In Vitro Cell.Dev.Biol.—Animal
DOI 10.1007/s11626-015-9906-y

Plant Posters

P-2000

ARS-MEDIA for Excel – An Excel Spreadsheet for the Calculation of Media Recipes Based on Ion-specific Constraints. R. P. NIEDZ. USDA-ARS-U.S. Horticultural Research Laboratory, 2001 South Rock Road, Ft. Pierce, FL 34945-3030. Email: randall.niedz@ars.usda.gov

An Excel spreadsheet was developed that eliminates the need to learn complex linear programming mathematics required for mineral nutrition experiments to determine the effects of ion type, concentration, and proportion. The spreadsheet is specifically designed for the formulation of nutrient media used in such applications as plant tissue culture, hydroponics, algal culture, fertilizer formulations, microbial culture, and any application that requires the definition of a specific culture media by its ion composition and the component salts necessary to make that culture media. ARS-Media for Excel utilizes Excel's internal linear programming optimization algorithm to determine the combination of salts, acids, and bases that satisfy any given target solution of ions. This solves the difficult problem of generating a recipe for a specific combination of ions; thus allowing for the construction of experimental designs that use ions, as opposed to salts, as statistical factors. Using a salt to determine the effect of a specific ion requires accepting the confounding effect of the counter-ion of the salt used to manipulate the ion(s) of interest. The result is that the effect of a single ion cannot be determined. Using the spreadsheet is simple. The desired ion composition is entered and the spreadsheet solves for the recipe and generates a report that lists the salts to use and in what quantities. Additionally, the user has the capability of specifying what salts to use or not use, and to assign weights to each salt. This has the advantage that a user can specify and/or weight specific salts, such as those available in the laboratory or more easily handled.

P-2001

Improving Growth of *Quercus palustris* Embryo Axes In Vitro: a DOE Approach. A. VANHOVE[1], R. Niedz[2], and V. C. Pence[1]. [1]Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220 and [2]USDA-ARS-U.S. Horticultural Research Laboratory, 2001 South Rock Road, Ft. Pierce, FL 34945-3030. Email: annecatherine.vanhove@cincinnatizoo.org

Storage in liquid nitrogen (LN) has great potential for the long-term storage of short-lived and/or recalcitrant seeds, but few reports exist on the actual effectiveness of this method. In preparation for retrieval of embryonic axes of *Quercus palustris* after 22 years of storage in LN, a Design of Experiments (DOE) approach was used to improve a growth medium for embryonic axes of this species. Six factors were tested and included NO_3, PO_4, BAP, IAA, and MS vitamins and the proportion of NH_4:K. Embryonic axes were sterilized and placed on 33 different treatments. Measured responses include quantitative measurements (presence, number, and length of roots, shoots, and leaves and presence of callus) and qualitative observations (color of the roots, quality of roots, shoots, and leaves, and overall look of the whole plant). Callus formation was enhanced at higher IAA and BAP levels. The BAP level was a negative main driving force for the presence and number of roots as well as for a healthy root color. The NH_4:K ratio was also a positive driver for the latter. Overall, root growth benefitted from lower BAP and N, but a higher NH_4:K ratio was beneficial. The tested factors did not significantly affect initial shoot development or total shoot length. The presence of some BAP promoted multiple shoot development. Shoot appearance was negatively affected by higher IAA and N. Higher BAP and N levels inhibited leaf expansion and negatively affected the overall appearance of the plants. P and vitamin levels appeared to have little or no effect on plantlet development. These results are providing guidance for an improved medium for the recovery of oak embryos after cryopreservation. (This work supported in part by IMLS grant # LG-25-12-0595).

P-2002

Using Low Sucrose Concentration to Extend Time Between Sweet Potato Transfers. THOMAS W. ZIMMERMAN and

Published online: 13 May 2015

K. M. Emanuel. University of the Virgin Islands Agricultural Experiment Station, RR#1 Box 10,000, Kingshill, VI 00850. Email: tzimmer@uvi.edu

Virus-free sweet potato plants are being maintained in culture to supply clean plantlets, for field plot trials, and require frequent transfers due to the rapid rate of growth. However, between trials a system was needed to control rate of growth and increase the intervals between transfers. Long-term in vitro maintenance, on MS medium containing 0-12% sucrose, was used to evaluate shoot growth over time on six sweet potato cultivars. Sucrose levels from 2-12% had no influence on controlling in vitro rate of growth and development over time. Having no sucrose in the medium resulted in minimal growth but was lethal to 50% or more of the cultures. The rate of growth and leaf development was greatly reduced on sucrose levels from 0.1-0.3%. These low sucrose levels controlled the rate of growth and extended the interval between transfers from monthly to 9 - 12 months. Explants recovered when transferred back to 3% after one year. Low sucrose concentrations can be used to control the rate of in vitro growth of sweet potato and extend the intervals between transfers. This project was developed through grant funding by the USDA-NIFA-Insular Tropical Grant funds and USDA Specialty Crops Block Grant administered by the VI Dept. of Agric.

P-2003

Variations In Micropropagation Ratio of Yam (*Dioscorea Spp*.) Genotypes at Different Subculture Cycles. M. O. BALOGUN[1,2], N. Maroya[1], and R. Asiedu[1]. [1]International Institute of Tropical Agriculture, P. M. B. 5320, Ibadan, NIGERIA and [2]Department of Crop Protection and Environmental Biology, University of Ibadan, NIGERIA. Email: m.balogun@cgiar.org, mo.balogun@ui.edu.ng

Seed alone constitutes more than 50% of production cost of yam, while pests are major constraints. Conventional Tissue culture techniques of meristem culture combined with heat therapy have been used to produce high-yielding virus-tested plantlets, which are later multiplied through micropropagation using nodes. However, the micropropagation ratio is low, compared to banana where more than 6 shoots were obtained in a 30-day cycle. To improve our current ratio of 4-5 nodes in a 60-day cycle, we investigated the effect of subculture cycle after first introduction *in vitro* on number of nodes produced every 10 weeks of culture for 4 cycles. Five genotypes were evaluated in a completely randomized design with 5 replicates. The number of nodes at first introduction ranged from mean of 5.8 in Dente to 8.3 in TDa 291. In all genotypes except Dente where there was no significant difference, the ratio reduced by nearly half in the second cycle of subculture, and ranged from 3.2 in Obiturugo to 5.5 in Dente. In the third cycle, the ratio either decreased or there was no significant difference from the second cycle. In the 4^{th} cycle, the ratio had stabilized, with no significant difference between it and the 3^{rd} cycle and was half the ratio recorded at first introduction. These suggest the need to review the cultural conditions of yam *in vitro*, including light quality, duration of exposure and intensity in addition to culture aeration so as to sustain the vigour recorded at first introduction and achieve high micropropagation ratio. In addition, success of hardening of more vigorous plantlets will be higher, reducing germplasm losses. Prior to this, there should be active maintenance of vine stocks in screenhouses for re-introduction *in vitro* after at most 3 cycles of subculture.

P-2004

Artemisia annua a Biomatrix for the Synthesis of Fluorescent Silver Nanoparticles and Its Antibacterial Effect. NAFEESA KHATOON and Meryam Sardar. Enzyme Technology Lab, Department of Biosciences, Jamia Millia Islamia, New Delhi-110025, INDIA. Email: nafisakhan912@gmail.com, msardar@jmi.ac.in

In recent years, green synthesis of silver nanoparticles (AgNPs) has gained much interest from chemists and researchers. In this concern, Indian flora has yet to divulge innumerable sources of cost-effective non-hazardous reducing and stabilizing compounds utilized in preparing AgNPs. This study investigates an efficient and sustainable route of AgNP preparation from medicinal Plant *Artemisia annua* leaf extracts. Plant based synthesis are cost-effective, ecological friendly and efficient alternative for the large-scale synthesis of nanoparticles. After exposing the silver ions to the leaf extract, the rapid reduction of silver ions led to the formation of fluorescent nano silver. The synthesized fluorescent silver nanoparticles were characterized by UV-VIS, TEM, EDX, XRD, FTIR, Fluorescent spectroscopy and Fluorescent microscopy. The antibacterial activity of the nanoparticles against gram negative [*E. coli* (MTCC-405) and *P. aeruginosa* (MTCC-424)] and gram positive [*S. aureaus* (MTCC-3160), *S. epidermidis* (MTCC-435) and *B. subtilis* (MTCC-736)] bacterial strains were studied. The antibacterial effect was studied by MIC, MBC and Disk diffusion assay. The stability in biological media of fluorescent silver nanoparticles was demonstrated with the resistance to oxidation by the addition of H_2O_2. TEM analysis of fluorescent silver nanoparticles treated bacterial cells reveals that the silver nanoparticles exert antibacterial activity by disrupting the cell membrane structure and integrity. The biosynthesized silver nanoparticles were biocompatible which were confirmed by checking the cytotoxicity against human erythrocytes. The silver nanoparticles showed significant fluorescence and antibacterial activity,

indicating that has the potential to be used in the development of value-added products in the biomedical and nanotechnology-based industries.

P-2005

Towards Targeting Multiple Expression Cassettes into a Pre-characterized Genomic Locus of Sugarcane for Predictable Transgene Performance. YANG ZHAO[1], Jae Yoon Kim[1], Je Hyeong Jung[1], Bhuvan Pathak[1], Bruce Williamson[1], Chunyang Fan[2], Wenjin Yu[2], Shujie Dong[2], Vibha Srivastava[3], and Fredy Altpeter[1]. [1]Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida - IFAS, Gainesville, FL; [2]Syngenta Biotechnology Inc., Research Triangle Park, NC; and [3]Crop, Soil and Environmental Sciences, University of Arkansas. Email: altpeter@ufl.edu

Sugarcane, a tropical C4 grass in the genus *Saccharum* (Poaceae), accounts for nearly 80% of sugar produced worldwide. Sugarcane is also an efficient and sustainable feedstock for commercial biofuel production. Sugarcane is a highly polyploid and frequently aneuploidy interspecific hybrid, making crop improvement by breeding and genetic transformation challenging. Future transgenic strategies will require gene stacking for incorporating several traits or entire pathways. However, transgenes integrate randomly into the genome and genomic regions that support high level and stable transgene expression seem to occur less frequent in sugarcane than in other plants. Therefore, it is highly desirable to develop strategies for site directed integration of transgenes into the sugarcane genome. Our goal is to enhance predictability of transgene performance by targeting transgene integration into a pre-characterized genomic locus by site-specific recombination (SSR). The series of experiments include: 1) Construct vector that introduces SSR target shielded by insulators into sugarcane. 2) Generate and characterize expression stability of single-copy target lines to select events for re-transformation. 3) Remove selectable marker from target line by SSR system. 4) Create and characterize elite events following SSR of donor vector mediated by recombinase and promoter trapping of promoter-less selectable marker. We will report on the generation of single-copy target lines with high and stable transgene expression, removal of the selectable marker gene and regeneration and initial characterization of potential elite events following re-transformation of target lines with the donor vector.

