitious Root Cultures of *Passiflora pohlii* Mast. M. SIMÃO, T. Merhy, R. Garcia, E. Mansur, and G. Pacheco. Rio de Janeiro State University, Rua São Francisco Xavier 524 PHLC sl 505 Maracanã, Rio de Janeiro, BRAZIL. Email: marielajsimao@gmail.com

Passiflora pohlii Mast. is a wild species of the genus *Passiflora* (Passifloraceae) native to Brazil, with ornamental and agronomic potential. Although there are no studies on the medicine potential of this species, recent pharmacological studies with different species of the genus showed analgesic, antioxidant and anti-inflammatory activities in extracts from different plant materials, especially roots, confirming their popular use. The aim of this work was the development of an efficient *in vitro* adventitious roots culture system for *P. pohlii*. Internodal and nodal explants were excised from *in vitro*-grown plants and inoculated on MSM medium supplemented with NAA (0.54; 2.65; 5.4 µM), IAA (0.57; 2.85; 5.7 µM) or IBA (0.49; 2.45; 4.9 µM) using the following culture systems: solid medium, liquid medium with or without agitation (100 rpm), and filter paper bridges over liquid medium. In addition, root segments were also excised from *in vitro*-grown plants and inoculated on liquid MSM medium supplemented with the same growth regulators. Cultures were maintained for 60 days at 25°C, in the presence or absence of light. The highest induction of adventitious roots from stem explants was observed from internodal segments cultured on solid medium supplemented with 2.65 µM NAA, and maintained in the dark. However, callus production was also observed from these explants. On the other hand, highest proliferative capacity was observed from *in vitro*-root segments in response to 2.85 µM IAA, when cultured in the dark, with the yield of 0,2027 g dry weight after a culture period of 60 days. Studies aiming at the phytochemical characterization of extracts from these roots are being undertaken.

P-3044

Rootstock-specific Expression of *VcFT* in Tobacco Is Insufficient to Alter Scion Flowering Regime. A. WALWORTH, R. Wickenheiser, and G. Song. Michigan State

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Plant flowering, a critical component of agricultural productivity, is controlled by both genetic and environmental signals. Studies on the flowering pathway genes in *Arabidopsis thaliana* have shown that *FLOWERING LOCUS T* (*FT*) is a major floral activator; and homologs have been identified in a variety of species. Our previous studies have shown that the blueberry (*Vaccinium corymbosum* L.)-derived *FT*-like gene, *VcFT*, hastens flowering when overexpressed in either blueberry or tobacco (*Nicotiana tabacum* L.). Here we report the results of a grafting study using *VcFT*-overexpressing (T) and wild type (WT) tobacco plants combined in all possible conformations as scion or rootstock (notated as scion:rootstock). Flowering time of WT:T was not significantly altered compared to the control WT:WT plants, indicating that a transgenic rootstock alone is insufficient for earlier flowering; and the fact that T:T plants did not differ from T:WT shows that a transgenic rootstock does not contribute to early flowering either. As expected, ungrafted T plants flowered earlier than any of the grafted plants due to growth delays resulting from graft wound healing. Taken together, these data suggest low production or low rootstock-to-scion transmission of VcFT when expressed in the tobacco rootstock parts.

P-3045

Expression Analysis of Salt Overly Sensitive 2 (SOS2) Gene Salt Stressed Tomato Plants. Ö. ÇELIK, B. Candar-Çakır, and S. Sinem. Istanbul Kültür Üniversity, Faculty of Science and Letters, Department of Molecular Biology and Genetics, Atakoy, Istanbul 01 34156, TURKEY. Email: ocelik@ iku.edu.tr