P-2006

Stacking of Intragenic Herbicide Resistant Genes in Sugarcane. T. N. SILVA, T. Oz, and F. Altpeter. Agronomy Department, Plant Molecular and Cellular Biology Program and Genetics Institute, University of Florida - IFAS, Gainesville, FL. Email: tallyta@ufl.edu

Sugarcane is a prime crop for commercial production of table sugar and fuel ethanol. The incorporation of herbicide tolerance genes into sugarcane by genetic engineering will significantly reduce costs of weed control and allow low or no-till practices to reduce soil erosion. Herbicide resistance genes can also serve as selectable markers to recover events that are co-transformed with additional target genes. Intragenic biotechnologies utilize gene sequences derived from the target species or a sexually compatible species (e.g. sorghum sequences for sugarcane). Intragenic biotechnology may facilitate regulatory approval and reduce the costs and time required for this process. Our objective is to develop intragenic sugarcane with resistance to two type of herbicides, glyphosate and ALS-inhibiting herbicides. This will allow for rotation of herbicides and minimize the development of herbicide resistant weeds. Acetolactate synthase (ALS) is a key enzyme in the biosynthetic pathway of branched-chain amino acids. It is the target of highly selective ALS-inhibiting herbicides including sulfonylureas and imidazolinones. 5-enolpyruvoylshikimate-3-phosphate synthase (EPSPS) is a key enzyme participates in the biosynthesis of the aromatic amino acids phenylalanine, tyrosine and tryptophan. It is the target of the broad spectrum systemic herbicide glyphosate. Tow vectors encoding a mutant ALS and a mutant EPSPS gene from *Sorghum* were constructed using a constitutive ubiquitin promoter and the HSP 3'UTR from *Sorghum*. Following the removal of the vector backbone minimal expression cassettes were co-introduced into sugarcane by biolistic gene transfer. A selection protocol was developed by identifying a suitable herbicide and concentration for recovery of intragenic events from bombarded tissue cultures. Data describing the optimization of the selection protocol as well as molecular characterization of the regenerated plants will be presented at the conference.

P-2007

Development of Agave as an Integrated Bio-factory for Biofuel, Bioenergy and High Profit Bioproducts. C. GONZALEZ-VILLARREAL[1], I. S. Curtis[1], R. McQualter[1], J. Huynh[1], A. Van Amerongen[1], J. I. Reyes-Diaz[2], R. M. Nava-Becerril[2], J. L. Piña-Escutia[2], A. M. Arzate-Fernández[2], and S. M. Brumbley[1]. [1]University of North Texas, Biological Sciences, 1155 Union Circle #305220, Denton, TX 76203 and [2]Autonomous University of the State of Mexico, Facultad de Ciencias Agrícolas, Campus Universitario "El Cerrillo" Carr. Toluca-Ixtlahuaca Km. 15 entronque a El Cerrillo Piedras Blancas, Toluca, Estado de MEXICO. Email: claudiagonzalezvillarreal@my.unt.edu

Agave species has a crassulacean acid metabolism (CAM) that maximizes water use efficiency (WUE), allowing it to grow in semi-arid or marginal lands where high salt concentrations prevent the growth of many plants (Bergsten 2013). Commercially, Agave has been used for the production of industrial and dietary fibers, syrups, fructans and alcoholic beverages such as tequila and mescal (Portillo et al., 2007, Bezazi et al., 2014, and Velazquez-Martinez et al. 2014). In a joint collaboration between University of North Texas (UNT) and the Autonomous University of the State of Mexico (UAEM), work is under way to establish a germplasm collection for breeding and genomics studies. A tissue culture system for production of high quality planting material was developed at UAEM. At UNT the tissue culture system is being adapted for Agave transformation and mass propagation. Mature embryos (n = 346) of *Agave angustifolia* Haw were extracted from dry seeds and cultured on media designed to induce somatic embryos. The induction efficiency from mature embryos was 28.76%. Susceptibility to antibiotics was tested and biolistic transformation was performed with fluorescent protein markers. Various hormones were tested for optimal shoot regeneration.

P-2008

RNAi Mediated Silencing of *Triticum Mosaic Virus* Coat Protein Gene Induces Resistance to Virus in Transgenic Wheat. JESSICA L. RUPP[1], 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 Hard Winter Wheat Genetics Research Unit, Center for Grain and Animal Health Research, Manhattan, KS 66506. Email: jrupp@ksu.edu

Triticum mosaic virus (TriMV) is one of three viruses of the wheat mosaic complex affecting wheat in the Great Plains of the United States. Currently, there are no resistant commercial varieties. The disease management strategy incorporates mite vector control and various cultural practices; however, it is not fully effective. As an alternative strategy, we evaluated the use of interference RNA to generate resistance to these wheat viruses. A RNAi expression vector was created from the sequence of the coat protein of TriMV and immature embryos of the wheat cultivar 'Bobwhite' were co-transformed by biolistic particle delivery system with the RNAi expression vector and pAHC20, which contains the *bar* gene for glufosinate selection. After tissue culture and plant recovery, putative transformed plants were analyzed through PCR for the presence of the appropriate RNAi TriMV CP gene. Transgenic T_1 seeds were collected and each line was tested for transgene expression via RT-PCR. To determine viral resistance, T_1 progeny were mechanically inoculated with TriMV. Viral presence was established by ELISA. In the T_1 generation, resistance was seen in up to 80% of the plants evaluated for the TriMV CP construct. These plants have undergone single plant selection up to the T_6 generation and continue to show high level of resistance when challenged with the virus. Crosses have been made with the virus susceptible winter wheat, 'Overley.' Real-time PCR results show a decrease in viral titer up to 20-fold in the T_6 transgenic lines, the F1 crosses, and the BC1F1 compared to control plants. This research provides evidence that this RNAi silencing construct can provide stable resistance to TriMV and has great potential benefits to both breeders and producers.

P-2009

Expression of *Cry1Ac* Gene Driven by a Green Tissue Specific Promoter Conferred Resistance to *Helicoverpa armigera* in Chickpea. S. ACHARJEE[1], N. Hazarika, B. K. Sarmah[1], P. A. Kumar[2], J. Armstrong[3], W. J. Moar[4], A. Moore[5], and T. J. V. Higgins[5]. [1]Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat 785013, INDIA; [2] Institute of Biotechnology, ANGRAU, Rajendra Nagar, Hyderabad 500 030 A.P., INDIA; [3]CSIRO Entomology, GPO BOX 1700, Canberra, ACT 2601, AUSTRALIA; [4]Monsanto Company, 800 North Lindberg Blvd, St. Louis, MO 63167; and [5]CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601 AUSTRALIA. Email: sumita.aus@gmail.com

We report the generation of transgenic chickpea (*Cicer arietinum*) using *Agrobacterium* mediated gene transfer of *Cry1Ac* gene to confer resistance to *Helicoverpa armigera*. The binary vector contains an *nptII* gene as the selectable marker and *Cry1Ac* gene (either truncated or full length) driven by the Arabidopsis rubisco small subunit (SSU) gene (*ats1A*) promoter. We generated 54 and 46 independent transgenic lines using truncated and full length versions of the *Cry1Ac* gene, respectively. Of these lines, twelve lines transmitted the truncated *Cry1Ac* transgene to the next generation at a 3:1 ratio indicating a single copy of transgene insertion while only two (57 and 87) full length Cry1Ac lines transmitted the gene to the next generation. A total of six lines (60A, 75G, 81G, 81P, 100B and 100E) expressed Cry1Ac protein > 40 ng/mg FW, however, only five lines accumulated > 10ng / mg FW full length *Cry1Ac* protein. Expression levels of this high quantity of Cry1Ac protein have never been reported in chickpea. Moreover, the phenotypes of lines expressing high levels of Cry1Ac protein were indistinguishable from their null segregants and controls. The lines, 81G, 100B, 100E and 87 showed complete resistance to pod borer larvae in insect bioassays. The truncated Cry1Ac lines, 100B and 100E are being back-crossed in different genetic backgrounds for field trials.

P-2010

Overexpression of a Candidate Gene from *Xenorhabdus nematophila* in a Heterologous System Leads to Pest Resistance. S. MAHMOOD, M. Kumar, P. Kumari, N. Banerjee, and N. B. Sarin. School of Life Sciences, Jawaharlal Nehru University, New Delhi, INDIA. Email:saquibmahmood51@gmail.com

Plants express a wide variety of genes in response to plant pathogen or infection by pests. The best characterized genes belonging to PR protein family are those that encode the lytic enzymes such as chitinase, which play a vital role in plant defense against fungal pathogens due to their ability to hydrolyse the chitin, a mojor constituent of the lining of gut membrane of insects and the cell wall of a number of plant pathogenic fungi. Chitinase from different sources has been used as an important means of biological control for fungal diseases. In the present study, we have isolated a ~1.9 kb of chitinase gene from *Xenorhabdus nematophila*, a Gram negative, entomopathogenic bacteria. The chitinase gene was cloned and a ~ 76 kD protein corresponding to the gene was expressed in *E. coli*. Its chitinolytic activity was checked by chitinase assay. The recombinant protein was orally insecticidal to the major crop pest, *Helicoverpa armigera* when 40 μg/g of chitinase protein was fed to the larvae of insects. The chitinase protein was also over expressed in tobacco plants via *Agrobacterium* mediated transformation. The stable integration and the gene expression were checked by Southern blot and RT-PCR analysis respectively. The transgenic lines were phenotypically normal and healthy. The insect assays showed 100% mortality of the larvae when fed on transgenic plant leaves over expressing the chitinase protein and also 60-70% reduction in plant damage as compared to untransformed control plants. Our results demonstrate that the chitinase isolated from *Xenorhabdus nematophila* is toxic to *Helicoverpa armigera* and can prove to be a potent candidate for pest control in plants in future.

P-2011

Ectopic Expression of Cytoslic Ascorbate Peroxidase (cAPX) Confers Synergistic Tolerance to Abiotic and Biotic Stress in Musa. spp. SHASHI SHEKHAR[1,2], Anjana Rustagi[3], Neera Bhalla Sarin[1], and Kapil Lawrence[1,2]. [1]School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, INDIA; [2]Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad-211007, INDIA; and [3] Department of Botany, University of Delhi, New Delhi, 110021, INDIA. Email: shashibiochemistry@gmail.com

Banana (*Musa spp.*) is one of the world's leading fruit crops, predicted to be highly vulnerable to abiotic and biotic stresses. The present study is aimed to develop stress tolerant Banana through genetic manipulation, to enhance the antioxidant capacity. The candidate gene Cytosolic *Ascorbate Peroxidase* (*cAPX*), cloned under CaMV 35S promotor was introduced into Banana genome through vacuum assisted transformation of multiple shoot clumps. The integration and expression of *cAPX* in putative transformants were confirmed by PCR, southern and RT-PCR analysis. Transgenic Bananaover-expressing *cAPX* showed enhanced APX activity and total ascorbate pool. Electrolyte leakage and malondialdehyde content were significantly lower in transgenic plants as compared to the untransformed control (UC) plants under drought, salt and heavy metal stress. The free proline and relative water content were found to be higher in transgenic plants compared to UC plants. The transgenic plants were also evaluated for biotic stress tolerance against *Fusarium oxysporum f. sp. cubense.* The transgenic plants showed higher retention of chlorophyll and leaf water content with substantially decrease in fungal hyphae growth as compared to UC plants when infected with the *Fusarium oxysporum f. sp. cubense* under *in vitro* conditions. There was significant reduction in stomatal density and aperture size in transgenic plants to lower the levels of water loss in comparison to UC plants. Together, this finding suggests the potential use of cAPX gene to engineer the crop plants for broad spectrum biotic and abiotic stress tolerance to improve crop productivity which could be harnessed for the food security and poverty alleviation in developing country.

P-2012

Overexpression of *hlyB* Gene Confers Halotolerance in the Cyanobacterium *Fremyella diplosiphon.* B. TABATABAI[1], S. Arumanayagam[2], B. L. Montgomery[3], and V. Sitther[1]. [1]Department of Biology, Morgan State University, Baltimore, MD 21251; [2]Department of Pathology, Methodist Hospital Research Institute, Houston, TX 77030; and [3]Department of Biochemistry and Molecular Biology, Michigan State University, E. Lansing, MI 48824. Email: viji.sitther@morgan.edu

Fremyella diplosiphon, a fresh water-inhabiting cyanobacterium, is a widely studied model organism that can thrive at low light intensity and optimal temperature. In the present study, halotolerance in *F. diplosiphon* was enhanced by overexpression of the *hlyB* gene, an ABC transporter derived from the naturally halotolerant cyanobacterium *Aphanothece halophytica*. An expression plasmid harboring the *hlyB* gene was constructed and transferred to *F. diplosiphon*. Integration of the gene was performed using electroporation-mediated transformation and confirmed by molecular and physiological evaluations. PCR analysis of genomic DNA in the transformant strain confirmed presence of the insert at an

expected size of 1200 bp, which was verified by Sanger sequencing. In further studies, RNA was isolated from the transformant and cDNA was measured using quantitative reverse transcriptase-PCR. A ten-fold increase in the level of *hlyB* gene expression was observed in the transformant. Physiological evaluation demonstrated excellent growth of the transformant in BG-11 liquid media supplemented with 35 g/L NaCl (0.528±0.020) that was comparable to wildtype grown in BG-11 (0.563±0.054). Results of the study confirmed integration and overexpression of halotolerance-linked *hlyB* gene in the transformant. These findings indicate that electroporation-mediated transformation is an efficient method to enhance halotolerance in *F. diplosiphon*. Further studies will aim towards evaluating differential protein expression in the transformant and wild type strains.

P-2013

Salt Tolerant Cell Lines of *Arachis Hypogaea* as Repertoire of Antioxdant Enzymes for Abiotic Stress Tolerance. NEELAM PRABHA NEGI[1], Divya Shrivastava[1], Vinay Sharma[2], and Neera Bhalla Sarin[1]. [1]School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, INDIA and [2]Department of Biotechnology, Banasthali University, Rajasthan, INDIA. Email: sapphirenegi@yahoo.com

The salt tolerant (ST) cell lines of *Arachis hypogaea*, stably thriving at 200 mM NaCl were developed using multistep selection procedure. The ST cells showed higher transcript level of the antioxidant enzymes, ascorbate peroxidase and superoxide dismutase under multiple abiotic stresses, including drought, salinity, cold, and oxidative stress treatment. A cDNA library of *Arachis hypogaea* was used for isolating the gene for these antioxidant enzymes. Since, superoxide dismutase (SOD) constitutes the first line of defense against reactive oxygen species by converting superoxide anion radicals to hydrogen peroxide, which is further converted to non-toxic water and monodehydroascorbate, it can serve as a protective function during oxidative stress. A 556 bp long cDNA was isolated by RT-PCR and was cloned in pGEM-T vector. The deduced amino acid sequence of *AhCuZnSOD* showed higher identity with the cytosolic CuZnSOD from other species. A functional role of *AhCuZnSOD* in alleviation of abiotic stress was further exploited through its overexpression in the model plant tobacco and subsequently used for the transformation of a highly important oilseed crop, *Brassica juncea* (Indian Mustard). Compared with wild type plants, transgenic plants survived under longer period of water deficiency and salinity stress and displayed improved recovery after rehydration. The enhanced levels of antioxidant enzymes in the transgenic plants correlated with higher relative water content, improved photosynthetic efficiency, less electrolyte damage, elevated accumulation of compatible osmolytes, less malondialdehyde as well as H_2O_2 accumulation and O_2^- accumulation under stress conditions compared to untransformed wild-type controls. Our results substantiate that increased levels of SOD activity brought about by overexpression of *AhCuZnSOD* gene may play an important role in ameliorating oxidative injury induced by various environmental stresses.

P-2014

In Vitro Selection and Regeneration of Spring Barley Overexpressing the *Atwbc19* Gene. L. OHNOUTKOVA[1], P. Krenek[1], J, Vaskova[1], and A. Mentewab[2]. [1]Palacky University in Olomouc, Faculty of Science, Centre of the Region Hana for Biotechnological and Agricultural Research, Olomouc 783 71, CZECH REPUBLIC and [2]Spelman College, Biology Department, Atlanta, GA 30314-4399. Email: ludmila.ohnoutkova@upol.cz

The *Atwbc19* gene encoding an *Arabidopsis thaliana* ATP binding cassette (ABC) transporter, confers resistance to kanamycin specifically and could be used as an alternative to bacterial antibiotic resistance genes. This gene was recently used for the transformation of *dicotyledonous* plants, and was tested for its ability to confer resistance to different aminoglycoside antibiotics. Transgenic tobacco was only resistant to kanamycin, whereas transgenic hybrid aspen also shoved resistance to three other aminoglycoside antibiotics. We have begun testing the selection effectiveness of the *Atwbc19* gene from *A. thaliana*, which has been cloned into two expression vectors suitable for transformation of monocot plants. In the first expression vector, the *Atwbc19A gene* was under the control of the *Ubi* promoter and in the second *Atwbc19A* gene was under the control of the 35S promoter. The constructs were used to transform 1020 immature embryos of the spring barley variety Golden Promise by *Agrobacterium*-mediated transformation. Following transformation, the explants were cultured on selection and regeneration induction media which contained four different antibiotics,kanamycin, neomycin, geneticin and paromomycin, each at four different concentrations. It was found that the concentration of antibiotic had no significant effect on callus induction. However, plant regeneration appears to be strongly influenced by the type and concentration of antibiotic added to the medium. We also found that transgenic calluses of spring barley Golden Promise do not produce green plant on medium with kanamycin and paromomycin. The greatest selection efficacy of the *Atwbc19A* transgene was observed for the antibiotic neomycin at a concentration of 200 mg/L. In 80% of the regenerated plants, we detected the presence of the *Atwbc19A* gene by PCR. The efficiency of the gene of interest was markedly influenced by the promoter. Greater transformation efficiency

was shown by the transgene *Atwbc19A* under the ubiquitous promoter (*Ubi*). This work was supported by MEYS Czech Republic, project KONTAKT II LH13069.

P-2015

Isolation and Functional Characterization of an *AGAMOUS* Homolog from Black Ash (*Fraxinus nigra*). J. LEE[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: lee1512@purdue.edu, ppijut@purdue.edu

Black ash is a commercially valuable hardwood tree species that also provides food and habitat for wildlife. The wood is used for cabinets and flooring, and is preferred by Native Americans for making splints for basketry. The emerald ash borer (EAB), an aggressive exotic wood-boring beetle from Asia, is threatening all North American ash species. Development of EAB-resistant transgenic ash trees is needed in order to stop this devastating invasive pest and save the ash trees. However, transgenic trees are not allowed to be routinely planted because of concerns of transgene flow. Reproductive sterility can serve as an important tool for gene containment to reduce the probability of transgene movement through pollen dispersal. *AGAMOUS* (*AG*) is a C-function floral organ identity gene responsible for stamen and carpel development. The *Arabidopsis ag* mutant is sterile with petals and new flowers instead of stamens and carpels. *FnAG*, an *AG* homolog from black ash (*Fraxinus nigra*) was isolated by conducting reverse transcription-polymerase chain reaction and rapid amplification of cDNA ends. The deduced amino acid sequence showed a typical MIKC structure of type II plant MADS-box proteins with a highly conserved MADS-box and K-box. Phylogenetic analysis revealed that *FnAG* has a close relationship with other *AG* homologs from green ash, poplar, black cherry, and Chinese chestnut. Expression of *FnAG* transcript was detected in the reproductive tissues (female and male flowers), but rarely detected in the vegetative tissues (leaves), consistent with the ABC model for floral development. A functional analysis was performed by ectopic expression of *FnAG* driven by the CaMV35S promoter in transgenic *Arabidopsis*. Transformed plants showed small rosette leaves and early flowering time compared to wild type plant. Phenotypic mutations were observed including flowers with short sepals and defective petals; stamen-like petal in the second whorl; and curled cauline leaves. These data suggested that the *FnAG* functions in the same way as *AG*.