Ion homeostasis is important to prevent toxic effects of natrium ions which disturb the K^+ nutrition in salinity soils. K^+/Na^+ selective transport system, one of the ion homeostasis parameters, is known to be affected by salinity due to changes in cell membrane stability. SOS pathway genes, which consist of SOS1, SOS2 and SOS3 genes, is responsible to mediate Na^+ transport in plants. SOS2 is an serine/threonin kinase and has main role in activating SOS1 which is an Na^+/H^+ antiporter and excluses Na^+ ions into apoplast. Increased SOS2 gene expression levels regulate Na^+/K^+ homeostasis in SOS scavenging pathway against salt stress. For this reason, in this study, we investigated the SOS2 gene expression levels between two different industrial tomato varieties (X5671R and 5MX12956) comparatively with cellular Na^+/K^+ uptake levels. We subjected 14 day-old tomato seedlings to two different (0, 100 mM and 200 mM) NaCl concentrations for 14 days. The leaves of the salt stressed and control plants were collected and used in analyses. Shoot lengths relative water contents, normalized difference vegetation index (NDVI), cellular Na^+/K^+ contents were estimated and SOS2 gene expression levels were determined with qRT-PCR. PCR conditions were standardized using gene specific primers for Elongation Factor 1-α and the relative expression levels were calculated by DDCt method. The photosynthetic index was decreased due to increasing salt concentration. Relative water content of salt stressed plants were increased with respect to control plants. While Na^+/K^+ content and the transcript level of SOS2 were increased in both tomato varieties in correlation with increasing salt concentrations. According to the results of growth parameters, Na^+/K^+ ratios, increase of SOS2 gene expression levels, 5MX12956 is found more salt tolerant than X5671R. The findings supports that the concomitantly evaluation of Na^+/K^+ ratio and increased transcript levels of SOS2 gene can be used as markers to select salt tolerant tomato varieties in breeding studies.

P-3046

Microencapsulation of *Urtica dioica (from Aydın Region of Turkey)* Leaf Extracts. Ö. ÇELIK and A. İnan. Istanbul Kültür Üniversity, Faculty of Science and Letters, Department of Molecular Biology and Genetics, Atakoy, Istanbul 01 34156, TURKEY. Email: ocelik@iku.edu.tr

Urtica dioica has usage in medicine, pharmaceuticals, cosmetics, dye, fibre and food industry. It is widely used in the cure of different diseases as animicrobial, antibacterial, antidiabetic and antiallergic, antienflamatuar and antiromatismal agent. It has a potent antioxidant effect due to its phenol contents. Therefore, an appropriate drug delivery system makes Urtica as a potential alternative drug fo several diseases. In this research, we studied for the development of chitosan based microspheres for leaf extract of Urtica which is collected from Aydın, Turkey. Chitosan microsphres were prepared by complex coaservation. Characterization of microspheres was also made by zeta potential, SEM and FTIR methods. Different encapsulation methods for evaluated to obtain the best efficiency results. By changing the pH of chitosan solution, encapsulation efficiency was changed from 89.1% to 94.1. Microsphere sizes are between 584.1-1395.3 nm. We observed burst effect in release profiles of chitosan microsphere formulations for Urtica extracts. To ameliorate these relase characteristics, new modifications should be evaluated.

P-3047

Molecular Marker Analyses of Salt Tolerant Soybean Mutants. Ö. ÇELIK, B. Candar-Çakır, and Ç. Atak. Istanbul Kültür Üniversity, Faculty of Science and Letters, Department of Molecular Biology and Genetics, Atakoy, Istanbul 01 34156, TURKEY. Email: ocelik@iku.edu.tr

The aim of this study was to determine the genetic distance between the salt tolerant soybean mutants and S04-05 control

soybean plants which were obtained from Black Sea Agricultural Institute, Samsun, Turkey, by molecular marker methods. The salt-tolerant mutant plants were generated by gamma radiation and were selected with relative to control plants. Selection studies were done due to in vivo survival under irrigation with nutrient solution containing 90 mM NaCl and in vitro callus formation ratios in MS medium containing 90 mM NaCl, relative to control plants. 8 mutant plants were used for the molecular marker analyses. These mutants were selected from M3 generation of S04-05 variety. 7 of these mutants were selected from plantlets which were irradiated by 150 Gy and one of the mutants were irradiated with doses of 250 Gy. They were grown under controlled conditions. Polymorphism rates between the mutants and control plant was evaluated due to the results of Random Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeats (ISSR) molecular marker methods. The genetic distance analyses were evaluated by using NTSYSpc version 2.1 programme. 68.43% polymorphism rate was obtained by using 105 random primers. The highest polymorphism ratio was observed in the OPJ primers (77.64%), while the lowest was observed in OPH primers (56.31%) by RAPD analyses.