P-2016

Comparative Asymbiotic and Symbiotic Seed Germination of the Ghost Orchid, *Dendrophylax lindenii.* N. H. HOANG[1], M. E. Kane[1], and L. W. Zettler[2]. [1]Environmental Horticulture Department, University of Florida, Bldg. 68, PO Box 110675, Gainesville FL 32611-0675 and [2]Department of Biology, The Illinois College, 1101 West College Ave., Jacksonville, IL 62650. Email: nhhoang@ufl.edu

In this study we explored seed germination and seedling development of the leafless Ghost orchid, *Dendrophylax lindenii* (Lindl.) Benth. *ex* Rolfe, under symbiotic (co-culture with mycobiont) and asymbiotic seed culture. The first goal of this study was to determine the morphological and anatomical features of asymbiotically cultured seedlings. Seed germination and subsequent seedling development on modified ¼X MS medium (asymbiotic culture) were monitored under light at 5-day intervals for 80 days. Ghost orchid germination/seedling development was divided into seven distinct stages: (0) no germination, viable embryo; (1) seed with swollen embryo; (2) enlarged green embryo with ruptured testa; (3) protocorm with trichomes or first crest; (4) protocorm with elongated first crest; (5) protocorm with emerging leaves or first root; and (6) seedling with elongated roots. These development stages were used to numerically compare germination and seedling development rates during symbiotic co-culture with one of two mycorrhizal fungi (*Ceratorhiza* sp. Isolate #394 or 379 isolated from Ghost orchid roots) on oatmeal agar medium with those cultured on asymbiotic culture medium. Visual observations and histological sectioning revealed a highly modified seedling shoot and root structure consisting of a central stem core. Rapid formation of fungal pelotons in protocorm cells under symbiotic culture conditions was verified. Our studies have allowed quantification and comparison of seedling development in this unique orchid species using different germination methods.

P-2017

Efficient Regeneration and *Agrobacterium*-mediated Transformation System for Various *Musa* Cultivars. JAINDRANATH TRIPATHI[1,2], Evans Nyaboga[1], Richard O. Odour[2], and Leena Tripathi[1]. [1]International Institute of Tropical Agriculture, KENYA and [2]Dept. of Biotechnology and Biochemistry, Kenyatta University, KENYA. Email: j.tripathi@cgiar.org

Banana and plantain (*Musa* spp.) are important staple food for rural and urban consumers and provide a source of income for resource poor farmers in the humid tropics of sub-Saharan Africa. In spite of the importance, the crop is vulnerable to

many pests and diseases. Most of the banana and plantain cultivars are triploids and difficult to improve through conventional breeding due to sterility, various level of polyploidy and long generation life cycle. Genetic engineering offers an alternative and effective approach for improvement of banana. Embryogenic cell suspension (ECS) is basic starting material for developing transgenic banana. We have investigated two different explants (immature male flowers and multiple buds) for developing ECS from several cultivars of banana and plantain. ECS were developed from 'Gros Michel', 'Cavendish Williams', 'Gonja manjaya' using multiple buds and ECS of 'Sukali ndiizi' was developed using immature male flowers. Regeneration efficiency of the developed ECS was investigated and the results showed that about 20,000-50,000 plantlets were regenerated from 1 ml of settled cell volume (SCV) of ECS depending on cultivar used. ECS were co-cultivated with *Agrobacterium* strain EHA105 harboring a binary vector pCAMBIA2301, followed by selection of kanamycin-resistant embryos and regenerated plantlets. Depending on the cultivar, 30-70 independent transgenic events per 1 ml SCV of ECS were regenerated on selective medium. Histochemical GUS assays confirmed the expression of *gusA* gene in transgenic tissues. Molecular characterization was performed by PCR, dot blot and Southern analysis to confirm the presence and integration of the transgene in transgenic plants. Here we report an efficient regeneration and *Agrobacterium*-mediated transformation protocol for stable integration of foreign genes into different cultivars of banana and plantain. This transformation system could be useful for future studies on transferring economically important genes into banana and plantain.

P-2018

Cryopreservation of Citrus Seeds Via Dehydration and Direct Immersion in Liquid Nitrogen. G. ALDEMIR[1], E. Kaya[2], E. Yilmaz-Gokdogan[1], F. V. D. Souza[3], and M. M. Jenderek[4]. [1]Mugla Sitki Kocman University, Faculty of Science, Biology Department, Kotekli, Mugla 48000, TURKEY; [2]Mugla Sitki Kocman University, Faculty of Science, Molecular Biology and Genetics Department, Kotekli, Mugla 48000, TURKEY; [3]Embrapa Cassava & Fruits, Caixa Postal 007, Cruz das Almas, BA 44380-000, BRAZIL; and [4]USDA-ARS National Center for Genetic Resources Preservation, Fort Collins, CO 80526. Email: ergunkaya@mu.edu.tr

Citrus germplasm is conventionally conserved in clonal orchards and greenhouses, where it is subjected to potential losses due to pests, diseases and climatic hazards. In recent years, many studies have dealt with cryopreservation of the genus Citrus. As a result, effective freezing protocols have been reported for various organs and tissues such as shoot tips, seeds, embryonic axes, somatic embryos, ovules, embryogenic callus and nucellar cells. An important alternative for plant germplasm conservation is offered by biotechnology-based approaches as cryopreservation refers to the storage of plant material at ultra-low temperatures in liquid nitrogen. An effective cryopreservation procedure of seed dehydration and direct immersion in liquid nitrogen was developed for polyembryonic seeds of different Citrus cultivars from Turkey. Seed dehydration was performed different exposure times, in the sterile air current of a laminar flow-hood. All cultivars showed the best adaptability to seed cryopreservation, after the seeds were appropriately dehydrated between 21.8% (*Poncirus trifoliata* Raf. x *C. sinensis* Osb.) and 17.6% (*C. limonia* Osb.). The optimum post-freezing germinability ranged from 73.3% (*Poncirus trifoliata* Raf. x *C. sinensis* Osb. and *Fortunella margarita* (Lour.) Swingle) to 93.3% (*C. jambhiri* Lush). All citrus cultivars benefited from the dehydration treatments in terms of germinability after cryopreservation, the cryopreserved seedlings had well-formed roots, and acclimatization to *in vivo* conditions was easy.

P-2019

Pectin Methylesterase Action Reduces Sugar Beet Pulp Water Binding in an In Vitro Functional Assay. MEGAN CEASE[1,2], Jose C. Tovar[1,2], Ningning Zhang[1,2], Jianfeng (Jay) Xu[2,3], and Brett J. Savary[2,3]. [1]College of Science and Mathematics, [2]Arkansas Biosciences Institute, and [3]College of Agriculture and Technology, Arkansas State University, Jonesboro, AR. Email: bsavary@astate.edu, megan.cease@smail.astate.edu

The North American beet sugar industry annually generates tens of millions of tons of wet pulp residue. A high cost (30% of the energy in a beet processing plant) is incurred by pressing and drying beet pulp to reduce its water content from 90 to 15%. Pulp drying is necessary to stabilize for storage and reduce weight for transport, before its final use as an animal feed ingredient. This biomass is highly hydrophilic because it is rich in pectin, a complex cell wall polysaccharide that entraps water. We are investigating two pectinases, a thermally-tolerant pectin methylesterase (TT-PME) and a thermostable endo-arabinanase (TS-ABN), for improved pulp pressing through calcium-mediated pectin cross-linking. Calcium is often used as a pressing aid in pulp processing. These enzymes maintain stable activity at the high temperature (70°C) of water used to diffuse sucrose from beet roots. These pectinases are thus candidates for genetically engineering in beet roots if they can provide an effective output trait for beet processing. We hypothesize that action by PME and ABN on pectin structures in beet root cell walls will modify pulp's functional properties, resulting in a reduced water-binding

capacity through enhanced calcium cross-linking. For this study we developed an *in vitro* functional assay to assess PME and ABN treatments supplemented with calcium on the water-binding capacity by beet pulp. We present evidence that PME action dramatically reduces water binding in the presence of calcium, having an effect about 3-fold greater than pulp treatments with calcium alone. In contrast ABN treatment (independent or in combination with PME and calcium) has no significant effect on water-binding. These results provide proof-of-principle for recombinant TT-PME expression in sugar beet roots. We are further evaluating the ABN to increase bioavailability of functional oligosaccharides for improving pulp's nutritional value in new food and feed applications.

P-2020

Modification of Terpenoid Biosynthetic Pathway for Biofuel Production in Sorghum. I. S. CURTIS[1], R. Nair[2], H. Lee[2], K. Bastola[3], R. Nandakumar[2], S. Carlson[2], S. Brumbley[1], P. Vadlani[3], and K. Davenport[4]. [1]University of North Texas, Biological Sciences, 1155 Union Circle #305220, Denton, TX 76203; [2]Chromatin Inc., 2109 S. Oak St., Suite 101, Champaign, IL 61820; [3]101C BIVAP Innovation Center, Kansas State University, 1980 Kimball Avenue, Manhattan, KS 66506; and [4]Chromatin Inc., 10 South LaSalle St., Suite 2100, Chicago, IL 60603. Email: icurtis@chromatininc.com

Sorghum is a highly desirable plant for producing biofuels due to its tolerance to drought and high yields of starch, sugar and lignocelluloses compared to other bioenergy crops. To avoid expensive downstream processing and production costs, our research aims to elevate the level of sesquiterpenes that function as precursor for biofuel molecules such as farnesane, by modifying the endogenous terpenoid biosynthetic pathway in sorghum. To achieve this goal, genes from the mevalonate (MVA) and methylerylthritol 4-phosphate (MEP) pathways were expressed. Sorghum is generally considered recalcitrant, compared to other cereals, in terms of transgenic event production. Despite this low efficiency, delivering single and multistack gene constructs into embryogenic calli have yielded plants with up to nine genes representing the entire MVA pathway. We have also performed chemical induction experiments to evaluate the effect on sesquiterpene production in sorghum. This presentation will update the progress made on the genetic modification of sorghum for engineering sesquiterpene production.

P-2021

Transformation of Soy (*Glycine max*) for Heightened Expression of the SIZ1 Gene. TIM DEMARSH, Thien Luu, and Peiyu Zeng. SUNY Cobleskill, State Route 7, Cobleskill, NY 12043. Email: demarst975@cobleskill.edu

SIZ1 is a member of the PIAS family of proteins. One of this enzyme's functions is to act as an E3 ligase, conjugating small ubiquitin-related modifiers (SUMOs) to various substrate proteins as part of the process called sumoylation. Sumoylation alters the chemical conformation and function of substrate proteins, affecting their interactions with other cellular constituents and their resultant physiological roles. At the molecular level, sumoylation affects myriad biochemical processes, ranging from DNA repair and regulation of the cell cycle to signal transduction, nuclear transport, and modification of transcription factor activity. Constitutive expression of the *SIZ1* gene in transgenic creeping bentgrass (*Agrostis stolonifera L.*) has been demonstrated to result in more robust growth and higher levels of photosynthetic activity than are seen in non-transformed controls; heightened *SIZ1* expression also results in increased levels of adaptive response to stressors including elevated ambient temperatures, drought conditions, and phosphate deprivation. With the current experiment, *Agrobacterium tumefaciens* is being utilized to transform soy (*Glycine max*) with an additional copy of the endogenous *SIZ1* gene; the aim is to develop improved soy cultivars whose elevated expression of *SIZ1* will result in increased resilience under such stress conditions as are increasingly experienced during the cultivation of this economically important plant.

P-2022

Engineering a Thermostable Endo-arabinase *In Planta* for Post-harvest Recovery of Functional Oligosaccharides from Cell Wall Polysaccharides. C. ELMS[1], N. Zhang[1], B. Savary[1,2], and J. Xu[1,2]. [1]Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR 72401 and [2]College of Agriculture and Technology, Arkansas State University, Jonesboro, AR 72401. Email: christop.elms@smail.astate.edu

Varieties of "sugar beets" (*Beta vulgaris* L.), while a table sugar resource, are being explored as alternative bioenergy crops. To make use of sugar beets in biofuel production economically feasible, an effective method of recovering value-added products from the beet pulp byproduct should be developed. Functional oligosaccharides, like feruloylated arabinooligosaccharides (FAOs), can be extracted from the beet pulp with hydrolytic enzyme processing. FAOs may be used in food and feed applications for healthful colon function as implicated by prebiotic, anti-inflammatory and mucosal immunomodulatory activities. The *long term* goal of this project is to engineer "energy beets" with *in planta* expressed glycosyl hydrolases for either *in vivo* or *post-harvest* modification of

cell wall polysaccharides, facilitating production of industrial sugars and recovery of functional oligosaccharides from regional crops. This project aims to provide a proof of concept by engineering, in tobacco, a novel designer polymer tag consisting of 18 tandem repeats of a "Ser-Pro-Pro-Pro-Pro" motif, or $(SP4)_{18}$ and attaching this to a thermostable endo-1, 5-α-L-endoarabinase (ABN) that can selectively cleave the arabinan chain of beet pulp polysaccharides to release FAOs. The $(SP_4)_{18}$ tag is able to direct extensive hydroxyproline-O-glycosylation with oligo-arabinosides in plants, and presumably functions as a molecular carrier to direct the accumulation of the expressed ABN in the cell wall matrix and protect the enzyme from proteolytic degradation. The *in plant*-expressedenzyme was characterized in terms of expression level, enzymatic activity, subcellular localization and phenotype.

P-2023

Agrobacterium-mediated Transformation of *Camelina sativa*, an Oilseed Biofuel Plant. O. ENITAN[1], B. Tabatabai[1], S. A. Dhekney[2], and V. Sitther[1]. [1]Department of Biology, Morgan State University, Baltimore, MD 21251 and [2]Department of Plant Sciences, University of Wyoming, Sheridan, WY 82801. Email: viji.sitther@morgan.edu

Camelina sativa, an oilseed species rich in poly-unsaturated fatty acids, has gained great importance as an industrial oil platform crop in recent years. In the present study, shoot apical meristems of three *C. sativa* cultivars (Pl650159, Pl650161, and Pl258367) were co-cultivated using *Agrobacterium* strain 'EHA 105' harboring the green fluorescent protein (GFP) and neomycin phosphotransferase II genes. After three days of co-cultivation in the dark, explants were transferred to MS medium containing 1.5 mg/L BAP and 0.5 mg/L NAA. Transgenic shoots were identified on the basis of green fluorescence and kanamycin resistance. Kanamycin at a concentration of 40 mg/L in the selection media suppressed growth of non-transformed cultures and enabled uniform recovery of non-chimeric transgenic explants. Additionally, shoot weight of all three cultivars exposed to 40 mg/L kanamycin was significantly lower ($p < 0.05$) compared to cultures grown in the absence of the antibiotic. Integration of the gene in transgenic shoots was validated by polymerase chain reaction (PCR). Quantitative real-time PCR confirmed the presence and copy number of transgenes in the genome of transgenic explants. In future studies, the three cultivars will be transformed with genes for abiotic stress tolerance and qualitative traits for successful *C. sativa* production in marginal land. Incorporation of value-added traits through genetic transformation will maximize the use of this biofuel crop.

P-2024

Sugarcane Biotechnology for Improved Photosynthesis and Biomass Production. R. KARAN[1], X. Zhang[1], A. Vilharinho[1], A. K. Grennan[2], B. Kannan[1], N. S. Jaikumar[2], R. Slattery[2], C. Cahill[2], D. Ort[2], Steve Long[2], and Fredy Altpeter[1]. [1]Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida, Gainesville, FL 32606 and [2]Department of Plant Biology, Department of Crop Sciences, University of Illinois, Urbana, IL 61801. Email: rkaran@ufl.edu

Sugarcane is one of the most productive biofuel crops. Due to C4 type metabolism for fixation of carbon, sugarcane is very well adapted to biomass production in tropical and subtropical regions. Genetic improvement of photosynthetic efficiency can be achieved by targeting higher photosynthetic rates/unit of leaf area and by developing the most photosynthetically effective canopy. We introduced several transgenes into sugarcane and will report on transgene expression and its effect on altered phenotypes as well as their consequences on photosynthesis and biomass production under controlled environment and field conditions.

P-2025

Generating a Novel Soybean (Glycine max) Cultivar with Resistance to Nematode Infection and High Performance in Adverse Conditions by Overexpressing Cysteine Protease Inhibitor 1. T. LUU, T. Demarsh, and P. Zeng. SUNY Cobleskill, State Route 7, Cobleskill, NY, 12043. Email: thienluu300190@gmail.com

Unique proteases secreted by pathogenic organisms have been identified to play important roles in these organisms' pathogenesis. These proteases allow pathogenic organisms to invade host organisms, re-appropriate host nutrients, and evade host immune systems. Among these peptidases, cysteine proteases have been identified as the most important players in the parasitic infection of plants. Cysteine protease inhibitors have been shown to be the most effective compounds produced by plants to help them prevent the infection by pathogenic microorganisms. Overexpression of soybean cysteine inhibitor 1 (GmCPI1) in Arabidopsis significantly enhances its resistance to pest infection. Excessively expressing GmCPI1 has resulted in the increase in the performance of transgenic Arabidopsis under many adverse conditions, such as drought and high salinity. In this project, we are attempting to use develop novel soybean cultivars that overexpress GmCPI1 by using Agrobacterium mediated plant transformation method. Such cultivars will potentially show increase resistance to pest infections and other unfavorable conditions.

This research may provide a promising strategy to improve the pest resistance of other economically important plants and food crops, as well as increase their performance under adverse conditions, resulting in increasing crop yields.

P-2026

Phytochemical and Biological Investigations of *Asparagus adscendens* Growing in Himalayas Region of Pakistan. ABDUL MANNAN[1], Kashif Maqbool Khan[1], Muhammad Arfan[3], Ihsan-ul-Haq[2] and Izhar Hussain[1]. [1]Department of Pharmacy, COMSATS Institute of Information Technology, Abbottabad 22060, PAKISTAN; [2]Department of Pharmacy, Quaid-i-Azam University, Islamabad 45320, PAKISTAN; and [3]Department of Chemistry, School of Natural Sciences, National University of Science and Technology, Islamabad 46000, PAKISTAN. Email: abdulmannan_ka@yahoo.com

The aim of the current research work was to carry out various phytochemical and biological investigations of five fractions prepared from the methanolic extract of roots of *Asparagus adscendens*. All the fractions were prepared by using column and thin layer chromatography. According to the results, high total phenolic contents and total flavonoid contents were found in the fraction AAMD (11.8μgGAE/mg and 209.54μg QE/mg, respectively). The highest DPPH free radical percentage scavenging and ferric reducing antioxidant power was also measured in the fraction AAMD (13.46% and 49.05μg AAE/mg, respectively) and highest total antioxidant capacity was found in the fraction AAMG (121.15μgAAE/mg). In brine shrimp lethality bioassay, the lowest LD_{50} was calculated for the fraction AAMF (0μg/mL). The most significant antifungal results were obtained by the fraction AAMD against *Aspergillus fumigatus* and *Mucor* species (18.5mm and 15.25mm, respectively) and mild results were observed against *Bordetella bronchiseptica*. While no significant results were obtained for antibacterial activities. Antileishmanial activity of various fractions was performed against *Leishmania tropica* at concentration of 200μg/mL and excellent results were observed against the fractions AAMC, AAMD and AAME with 0% survival. All results showed that plant has good medicinal potential. This is a first report on phytochemical and biological evaluation of *Asparagus adscendens* of growing in foot hills of Himalayas region of Pakistan.

P-2027

Evaluation of Lox-flanked Pollen-specific Gene Excision from the *Arabadopsis thaliana* and *Nicotiana tabacum* Genomes by Cre Activity. E. PRUETT, J. Underwood, and V. Srivastava. University of Arkansas, Department of Crop, Soil and Environmental Sciences. Email: exp009@uark.edu

The heritability of transgene escape is undesirable, emphasizing the need for an efficient method for prevention by transgene excision or pollen mortality. With this study we strategize, through the use of the Cre-lox recombination system, which will excise the gene, if successful. If the Cre-lox system is not successful, pollen ablation will occur through the use of a pollen-specific promoter followed by expression of the diphtheria toxin A-chain (DTA) gene. The vector construction and *Agrobacterium* transformation in *Arabidopsis* and *Nicotiana* has been completed. We included GFP in the construct, which allows screening for pollen that contains the transgene. This shows the efficacy of either transgene excision or pollen ablation. Data on the development of transgenic plants will be presented.

P-2028

Genetic Transformation of Specialty Crops. GUO-QING SONG. Plant Biotechnology Resource and Outreach Center, Department of Horticulture, Michigan State University, East Lansing, MI 48824. Email: songg@msu.edu

Specialty crops are a group of mostly cash crops including fruits, tree nuts, vegetables, culinary herbs and spices, and medicinal plants. These economically important crops are highly valuable for human nutrition, health, and food security. Moving forward in the genomic era, reliable biotechnology tools will play an important bridge role in using functional genomics and genome editing to improve specialty crops. The Plant Biotechnology Resource and Outreach Center (PBROC) of Michigan State University (MSU) is providing service for transformation of atropa, canola, celery, petunia, rice, tobacco, and tomato. The Center has also developed workable protocols for transformation of apple, blueberry, camptotheca, cherry, rutabaga, strawberry, sweet potato, and switchgrass (http://www.ptc.msu.edu/index.html); contract collaboration for transformation of these plants, as well as development of efficient protocols for micropropagation or transformation of other plant species, are available. In addition, the PBROC provides training on gene cloning, plant micropropgation and transformation.

P-2029

Engineering Designer Biopolymers for Reconstructing Plant Cell Walls to Improve Biomass Processability. JIANFENG XU[1,2], Hong Fang[2], Ningning Zhang[1], Gregory Phillips[2], and Brett Savary[1,2]. [1]Arkansas Biosciences Institute, [2]College of

Agriculture and Technology, Arkansas State University, Jonesboro, AR 72401. Email: jxu@astate.edu

Efficient conversion of lignocellulosic biomass to biofuels and biobased products has been hampered by recalcitrance of the plant cell wall to biochemical deconstruction. This project aims to leverage hydroxyproline (Hyp)-*O*-glycosylation – a process unique to plant cell wall glycoproteins – as an innovative technology for *de novo* design and engineering of novel "designer biopolymers" (DBPs) that can facilitate plant cell wall reconstruction. This strategically designs Pro/Hyp-rich repetitive peptides, such as tandem repeats of "Ser-Pro" and "Ser-Pro-Pro-Pro-Pro" motifs as the DBP peptide backbone, which direct extensive Hyp-*O*-glycosylation in plants with arabinogalactan polysaccharides and oligoarabinosides, respectively. Such Hyp-*O*-glycosylated DBPs are proposed to be able to function as a *molecular carrier* for *in planta*-expressed cell wall-modifying (CWM) enzymes, targeting the enzymes to the cell wall matrix and stabilize the enzymes. Two types of DBP design, $(SP)_{32}$ comprised of 32 tandem repeats of "Ser-Pro" motif and $(SP4)_{18}$ comprised of 18 tandem repeats of "Ser-Pro-Pro-Pro-Pro" motif, was each engineered into tobacco plants as fusion with a reporter protein, enhanced green fluorescence protein (EGFP). The $(SP4)_{18}$ tag showed a better effectiveness on improving the accumulation of the tagged EGFP in cell wall matrix than the $(SP)_{32}$ tag. A thermostable CWM enzyme, E1 endoglucanase from *Acidothermus cellulolyticus*, was then engineered into tobacco as a fusion with the $(SP4)_{18}$ tag. The enzyme activity and subcellular localization of the enzyme, as well as phenotype and enzymatic digestibility of the plants were investigated. These DBP tags are also being engineered into the bioenergy crop−switchgrass to study their function as a molecular carrier in a monocot plant.

P-2030

In Vitro and In Planta Expression of Thermostable Endo-arabinase for Generating Functional Oligosaccharides from Plant Cell Wall for Colon-specific Health Benefits. NINGNING ZHANG[1], Brett Savary[1,2] and Jianfeng Xu[1,2]. [1]Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR 72401 and [2]College of Agriculture and Technology, Arkansas State University, Jonesboro, AR 72401. Email: Ningning.zhang@smail.astate.edu, jxu@astate.edu

Lignin-deficient (LD) plant fibers, e.g., sugar beet pulp and rice bran, are rich sources of functional cell wall polysaccharides. Used primarily as low-value animal feed, these established feedstock can be capitalized on for generation of value-added bioproducts, which will improve the economic viability and competitiveness of sugar beet and rice industry. The overall *goal* of this project is to develop an efficient enzymatic platform for generating functional oligosaccharides, specifically, feruloylated arabino-oligosaccharides (FAOs), from LD plant fibers. FAOs may be used in food and feed applications for healthful colon functioning through prebiotic, anti-inflammatory and mucosal immunomodulatory activities. FAOs of defined structure can be released from LD fibers through selectively cleaving the arabinan chain of cell wall polysaccharides by a key glycohydrolase - arabinase (ABN). In this study, a thermostable endo-1,5-α-L-ABN from *Bacillus thermodenitrifican* was expressed in yeast (*Pichia pastoris*) to produce significant quantity of enzyme for testing its activity of digesting plant fibers. Recombinant enzyme was secreted into culture media at a yield of ~70 mg/L and showed a specific enzymatic activity of 350 U/mg. ABN was also expressed *in planta* (in tobacco) to assess its function in *post-harvest* modification of cell wall polysaccharides to facilitate *in situ* FAOs releasing. Transgenic tobacco accumulated ~20 μg/gFW active ABN, and were not phenotypically different from wild-type plants. Finally, T84 epithelial cell culture was established to assay the anti-inflammatory activities of FAOs. Preliminary study indicated that FAOs isolated from rice bran significantly enhanced the expression of occludin, a biomarker of epithelial cells' immune response to FAOs.

P-2031

Plant Transformation Services. HYEYOUNG LEE, Liwen Zhou, Neng Wan, Xiaoyan Yin, Hanbing Li, Muruganantham Mookkan, Kaixuan Duan, Hua Liu, Michelle Folta, Phat Do, Chu Wu, Zixiao Zhao, Chris Willig, and Zhanyuan J. Zhang. Plant Transformation Core Facility, Division of Plant Sciences, University of Missouri, Columbia, MO 65211. Email: leehye@missouri.edu, zhangzh@missouri.edu

University of Missouri (MU) Plant Transformation Core Facility has been providing state-of-the-art plant transformation services over the past 14 years. The facility is aiming at fostering plant science research by providing transformation services worldwide. The services are on fees for cost recovery only, not for profit. The facility staff is dedicated to providing various types of transformation services with a focus on maize (*Zea mays*), soybean (*Glycine max*), switchgrass (*Panicum virgatum)*, sorghum (*Sorghum bicolor*), wheat (*Triticumaestivum*), alfalfa (*Medicago truncatula*), as well as *Setaria viridis*. The service categories include both standard and customized transformation. Transformation systems for all crops utilize *Agrobacterium*-mediated approaches and somatic embryogenesis processes except for soybean and *Medicago*. The *Agrobacterium*-mediated cot-node transformation system coupled with organogenesis regime is

employed for soybean and *Medicago* transformation. The facility is also ready to take on new service projects to transform new plant species as user's requests. Research activities are geared towards developing high-throughput transformation systems, effective small RNA-mediated gene silencing, gene stacking through coordinated transgene expression, and precise genome modifications to meet the needs of crop improvement and genome discoveries. More details on the facility can be found at http//www.plantsci.missouri.edu/muptcf.

P-2032

Expression of a Cystatin Transgene Can Confer Resistance to Root Lesion Nematodes in *Lilium longiflorum* cv.'Nellie White'. K. KAMO[1], P. Vieira[1], S. Wantoch[1], C. Lilley[2], D. Chitwood[3], and H. Atkinson[2]. [1]U.S.D.A.,10300 Baltimore Ave., Bldg. 010A, Floral and Nursery Plants Research Unit, U.S. National Arboretum, Beltsville, MD 20705; [2]Centre for Plant Sciences, University of Leeds, Leeds, UK; and [3]U.S.D.A., Nematology Laboratory, Beltsville, MD. Email: Kathryn.Kamo@ars.usda.gov

Lilium longiflorum cv. 'Nellie White' is an important flower, being one of the most valuable species in the US (annual wholesale pot plant value above $20,000,000). The root lesion nematode (RLN) *Pratylenchus penetrans* is a major pest of lilies due to the significant root damage it causes. Our efforts to control this pest have focused on generating soybean hairy roots (as a transient test model) and stable transgenic lilies overexpressing a modified rice cystatin (Oc-IΔD86) transgene and challenged with root lesion nematodes. Lily transformation was achieved by gene gun co-bombardment using both a pBluescript-based vector containing the cystatin gene and pDM307 that contains a bar gene for phosphinothricin selection. Both soybean hairy roots and lilies overexpressing the OcIΔD86 transgene exhibited enhanced resistance to RLN infection, showing nematode reduction up to 75%. In addition, lily plants overexpressing OcIΔD86 displayed an increased plant mass and better growth compared to wild-type plants, thereby demonstrating an alternative strategy for increasing the yield and reducing nematode damage to this important floral crop.

P-2033

The Yellowing of Dry Powder Plant Tissue Culture Media. DAVID S. HART, Gary R. Seckinger, and Kenneth C. Torres. [1]*Phyto*Technology Laboratories, 9245 Flint St., Overland Park, KS 66214. david@phytotechlab.com

Dry powder plant tissue culture media are exposed to a variety of conditions when shipped around the world often because of the receiving countries time in customs. Evidence from our studies on the yellowing of plant tissue culture media suggests the color change is a result of oxidation of ferrous (Fe^{2+}) ions to ferric ions (Fe^{3+}). We induced pristine-white, dry powder MS media to yellow through direct exposure to relative humidity (20-40% RH) and 40-44°C up to 24 hrs. We then tested the growth of a number of species and found no statistically significant differences in growth between plant tissue grown on white pristine media and yellow media.

P-2034

Elevating the Lipid Content in Vegetative Sugarcane Biomass by Metabolic Engineering. SAROJ PARAJULI[1], Georgina Sanahuja[1], Ratna Karan[1], Hui Liu[2], John Shanklin[2], and Fredy Altpeter[1]. [1]Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida, IFAS, Gainesville, FL and [2]Biosciences Dept., Brookhaven National Lab 463, 50 Bell Ave, Upton, NY 11973. Email: altpeter@ufl.edu, sparajuli@ufl.edu

Sugarcane is one of the highest yielding biomass crops used for production of table sugar and fuel ethanol. Diverting carbon flux for the production of oil in vegetative biomass has emerged as a promising strategy for increasing energy density and biofuel yield. Storage lipids are mainly composed of energy dense glycerol esters of fatty acids, also known as triacylglycerol (TAG). We investigated the TAG accumulation in vegetative tissues of sugarcane following over-expression and, or RNAi suppression of several candidate genes. Single or multiple gene expression/suppression cassettes were co-delivered with the selectable *nptII* expression cassette by biolistic gene transfer into sugarcane callus. Plants were regenerated on geneticin containing culture medium and analyzed for presence and expression of target constructs by PCR and RT-PCR, respectively. Plants expressing single or multiple constructs or displaying suppression of target genes were analyzed for TAG content by GC-MS. These results demonstrate the feasibility of engineering sugarcane for accumulation of lipids in vegetative biomass and will open new prospects for biofuel applications.

P-2035

Developing Improved Micropropagation Medium for Hazelnuts (*Corylus avellana* L.) Based on Ion Concentrations. MELEKSEN AKIN[1], Randall P. Niedz[2], and Barbara M. Reed[3]. [1]Oregon State University, Department of Horticulture, ALS 4017, Corvallis, OR 97331; [2]USDA-ARS, U.S. Horticultural Research Laboratory, 2001 South Rock Road, Ft. Pierce, FL 34945-3030; and [3] USDA-ARS, National Clonal

Germplasm Repository, 33447 Peoria Rd, Corvallis, OR 97333. Email: akinm@onid.oregonstate.edu

Hazelnuts are one of the most challenging crops to propagate because of the varied nutritional requirements of the genotypes. Initial steps to improving growth medium determined needed changes to the minor nutrient components. Major mineral nutrients are not yet optimized. To develop a medium optimal for a wide range of genotypes, ion concentrations of NH_4^+, Ca^{+2}, Mg^{+2}, PO_4^{-2} and SO_4^{-2} were tested at a range from 0.5x to 2x those of DKW medium with revised minor nutrients. These ions were derived from stock solutions of KH_2PO_4, K_2SO_4, $MgSO_4 * 7H_2O$, $Mg(NO_3)_2 * 6H_2O$, $Ca(NO_3)_4 * 4H_2O$, NH_4NO_3, $(NH_4)_2SO_4$ and $NH_4H_2PO_4$. KNO_3 as K^+ and $NO3^-$ was used for pH adjustment. Response surface statistical software assigned 24 treatments and a DKW control was added. *Corylus avellana* hazelnut cultivars Dorris, Dundee, Jefferson, Wepster and Zeta shoots were tested. The most significant ion factor affecting plant quality was NH_4^+. All of the cultivars required much higher levels of NH_4^+ for better growth. Plant quality for most of the cultivars was also better with higher concentrations of PO_4^{-2}, Mg^{+2} and SO_4^{-2}. Requirements were low to medium for Ca^{+2} for most. Only 'Jefferson' required low PO_4^{-2} and high Ca^{+2} concentrations. Using ions as factors is a promising approach to tissue culture medium formulation. This first step indicated the general concentrations needed for an improved medium however, further studies are required for better growth of hazelnuts.

P-2036

Development of Efficient Protocols for Somatic Embryogenesis and Regeneration of Fluted Pumpkin (*Telfairia occidentalis* Hook F.). D. O. AWOSIKA[1], E. E. Uchendu[1], M. O. Balogun[2], and V. O. Adetimirin[1]. [1]Dept. of Agronomy, University of Ibadan, Oyo State, NIGERIA and [2]Dept. of Crop Protection and Environmental Biology, University of Ibadan, Oyo State, NIGERIA. Email: dotskulls@gmail.com, esteruchendu@yahoo.co.uk

Fluted pumpkin (*Telfairia occidentalis* Hook. F.) is a leafy vegetable popularly consumed in Africa. It is traditionally propagated by seeds that are characterized with sporadic germination, and poor rooting of plants. Efficient protocols were developed for the induction of somatic embryos (SE) and its regeneration into plantlets using cotyledons from mature seeds. This study specifically evaluated the effect of plant growth regulators (mg/l); 2,4-D (0, 0.01, 0.05, 0.1 and 0.5) and Kinetin (0, 0.1, 0.5, 1 and 2) on the induction of SE, and IAA (0 and 0.01), 2,4-D (0 and 0.01) with Kinetin (0, 0.02 and 0.1) on its regeneration into plantlets. A significantly ($p < 0.001$) higher number of SE (381) were formed on MS medium with 0.5 mg/l 2,4-D and 0.1 mg/l Kinetin after 4 weeks of culture. All SE were obtained through an intermediary callus. For the production of SE-derived plantlets, treatments with 0.01 mg/l IAA + 0.02 mg/l Kinetin) had significantly ($p < 0.001$) higher plantlets than other treatments. With such a high number of SE achieved through this study, and its regeneration into rooted plants, it is now possible to mass produce *Telfairia*, a genus with narrow genetic diversity. This is the first report of its kind to the best of our knowledge. The regenerated plantlets which had broad leaves and high vigour were morphologically similar to the parent. A cryopreservation technique for somatic embryos can now be developed for the long term storage of this species to reduce the problems associated with erratic germination of seeds in storage.

P-2037

Studying Micropropagation Response of *Mentha x piperita* in Solid and Liquid Culture Systems. A. S. ALHASAN[1], D. R. Bergey[1], S. L. Bieber[2], V. Sitther[3], Z. T. Li[4], D. J. Gray[4], and S. A. Dhekney[1]. [1]University of Wyoming, Sheridan Research and Extension Center, Sheridan, WY 82801; [2]University of Wyoming, Department of Statistics, Laramie, WY 82801; [3]Morgan State University, Department of Biology, Baltimore, MD 21251; and [4]University of Florida, Mid-Florida Research and Education Center, Apopka, FL 32703. Email: aalhasan@uwyo.edu

Mentha x piperita (peppermint) is an important species cultivated worldwide for its essential oil that is highly valued in the pharmaceutical and food industry. Peppermint regeneration protocols have practical applications for rapid propagation of disease-free planting material and crop improvement using genetic engineering. In the present study, peppermint regeneration was compared using solid and liquid culture. Shoot tips of cultivar 'Black Mitcham' were obtained from field-grown plants and surface sterilized in 25% bleach solution. The apical meristem was then isolated and placed on solid medium containing MS or C2D macronutrients and vitamins along with varying levels of BA, kinetin and 2iP. After 6 weeks, proliferating shoots were transferred to fresh medium and the number of shoots was recorded during transfer. The treatment exhibiting the highest shoot proliferation was used to study growth in liquid medium using Liquid Lab Rocker (LLR) vessels. Five apical meristems were transferred to each vessel containing 50 ml liquid medium and grown on a rocker shaker for 6 weeks. To induce rooting, shoots were transferred to medium containing MS or C2D macronutrients and vitamins along with varying levels of IBA and NAA. Well-developed plants were transferred to potting mix, acclimatized and then transferred to a greenhouse. A wide variation in shoot proliferation was observed among various treatments. The

maximum number of shoots (40.7) were produced on C2D medium with 4.0 μM BA (C2D4B) followed by MS medium with 4.0 μM BA (32.2). Among the rooting treatments, shoots on MS medium with 1.0 μM IBA produced the maximum number of roots (14.4). The number of shoots produced in liquid C2D4B medium (103.4) was significantly higher than those produced on solid medium (40.7). We are currently comparing growth rates of additional *Mentha* species. The development of micropropagation protocols for *Mentha* species will enable rapid availability of disease-free planting material.

P-2038

Studying Micropropagation Response of Cold-hardy Grapevine Cultivars. S. A. DHEKNEY[1], R. Kandel[1], D. R. Bergey[1], H. Shafer[2], V. Sitther[3], Z. T. Li[4], and D. J. Gray[4]. [1]University of Wyoming, Sheridan Research and Extension Center, Sheridan WY 82801; [2]Sheridan College, Biology Program, Sheridan WY 82801; [3]Department of Biology, Morgan State University, Baltimore, MD 21251; and [4]University of Florida, Mid-Florida Research and Education Center, Apopka FL 32703. Email: sdhekney@uwyo.edu

Grape production in temperate regions is limited by freezing temperatures, which makes it difficult to grow *Vitis vinifera* cultivars. The development of cold-hardy *Vitis* hybrids has enabled grape production in temperate regions worldwide. Although cold-hardy cultivars have been rapidly developed, access to such cultivars remains limited due to inadequate planting material. Micropropagation serves as an alternative to vegetative propagation for increasing the amount of planting material. To study the micropropagation response of grape cultivars 'Elvira' 'Frontenac', 'Glenora', 'Jupiter', 'Marquette', 'Marquis', 'Neptune', 'Thomcord' and 'Vanessa', shoot tips were obtained from field-grown vines and surface-sterilized using 25% bleach solution. The apical meristem was isolated by excising the subtending leaves and placed on C2D medium containing 4.0 μM BA (C2D4B) and 20 mg l^{-1} rifampicin. Proliferating shoots were transferred to fresh medium at 4 weeks intervals and the number of shoots was recorded during transfer. After 8 weeks, shoots were transferred to MS medium containing 0.5μM NAA for rooting. Well-developed plants were transferred to sterile potting mix, acclimatized for 4 weeks and then transferred to a greenhouse. A wide variation in shoot proliferation rate was observed among grape cultivars. The number of shoots obtained after four weeks of culture ranged from 20 – 48 per explant. A rapid increase in proliferation rate was observed following transfer of cultures to fresh C2D4B medium. The total shoot number obtained after 8 weeks of culture ranged from 126 – 480 among various cultivars. A high rooting percentage (>90%) was observed in all cultivars. We are currently studying the micropropagation response of additional grape cultivars. Optimizing micropropagation protocols will ensure availability of disease-free planting material in a short period of time.

P-2039

Comparison of Orchid Seed Germination and Development on Agar-solidified and Liquid Media. B. A. HUGHES and M. E. Kane. Environmental Horticulture Department, University of Florida. 2043 IFAS Research Drive, Gainesville, FL 32611. Email: benhughes@ufl.edu

Florida is home to approximately half of the orchid species found in the United States, the majority of which are classified as either endangered or threatened. Asymbiotic seed germination on agar-solidified media is often used as an effective means of producing genetically diverse orchid seedlings for conservation and restoration efforts. Utilizing liquid media bioreactors in this process offers the potential of increased propagation efficiency over current methods. Preparing a seedling inoculum for orchid bioreactor culture allows seeds to germinate in a low-salt medium prior to immersion in the large volume of liquid medium found in a bioreactor. Determining whether a liquid or agar-solidified medium provides the fastest and most uniform germination rates will be critical for developing an efficient orchid bioreactor culture protocol. Seeds of *Bletia purpurea*, a threatened Florida native orchid, were sown in both liquid and agar-solidified Knudson C medium supplemented with 10% coconut water. Comparative percent germination and seedling development stage under the two culture conditions were evaluated every 2 weeks over a 6 week period.

P-2040

Somatic Embryogenesis in *Zanthoxylum armatum* DC-an Endangered Medicinal Plant of the Himalayan Region. SUMIT PUROHIT, Kuldeep Joshi, I. D. Bhatt, and S. K. Nandi. G. B. Pant Institute of Himalayan Environment and Development, Kosi-Katarmal, Almora - 263643, Uttarakhand, INDIA. Email: sumit.biotech2@gmail.com

Zanthoxylum armatum DC (family-Rutaceae; Common name-Prickly ash), an important medicinal plant, has gained tremendous importance in recent times in Indian system of medicine as carminative and stomachic agent. The essential oil of the species is known for antibacterial, antifungal activities, and thus accelerated its demand in pharmaceutical industries. As a consequence reckless harvesting & overexploitation from the wild has increased, and this species has currently

found place in endangered category. Hence, the situation demands sustainable utilization and conservation of this species. The present study, therefore, aims to develop *in vitro* techniques for multiplication of this species through somatic embryogenesis using leaf explants. Leaf explants were cultured on MS medium supplemented with various concentrations of plant growth regulators. In the first set of experiments, the *in vitro* raised excised leaves were soaked in MS liquid medium supplemented with three different concentrations of Thidizuran (15, 25 and 50 μM) for different time periods (12, 24 and 48 h). After pre-treatment, explants were incubated in to an agar solidified MS medium supplemented with TDZ (0- 10 μM) and NAA (0.5-1.0 μM). After 2 months callus formation was started in all the combinations, and best callus was observed in the combination of TDZ 6.0 μM and NAA 0.5 μM. Callus was then transferred into different concentration of BAP; 5-15 μM, IAA; 0-1 μM & GA_3; 0.5 μM for shoot organogenesis. Maximum shoot number (8.0±0.82) and shoot length (4.6±0.15 cm) were observed in a combination of 4 μM BAP, 1 μM IAA and 0.5 μM GA_3. Shoots transferred in to ½ MS medium containing 50μM IBA for 24 h, 100% rooting was achieved followed by subsequent culture on the basal medium. The plants regenerated through this method and transferred to field for acclimatization where 80% plants survived. This method can be utilized for obtaining large number of planting material for conservation as well as commercial cultivation of the *Z. armatum*.

P-2041

Factors Influencing Dormancy and Sprouting of *In Vitro* Produced *Sagittaria latifolia* Corms. P. QUIJIA and M. E. Kane. Environmental Horticulture Department, University of Florida, Gainesville, FL. Email: paulinaquijial@ufl.edu

In vitro culture conditions, media composition and plant growth regulators were evaluated to determine their effects on dormancy and sprouting capacity of *in vitro* produced corms in the wetland species *Sagittaria latifolia*. Intact *in vitro* produced corms exhibited varying degrees of dormancy depending on the corm induction treatment. Corms produced in medium supplemented with 190 mM sucrose under short days (SD) at 20 °C exhibited the least dormancy (47.8% shoot sprouting). Corms produced in either the presence of 50 μM ABA or under SD at 20 °C for 8 weeks displayed greater dormancy. Breaking corm dormancy was achieved by corm cutting treatments or removal of corm lateral bracts. Longitudinally or latitudinally cutting of corms promoted *in vitro* sprouting. Cutting corms disrupts dormancy by exposing internal tissue and decreasing the inhibited effect of an internal dormancy factor such as ABA content. Removal of the lateral bracts on intact corms, produced in the presence of ABA under LD, resulted in 98% sprouting with 3 leafy shoots per corm. Lateral bract removal on corms produced under SD at 20 °C resulted in 82% sprouting with 2 leafy shoot per corm. Results of this study support the suggestion that factors affecting induction of *in vitro* corm formation can also influence subsequence corm dormancy and sprouting capacity. The ability to enhance corm spouting increases production efficiency three-fold. This study also demonstrates the potential application of *in vitro* plant culture for producing wetland plants for restoration and conservation purposes.

P-2042

In Vitro Micropropagation of Cacao by Leaf and Nodal Segments. OLUFEMI SOBOWALE[1], Esther E. Uchendu[1], Felix Oluwasegun Alao[2], and Victor O. Adetimirin[1]. [1]Department of Agronomy, University of Ibadan, Ibadan, NIGERIA and [2]Department of Biological Sciences, Bells University of Technology, Ota, NIGERIA. Email: sobowale2006_amin@yahoo.com; esteruchendu@yahoo.co.uk

Theobroma cacao L. is a crop of global economic importance. It is traditionally propagated by rooted cuttings and graftings but these methods have low and inefficient propagation rates. A high percentage is grown from seeds resulting in yield variations due to its heterozygous genetic background. An *in vitro* micropropagation method using somatic embryos is common but the length of time to somatic embryo production is long and many genotypes are unable to induce somatic embryos. The major challenges associated with cacao tissue culture is lack of clean materials due to its high endogenous bacteria contaminants and poor growth.This study aimed to develop an alternative tissue culture protocol for clonal micropropagation of five cacao improved cultivars (CRIN TC I, II, V,VIII and F3 Amazon) using leaf and nodal segments i.e. micropropagation by axillary buds. To tackle the problem of endogenous bacterial contaminants, experiments were set up to standardize the disinfection procedure. Treatment in 7% (v/v) sodium hypocloride solution for 10 min followed by 70% ethanol (v/v) for 2 min and subsequent transfer into Murashige and Skoog (MS) basal medium with 0.5 mg/l 2,4-D, 3% sucrose and 5μg/l ciprofloxacin at pH 5.7, was most effective for producing clean shoots from nodal segments. Clean leaf cultures were also obtained, but without *in vitro* regeneration. The controls for both explants (without antibiotics) had zero regeneration and high levels of bacterial contamination. The predominant bacterial contaminant isolated from all 5 cultivars was a Gram negative rod, *Ochrobactrum anthropi*. This organism was also susceptible toofloxacin (5μg/l) and gentamicin (10μg/l); however, it showed high resistance to cefixime (5μg/l), ceftazidime (30μg/l), cefuroxime (30μg/l), nitrofurantoin (30μg/l), and

augmentin (30μg/l). We recommend adding an antibiotic into the culture medium for *in vitro* regeneration of cacao.

P-2043

In Vitro Selection, Greenhouse and Field Evaluation for Assessment of Salt Tolerance in Taro and Sweet Potato. VIRENDRA M. VERMA. Micronesia Plant Propagation Research Center, Kosrae Agricultural Experiment Station, Cooperative Research and Extension, College of Micronesia-FSM, Kosrae, MICRONESIA. Email: vmv_vmv@hotmail.com

Salinity, an abiotic stress that combines elements of water deficiency and sodium toxicity is among the most serious and widespread of agricultural problems on islands resulting in lost crop yield and arable land. Therefore, the efforts to develop salt-tolerant plants are of immense importance to increase crop productivity. In recent years, tissue culture based in vitro selection has emerged as a feasible and cost-effective tool for developing and/or screening salt tolerant germplasm. Taro and sweet potato are most important staple food crops in the Pacific Region for local consumption as well as for export. These crops contribute significantly to the socio-economics and provide livelihood to almost all island people and thus are crucial for ensuring nutritional and economic security. Both taro and sweet potato are placed on high agricultural priority but limitations in availability of salt tolerant germplasm, and disease-free and elite seedlings, is a major bottleneck in production. Therefore, the study was undertaken for assessment of salt tolerance in taro and sweet potato through in vitro selection, followed by greenhouse and field evaluation. To establish aseptic cultures for collected germplasm of taro and sweet potato, various experiments were performed. Different concentrations of sodium chloride were used for in vitro selection of salt tolerant germplasm. This in vitro selected germplasm was further evaluated for salt tolerance in the greenhouse and finally was field evaluated at eight sites in four replications. Some varieties of taro and sweet potato performed very well at coastal sites. Results based on various physiological and morphological parameters collected during the research are presented in this paper.

P-2044

Effect of Roots on Artemisinin and Flavonoid Production in Shoots of *Artemisia annua.* S. WANG, P. J. Weathers, and M. J. Towler. Worcester Polytechnic Institute, Dept. of Biology and Biotechnology, 100 Institute Road, Worcester, MA 01609. Email: swang6@wpi.edu; weathers@wpi.edu

Artemisinin is a potent antimalarial sesquiterpene lactone produced and stored in the glandular trichomes (GLTs) of *Artemisia annua* L. Although they produce no artemisinin, nor any of the precursor compounds, *A. annua* roots appear to have a regulatory effect on production of the terpene in leaves. However, more information is needed to define the role of the roots in artemisinin production in the plant. We used grafting among 3 cultivars to measure phenotypic responses: SAM and #15 cultivars both have GLTs, but produce artemisinin at 1.4 and 0.8% DW, respectively; GLS cultivar produces neither GLTs nor artemisinin. Compared to ungrafted plants, all self-grafts, e.g. SAM/SAM (scion/rootstock), increased scion artemisinin probably from grafting stress. SAM/#15 grafts yielded less artemisinin than SAM/SAM, but more than either #15/#15 or ungrafted #15 and SAM suggesting rootstock inhibition of the scion. SAM/SAM also had more artemisinin than #15/SAM, which was also greater than either #15/#15 or ungrafted #15 and SAM. The #15/SAM graft also produced more artemisinin than SAM/#15, and with the other grafting results suggested that SAM roots were stimulating artemisinin production in the #15 scion. There was no appearance of either GLTs or artemisinin when GLS scions were grafted to SAM indicating that GLTs had to be present to receive putative signals from SAM rootstocks. Other artemisinic metabolites, total flavonoids, and GLTs numbers were also measured. The various phenotypes were analyzed several months after grafting indicating a persistent change and suggesting a possible epigenetic alteration of the scion. This study will provide valuable information regarding the role that roots play in the production of artemisinin in the shoots.

P-2045

In Vitro Recovery of Embryonic Axes of Temperate Trees after 11-23 Years of Storage in Liquid Nitrogen. DANIEL BALLESTEROS and Valerie C. Pence. Center for Conservation and Research of Endangered Wildlife (CREW), Cincinnati Zoo & Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220. Email: Daniel.Ballesteros@uv.es

Liquid nitrogen (LN) storage has been recommended for the long-term conservation of plants with seeds that do not survive traditional seed banking, such as short-lived or recalcitrant seeds, but there are few reports confirming its effectiveness for these species. In this study, embryonic axes of *Juglans nigra* (short-lived), *Aesculus hippocastanum* and *A. glabra* (recalcitrant) stored for 11-23 yrs in LN were thawed, cultured in vitro, and evaluated for survival. Excised axes were originally dried under the air flow of a laminar flow hood to moisture levels of 5% (*J. nigra*) to 30% (*Aesculus* spp.), transferred to cryovials, and cryopreserved by direct immersion of the cryovials in LN. At the time of storage, survival of a cryopreserved control was evaluated by culturing

the axes on MS medium with 2 mg/L each of BAP and IAPhe, but studies with freshly harvested axes and axes removed after 9.5 yrs indicated that growth regulators were not needed, and in this study, samples were cultured without hormones. Embryos were judged to be alive when swelling or growth had occurred after two weeks of culture. Axes of *J. nigra* stored for 23 years in LN maintained the high viability (>80%) that had been observed both initially and after 9.5 years. Similarly, embryos stored for 11 yrs showed high viability (60-100%), depending on the accession. The normal-appearing plantlets from the 23 year-old axes were successfully acclimatized to soil, although conditions in the lab/greenhouse did not support continued growth of all but one of the plantlets. Axes *of A. glabra* showed a decline in viability to 33% after 23 years in storage, a decline from 75% after 9.5 yrs. However, viability of *A. hippocastanum* axes remained high (90%) after 23 years in LN. Surviving *Aesculus* axes did not produce plants, but did produce somatic embryos or axillary shoots. These results demonstrate that LN storage can preserve embryo axes of *J. nigra* and *Aesculus* spp., for at least two decades, providing a workable conservation tool for these species that cannot survive in traditional seed banks. This work supported in part by IMLS grant # LG-25-12-0595.